

QUALITY CHANGES INDUCED BY MECHANICAL STRESS ON ROMA-TYPE
TOMATO AND POTENTIAL ALLEVIATION BY 1-METHYLCYCLOPROPENE

By

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This document is dedicated to the graduate students of the University of Florida.

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Abstract of Thesis Presented to the Graduate School
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By

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Roma-type tomato (*Lycopersicon esculentum Mill.*) at different ripeness stages was subjected to a range of impact forces simulating commercial handling conditions to evaluate the effect of impacts on fruit quality during ripening. A single drop from 60 cm increased CO₂ and ethylene production for roma tomato at mature-green, breaker, or pink ripeness stages while there was no significant difference in TTA, SSC, and pH. Compared with 20-cm or 40-cm impact, a single impact equivalent to 60-cm drop height caused more injury symptoms such as increased respiration rate, ethylene production and ripening rate on roma tomato at breaker stage.

After the first experiment, a pendulum impactor was adopted to provide more accurate impacts for subsequent tests. Double impacts equivalent to a 40-cm drop height induced significant increases in respiration rate and ethylene, and promoted softening at breaker than pink or light-red stage. SSC, TTA, pH and sugar/acid ratio of roma tomato were rarely affected by impact force or by fruit maturity when fruit was impacted.

In addition, 1-MCP was applied to breaker-stage fruit (<10% red) prior to impacts and the effect on stressed-fruit quality was assessed. 1-MCP treatment ($1 \mu\text{l}\cdot\text{l}^{-1}$ for 24 h at about 22°C) delayed softening, color development and the increase in PG activity 2.5 times over non-1-MCP treated control; 1-MCP also decreased the respiration rate. Double impacts of 40 cm or 80 cm did not increase CO_2 production in 1-MCP-treated fruit, while 1-MCP did not reduce the acceleration in ethylene production, softening and color development due to impacts. Double impacts of 80 cm caused a rapid increase in PG activity, while double impacts of 40 cm did not. The variation in injury caused by impact force, fruit maturity, or 1-MCP treatment has to be considered to minimize the loss of quality in roma-type tomato induced by impact during commercial handling procedures.

CHAPTER 1 INTRODUCTION

The second largest fresh vegetable in the U.S. for export, tomato (*Lycopersicon esculentum Mill.*) ranked number three in consumer preference of vegetables at the retail level. Only potatoes and lettuce are rated higher. The per capita consumption of tomatoes in the United States is approximately 39.8 kg per year, 8.2 kg of fresh tomato, and 31.6 kg of processing tomato (ERS USDA, 2004). Tomato's pigment, lycopene, was recently reported to protect against prostate cancer, which encourages consumption of tomatoes. In addition, people love tomato as a good source in diets because of its low calories (24 cal/100g fresh wt.).

In mid-1990's, consumer demand for fresh flavor tomatoes increased, which introduced several specialty tomatoes such as roma, cherry, yellow, cluster, grape, ugly and mini-pear types in the food market. Specialty tomatoes have unique color, shape, and flavor. Roma-type tomato, also called Italian tomato or plum tomato, is one of the most popular specialty tomatoes. This small, elongated, pear-shaped fruit is very prolific and it has less water content and good flavor, which makes it good for canning, puree, or paste. In addition, the roma-type fruit was reported to be so firm that cracking was not a problem (Clark et al., 1999). Thus, this specialty tomato occupies a corner on the specialty tomato market. Its high acceptance was reported by Grassbaugh et al. (2000).

In 2003, 113 million Mt tomatoes were produced in the world, and the U.S. produced about 13 % of total production. Tomato was planted in 166,100 hectares and about 10.4 million Mt tomatoes were produced in the U.S. (FAOSTAT, 2004). Of this

amount, roma-type tomato produced in the U.S. was about 0.12 million Mt. The U.S. imported 0.86 million Mt tomatoes and 0.18 million Mt tomatoes were exported in 2002 (FAOSTAT, 2004). Seven percent of the U.S. fresh tomato exports in 2003 and 26% of the U.S. fresh tomato imports in 2002 was roma-type tomato (Foreign Agricultural Service, 2004).

Florida produces virtually the entire fresh market field grown tomatoes in the United States from December through May each year, and accounts for about 50% of all domestic fresh tomatoes in the U.S. each year. California is the number one state in processing tomato production (95%). Tomatoes for processing were planted in 125,000 hectares and harvested as 8.9 million Mt in the U.S. in 2003 (FAOSTAT, 2004).

Tomato ripeness stage is divided into six stages based on the percentage of the external color: mature green (no external red coloration), breaker (<10% red color at blossom end), turning (10% to 30% of fruit surface having some red color), pink (30% to 60% of fruit surface having some red color), light red (60% to 90% of fruit surface having some red color development), and red (at least 90% of fruit surface having red color).

Tomatoes are transferred at least 15 times before packing. Damage from bruising during improper handling and shipping is one of the major causes of poor quality of fresh tomatoes. Fixed pressure, impacts, or vibrations bruise tomatoes. Halsey (1955) reported the bruising in tomatoes was caused more by impacts rather than by fixed pressure. Impact bruising developed from dumping, sorting, tossing or dropping onto hard surfaces. Bruising is easily overlooked because much of it is hidden damage, but internal

bruising symptoms are severe: water-soaking tissues, cloudy and watery gel, and seed detachment from the placenta (Halsey, 1955; Hatton and Reeder, 1963).

Incidence of bruising injury was influenced by the kind of damaged tissue, by the stage of maturity, and by the severity of the treatment. Moretti et al. (1998) reported locules are the most visibly affected and vulnerable tissue to bruising. Also, it was reported by McColloch (1962) that damage increased with drop height and maturity, and turning-stage fruits got bruising four times as mush as green-stage tomatoes (Halsey and Showalter, 1953). Sargent et al. (1992b) mentioned tomato dropped at breaker ripeness stage developed more internal bruising than those dropped at green stage. Also, there were cultivar differences in impact damage susceptibility (Halsey, 1953; MacLeod et al., 1976).

In the study of impact injury, how to simulate the impact during handling is the most important and difficult part. Most common methods to simulate impact were dropping fruits from different heights, dropping certain mass onto the fruits, or using a pendulum impactor with a known mass. Dropping individual fruit from different heights onto a solid surface (Brusewitz et al., 1991; Fluck and Halsey, 1973; Kunze et al., 1975; Maness et al., 1992) is the method that most simulates actual impacts during harvest, dumping, and packing procedures. However, Kunze et al. (1975) reported that the height of the drop may be uniform, but the weight of the specimen fruit varies, causing the impact energy to vary from fruit to fruit. A modified method of this fruit falling system, certain mass was dropped from predetermined heights onto the fruits placed immediately below the constraining fruit. This method is an appropriate simulation method since impacts can be defined and this method is repeatable.

The pendulum impactor is the most commonly used method in the test of mechanical injury (Bajema and Hyde, 1995; Desmet et al., 2004; Hang and Prussia, 1989; Marshall and Burgess, 1991; Molema et al., 1997; Noble, 1985; Sober et al., 1990; Topping and Luton, 1986; Zeebroeck et al., 2003). Finney and Massie. (1975) used an instrumented pendulum to improve the understanding of impact characteristics and the damages to fruit. Fluck and Ahmed (1973) and Molema (1999) also mentioned that the pendulum impactor has advantages that impacts energy can be quantified and the impactor arm can be immobilized after impact to avoid repetitive impacts. Another advantage of using the pendulum is repeatability, the same as known mass impact method. Bajema and Hyde (1998) cited that impact energy is dissipated in more than one place in usage of an impact device, while the impact energy is predictably absorbed in one area of the specimen in the method of dropping a specimen onto a rigid surface.

Correlation between laboratory studies and commercial procedures have been proved by studies on the incidence of bruises produced by moving through postharvest operations (Grant et al., 1986; O'Brien et al., 1978; Sargent et al., 1987) and by mathematical modeling (Holt and Schoorl, 1985).

Researchers have spent a lot of time and effort to reduce horticultural losses induced by mechanical injury. At the end of the 1980's, pseudo-fruits were used to measure impacts in commercial packing lines in the U.S. Impact data loggers such as IS 100 measure accelerations in three directions. Research using the IS was carried out on apple, citrus fruit, kiwifruit, pear, potato, onion, tomato and peach packing lines. This electronic sphere is now used worldwide in harvesting and processing operations to locate high risk zones for bruising (Molema, 1999). Another way of using the IS to

analyze impacts during handling is by dropping the IS onto various surfaces. The relationship between the impact and the size of the bruise was verified using apples dropped from the same distance onto the same surface (Sober et al., 1990). Also, Marshall and Burgess (1991) used IS as a pendulum, which has been proven to be a valuable tool in evaluating bruise damage criteria.

Ethylene plays a central role in initiating and acceleration of ripening-related processes in fruits (Nagata et al., 1995; Theologis et al., 1992). To maintain quality and delay over-ripening and senescence, much research has focused on the inhibition of ethylene action. As a result, 1-methylcyclopropene (1-MCP) as an effective ethylene antagonist was found to be a good derivative for practical use since it is less volatile, nontoxic, odorless, and effective. Sisler and Serek (1997) showed that low concentration ($0.5 \text{ nL}\cdot\text{l}^{-1}$) of 1-MCP could inhibit ethylene action and proposed a model of how 1-MCP reacts with the ethylene receptor, since the affinity of 1-MCP for the receptor is approximately 10 times greater than that of ethylene. 1-ethylcyclopropene (1-ECP) and 1-propylcyclopropene (1-PCP), two structural analogues of 1-methylcyclopropene (1-MCP), were also found to inhibit ethylene action.

1-MCP has been shown to have the following effects on the physiology of fruits and vegetables: less ethylene production, less respiration rate, reduced color change. 1-MCP also reduce levels of phenolics, dimethyl trisulfide production, α -farnesene accumulation and oxidation, increase of (alpha)-L-arabinofuranosidase (AF-ase) activity (Xu et al., 2004) and cell-wall degradation (Botondi et al., 2003; Jeong et al., 2002; Mao et al., 2004). The effectiveness of 1-MCP treatment differs by the concentration, treatment time, treatment temperature of 1-MCP, and ripeness stage of treated fruit.

There are several reports about 1-MCP application on tomato. Serek et al. (1995) reported that 1-MCP applied before the initiation of ripening can prevent tomatoes from responding to applied ethylene for a period of several days. Furthermore, 1-MCP can arrest tomato ripening at various stages of ripeness (Hoeberichts et al., 2002; Mir et al., 1999; Rohwer and Gladon, 2001; Wills and Ku, 2002). As a biochemical approach, 1-MCP in tomato can negatively regulate ethylene production through the inhibition of the accumulation of ACC synthase and ACC oxidase mRNA (Nakatsuka et al., 1998). Negative feedback regulation of ethylene production is mediated through the repression of certain ethylene biosynthetic pathway genes expressed in the early stages of ripening (system I) while other genes are not affected by ethylene (Nakatsuka et al., 1998). Hoeberichts et al. (2002) cited that 1-MCP mediated decrease in transcript levels of ripening-related genes in tomato may account for its effect on ripening.

So far, not much research has investigated the quality of roma-type tomato subjected to impact. The primary factors affecting susceptibility of roma tomato to mechanical injury are the ripeness stage of the fruit and impact force. In the addition, the potential effectiveness of 1-MCP on impacted roma-type tomato is attractive to tomato growers and shippers.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Tomato Production

2.1.1 Consumption and Production of Round Tomato

The tomato originated in South America, and has been cultivated in Europe and the United States for over 200 years. Today there are about 400 varieties of tomatoes grown. More suitable varieties in certain areas are selected based on the following qualities; yield, disease resistance, horticultural quality, adaptability, and market acceptability (Olson et al., 2004). Characteristics of withstanding disease and physical stresses during harvest, packing, and transportation were importantly concerns since early times.

The second largest fresh vegetable of U.S. export, tomato (*Lycopersicon esculentum Mill.*) ranked number three in consumer preference of vegetables at the retail level. Only potatoes and lettuce exceed them. The per capita consumption of tomatoes in the United States is approximately 39.8 kg per year, 8.2 kg of fresh tomato, and 31.6 kg of processing tomato (ERS USDA, 2004). Tomato's pigment, lycopene, was recently reported to protect against prostate cancer, which encourages consumption of tomatoes. Also, people love tomato as a good source in diets because of its low calories (24 cal/100g fresh wt.).

In 2003, 113 million Mt tomatoes (e.g., round, roma, cherry, grape, pear-type tomato) were produced in the world, and the U.S. produced about 13 % of total production. Tomato was planted in 166,100 hectares fields, market value of tomato in the U.S. was 1.2 billion dollars and about 10.4 million Mt tomatoes were produced in the

U.S. (FAOSTAT, 2004). Based on the FAO reports, 0.86 million Mt tomatoes were imported, 0.18 million Mt tomatoes were exported and 0.57 million Mt tomatoes were wasted in the U.S. in 2002 (FAOSTAT, 2004).

Competition in world tomato markets has become more severe because of increasing suppliers. The top fresh tomato exporters are Spain, Mexico, Canada, the United States, Italy, France, and Turkey (Foreign Agricultural Service, 2004). While Canada, the United States, Italy, France, and Turkey supplied similar amounts of tomatoes every year from 2000 to 2002, exports from Spain and Mexico were dominant and sharply increased. The major market for fresh tomatoes is the United States, importing 800 million dollars tomato in 2002. Other major importers are France, Canada, Italy, Mexico, and Japan. In processing tomato market, Italy is dominant. In 2002, Italy accounted for approximately 80 percent of the world's canned tomato exports. Since 2000, China's exports of tomato products have grown immensely. In particular, China became the second largest producer of tomato paste in 2002. In 2003, China exported tomato paste three times as much as that in 2000 (Foreign Agricultural Service, 2004).

The United States is the world's second leading producer of tomatoes, after China. In 2003-2004, the U.S. exported 73 thousand Mt, 14% increase and imported 593 thousand Mt, 6% decrease (Foreign Agricultural Service, 2004). Tomato exports increased 20 percent from 1999-2003. Main export markets of U.S. are Canada and Mexico. The U.S. exported \$127 million of tomatoes (84%) to Canada and 9 million dollars of tomatoes (6%) to Mexico. In 2002, the U.S. imported 800 million dollars of tomatoes, consisting 560 million dollars of tomato (70%) from Mexico and 170 millions of tomato (20%) from Canada (Foreign Agricultural Service, 2004).

Florida produces virtually the entire fresh-market, field-grown tomatoes in the United States from December through May each year, and accounts for about 50 percent of all of the domestically produced fresh tomatoes in the U. S. each year. Florida tomatoes are exported primarily to Canada. Florida competes with imports from Mexico during most of the season while exports to Mexico are minimal. Florida also supplies tomatoes for the foodservice industry in Japan. Growers in Florida tried to produce more tomatoes from November to January since the price of tomatoes during this period is the highest during the year. Average value per carton was 13.30 dollars in December while it was 5.93 dollars in May, 2003 (USDA, 2003). Tomatoes for processing were planted in 125 thousands hectares and harvested as 8.9 million Mt. California is the number one state in processing tomato production, producing 95% of the total.

Tomato is a climacteric fruit, which shows the peaks in the respiration rate and ethylene production during ripening. The respiration rate and ethylene production of standard round tomato are moderate (10-20 mg/hr and 1.0–10.0 µl/kg-hr, respectively). Recommended storage temperature for maintaining good quality of tomato is 13–18 °C. Since tomato is sensitive to the chilling injury, it should not be stored below 13 °C during ripening.

There are six ripeness stages based on the percentage of the external color as the following table according to the U.S. Dept. of Agric. Grade Standards.

Table 2-1. Tomato ripeness stage.

Ripeness stage	Surface-Color change
1 (green)	green
2 (breaker)	< 10% red
3 (turning)	10 - 30% red
4 (pink)	30 - 60% red
5 (light-red)	60 -90% red
6 (red)	> 90% red

Tomatoes are often harvested at green stage to extend handling and transportation. Mature-green tomatoes consist of four stages of maturation, M1 to M4, based on locular gel development (Kader and Morris, 1976); M1 with immature, white-color seeds and no gel in the locule, M2 with mature, tan seeds and gel formation in at least two locules, M3 with gel formation in all locules and still green, internal color and M4 with watery gel, and red color gel and pericarp tissue. Tomatoes harvested at immature-green stage ripen poorly, resulting poor color and poor taste (Kader et al., 1978; Maul et al., 1997, 1998a, 1998b). To get good quality of tomatoes, only mature-green tomato should be picked. Unfortunately, immature green fruit can comprise an average of 31% to 49% of fruit intended to be harvested at the mature-green stage because of the difficulty in distinguishing them from the mature green based on the external color (Maul et al., 1998b). A nondestructive method to select mature green fruit without cutting would be useful.

Tomatoes are graded to meet the requirements for U.S. No. 1, U.S. Combination or U.S. No. 2 of the U.S. Standards for Grades of Fresh Tomatoes. When not more than 15 percent of tomatoes in any lot fail to meet the requirements of U.S. No. 1 grade and not more than one-third of this 15 percent (or 5 percent) are comprised of defects causing very serious damage, including not more than one percent of tomatoes which are soft or affected by decay, such tomatoes may be shipped and designated as at least 85% U.S. No. 1 grade. After grading, tomatoes are packed in the 11.4 kg (25 lb) cartons.

2.1.2 Consumption and Production of Specialty Tomato Types

In mid-1990's, consumer demand for fresh-flavor tomatoes increased, which induced introductions of several specialty tomatoes such as roma (plum), cherry, yellow, cluster, grape, heirloom and mini-pear types in the food market. Unique color, shape, and

flavor of specialty tomatoes satisfy not only the eyes, but also taste of consumers. In summer of 2001-2002, Maynard (2002) reported 46% of weekly sales of tomato in Bloomington, Indiana, were in specialty tomatoes. The fact, the U.S. shipment change in second the quarter of 2004 was 8.7% in tomato and 30.3% in cherry-type tomato over 2003, revealing the increasing demand for specialty tomatoes (Agricultural marketing service, USDA).

Despite the growing demand, profitability of specialty tomatoes was limited because of damage and disease problems. Vigneault et al. (2000) reported the small size of cherry, grape, and mini-pear types would appear to exacerbate decay, because their irregular surfaces might make water removal difficult after the washing. In addition, an inconsistent supply might cause customers to hesitate to buy specialty tomatoes. Maynard (2002) cited that Midwest consumers would purchase more specialty tomatoes if these tomatoes were available in central Indiana and the greater Chicago area.

Most research has been conducted on round tomatoes, and there is currently, little reliable postharvest information on high-flavor, specialty tomatoes. Sargent et al. (1999) studied flavor and postharvest life of seven cluster tomatoes and Licher et al. (2002) reported cracking of cherry tomatoes was affected by calcium treatment, storage, solution acidity and harvest time. Two laser-based detection systems constituting photothermal deflection and the other photoacoustics have been qualified as useful methods to detect ethylene release from ripening cherry tomatoes subjected to mechanical injury (De Vries et al., 1995). Mass transfer kinetics of osmotic dehydration in cherry tomato was studied by Azoubel and Murr (2004).

2.1.3 Roma Tomato

Roma-type tomato, also called Italian tomato or plum tomato, is one of most popular specialty tomatoes. It is a hybrid of tomato which originates from inter-specific crossbreeding among *Lycopersicon lycopersicum*, *Lycopersicon pimpinellifolium* (Red Currant type) and *Lycopersicon chesmanii* (Muratore et al., 2004). The acreages of roma varieties is increasing, but is still relatively small compared to regular tomato. Hybrid 882, Yaqui, and Monica are the most common roma tomato varieties in California and Marina, Plum Dandy, Spectrum 882, Supra and Veronica are commonly grown in Florida (Olson, S.M et al, 2004).

Roma-type tomato production in the U.S. was about 0.12 million Mt. In 2003, the U.S. exported 10 million dollars of roma tomato, which was 7% of U.S. fresh tomato exports; 26% of U.S. fresh tomato imports in 2002 were roma-type tomato (Foreign Agricultural Service, 2004).

This small, elongated, pear-shaped fruit is very prolific and it has less water content than regular tomato and good flavor, so good for canning, puree, or paste. In addition, the Roma-type fruit was much firmer and cracking was not a problem (Clark et al., 1999). Its high acceptance by buyers was reported by Grassbaugh et al. (2000). The demand for roma-type tomato has rapidly increased in the recent years due to both its sensory characteristics and high concentrations of bioactive constituents, such as vitamin C, carotenoids, lycopene and polyphenols (Muratore et al., submitted for publication). Thus, this specialty tomato occupies a corner of the market.

Despite increasing demand, susceptibility to disease in the field decreases yield of roma-type tomato. Mullen and Mickler (2003) reported that water stress coupled with heat caused serious blossom-end rot in roma-type tomato, but only minimal rot in the

round-type line. While roma-type tomato is often displayed without any film or container on the shelf at markets in the U.S., Muratore (2004) showed the polymeric materials used for the container must be appropriately chosen in order to slow down all the detrimental phenomena responsible for the decay during storage. To satisfy consumers demand, more researches on better varieties with disease resistance and increased flavor are required. Handling and storage information is also necessary to reduce postharvest quality losses.

2.2 Mechanical Injury

2.2.1 Extent of Mechanical Injury

Injury induced by mechanical stress is one of the major causes of postharvest losses in the world (FAO, 1989). Wright and Billeter (1975) reported that mechanical injury was the leading cause of quality loss at wholesale and retail levels for apples, peaches, potatoes, and strawberries. Peleg (1984) noted that loss of the yield (about 30%) in some fruit and vegetable crops might be caused by bruising. Annually, the \$2.5 billion U.S. potato industry loses an estimated \$300 million by bruising (Brook, 1996), and Storey and Davies (1992) noted internal damage induced by impacts on potato tubers may cause losses in excess of 20%. The U.S. \$1.2 billion apple and the \$280 million pear industries also suffer substantial losses each year due to bruising (USDA, 1999). Annually, researchers make efforts to reduce bruising in fruits and vegetables.

Mechanical injury, such as cutting, bruises, and abrasions, causes marked deterioration in fruit quality (Quintana and Paull, 1993; Shewfelt, 1993). The use of mechanical equipment for the harvesting, packing and transportation of fruits has increased; the mechanical injury on fruits and vegetables has become a very important problem (Brusewitz and Bartsch, 1989; Marshall and Brook, 1999). O'Brien et al. (1969) reported that up to 40% of peaches were damaged on the road journey of 260 km.

Maindonald and Finch (1986) also noted up to 16% of apples in bulk bins at various locations on two different trucks got appreciable damage. Mechanical injury is cumulative (Bollen et al., 2001; Sargent et al., 1989). The proportion of damaged tomatoes increased from 15 % before to 35 % after dumping. (O'Brien et al. 1972) and internal bruising on tomato increased from 5.2 % to 23.8 % from the float tank to the grading table (Sargent et al., 1989).

Mechanical injury is generally caused by impact, compression, or vibration (Brusewitz et al., 1991; Vergano et al., 1991). Garcia et al. (1988) identified impact as “the most important cause of damage and losses in fruits during harvesting, handling, and transportation operations”. Impact bruising develops when a fruit falls onto a hard surface or onto other fruits during harvesting, packing, or transportation. When an abject falls onto the fruits, bruises can also happen (Crisosto et al., 1993). Sweetpotato showed a 72% increase in the respiration rate after physical impact (Saltveit and Locy, 1982). Sommer et al. (1960) also reported that stone fruits and pears are more susceptible to impact pressure than to vibration pressure.

When fruits are compressed by other fruits or other objects, pressure bruising also can be induced (Crisosto et al., 1993). This was shown by the fact that the amount of initial damage was 26% of harvested sweet cherries in the bottom half of the bins compared to 16% of those in the top. Lulai et al. (1996) also found that pressure-induced bruising in potato tuber elicited little or no biological wound-healing response compared to the relatively higher changes reported for cut and skinned tubers. A cause of quality loss on potatoes, blackspot formation is a symptom of pressure-bruising inducing water loss (Lulai et al., 1996). Strawberries and apples showed more injury when subjected to

compression than impact (Guilou, 1964; Holt and Schoorl, 1976; Holt and Schoorl, 1982).

Another cause of mechanical injury, vibration, usually occurs during transportation, which caused high percentages of losses (Jones et al., 1991). Vibration during transportation can be a serious problem for tomatoes. Vibration pressure can result in about 20% losses of their round shape, decreased firmness, and increased decay rates (Singh et al., 1992), and citric and malic acids in vibration-pressured tomato were increased (Nakamura et al., 1977).

2.2.2 Bruising

Bruising is major cause for fruit rejection (Brusewiz and Bartsch, 1989). For apple, grade is influenced by bruise diameter, depth, and the number of bruise (USDA, 1978). Eighty-nine percent of the ‘Golden Delicious’ apples were bruised during packinghouse operations (Bartram et al., 1983), and approximately 23% of ‘Golden Delicious’ apples fail to meet the requirements of highest quality because of bruising (Held et al., 1974).

Bruising is initiated by the breakage of cell membranes (Scoorl and Holt, 1983), leading cytoplasmic enzymes to react with vacuolar contents (Rouet-Mayer et al., 1990; Shewfelt, 1993). Partington et al. (1999) reported that bruised tissue undergoes cell death in contrast to wounded tissues showing continued induction of defense proteins, tissue repair and no cell death. About 3 h after impact, cell death was initiated and coincided with de-compartmentalization, the appearance of lipid peroxides and melanin production. Finally, bruising induces death and dehydration of all the cells, which leave a large space indicating loss of effective barrier against infection (Partington et al., 1999).

McColloch (1962) reported that much of the bruising injury is internal, and is not visible on the outside of the tomato. Externally bruised fruits can be eliminated during

processing, but internal bruising is easily overlooked. Although internal bruising is hidden damage, its symptom is severe. Internal bruising of tomato was described by Halsey (1955) as “water-soaked cellular breakdown of crosswall and locular areas” and by Hatton and Reeder(1963) as “ broken-down of the locular gel from the normal, clear pink to a cloudy, yellowish, stringy, collapsed, disorganized and gelatinous tissue.”

2.2.3 Quality Changes Caused by Mechanical Injury

Mechanical injury has been correlated with metabolic and quality changes in fruits and vegetables. MacLeod et al. (1976) reported that C₂H₄ production increased within 1hr after drop impacts in tomatoes. In winter squash, this was related to increases in 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase (Hyodo et al., 1993). Increase in respiration rate was reported in pea and bean (Tewfik & Scott, 1954), apple (Dewey et al., 1981), cherry (Mitchell et al., 1980), sweet potato (Saltveit and Locy, 1982) and tomato (MacLeod et al., 1976). Also, soluble solids content decreased in grape (Morris et al., 1979), firmness decreased in cucumber (Miller et al., 1987), and aroma volatiles changed in tomato (Moretti et al., 2002b). Panelists in a sensory test described the internally bruised tomato as having “watery,” “bland” flavor (Moretti et al., 1998). In cucumber, mechanical stress induced biochemical and morphological changes in major tissues and polygalaturonase activity significantly increased in all tissues (Miller et al., 1987). Lee et al. (2004) also reported that polyphenol oxidase activity increased more rapidly in the bruised persimmon than in the non-bruised fruit during storage. Furthermore, mechanical injury affects vitamin C content. Bruised locular tissue of tomato showed about 15% lower vitamin C content than non-bruised tomatoes (Moretti et al., 1998). Increases in total glycoalkaloids (TGA) levels (Dale et al., 1998) and increased

levels of phenols, glycoalkaloids and reduced level of ascorbic acid (Mondy et al., 1987) were observed in potato in response to the damage.

Incidence of mechanical injury was influenced by the kind of damaged tissue. Moretti et al. (1998) reported locules are the most visibly affected and vulnerable tissue to bruising in tomato. Moretti et al. (2002b) explained tightly bound pericarp cells might have a crucial role in the transmission of impact energy to the underlying locule tissue. Szczeniak and Smith (1969) also reported that commodities with large cells suffer more injury than those with small cells.

Another factor influencing the susceptibility to mechanical injury is the stage of ripeness. For example, riper tomatoes were more susceptible to mechanical damage (Olorunda and Tung, 1985). “Turning” stage developed four times as much bruising injury as mature-green tomato, and eight times as much as immature-green tomato (Halsey and Showalter, 1953). Internal bruising was more pronounced on breaker-stage than mature-green stage tomatoes (Sargent et al., 1989). Mature peaches also had larger bruise volumes and were more susceptible to bruising than less mature peaches (Hung and Prussia, 1989). Oppositely, Vergano et al. (1991) reported green peaches were more susceptible to bruising than ripe ones. As apples ripened, bruising decreased (Diener et al., 1979).

Fruit turgidity and firmness also have been shown to influence impact bruise susceptibility in apple and pear. Analysis of impact response showed that stresses in the tissues were higher in turgid apple or pear, so turgid fruit was more susceptible to bruising (Garcia et al., 1995). Horsfield et al. (1972) noted that desiccating the fruit to reduce turgor decreased bruise damage. In general, turgor (hydration) has more effect

than temperature on bruising for apples. Slight reductions in hydration (2 to 3 % mass loss) can reduce turgor enough to double the bruise threshold (Hyde et al., 2001).

Temperature can influence the tissue resistance to bruising by affecting the cell turgor. Warm sweet cherry developed fewer bruises than cold ones (Lidster et al., 1980). While bruising of apple increased with increasing fruit temperature at impact (Saltveit, 1984), Klein (1987) found no relationship between apple temperatures at impact and bruise susceptibility. Baritelle and Hyde (2001) have shown that the failure strain of the tissue increased as handling temperature increased for both potato and pear. In kiwifruit, reducing the temperature soon after impact decreased ethylene production and appearance of injury symptoms (Mencarelli et al., 1996). Heating papaya at 48 °C for approximately 6 hours or until fruit core temperature (FCT) reached 47.5 °C worsened the severity of skin mechanical injury (Quintana and Paull, 1993).

Susceptibility to mechanical injury was dependent on cultivar (Fridley and Adrian, 1966; Kunze et al., 1975; Vergano et al., 1991). In peach, Schulte-Pason et al. (1990) reported drops from 5 cm caused bruising to 80% of specimens while Kunze et al. (1975) reported that using a higher drop caused a much lower bruising: 25% for a 40 cm drop, 90% for a 130 cm drop. Also, chlorogenic acid levels increased in two cultivars but not in the others in potato tubers of five cultivars (Dale et al., 1998).

Bruising is linearly related to impact energy (Chen and Sun, 1981; Hung and Prussia, 1989; Pang et al., 1992). It was reported by McColloch (1962) that the damage in tomato increased with increased the drop height, and two drops caused more internal bruising than single drop. Mathew and Hyde (1997) also showed that drop height influenced not only the amount, but also the type of bruise damage in whole potato

tubers. Although there is a good correlation between energy absorbed and external damage (Grant and Hughes, 1985), the relationship with internal damage is much less clear and is affected by impact duration and the extent of deformation (Hemmat, 1987).

2.2.4 Impact Sensitivity

Other factors also can influence tissue resistance to bruising. McGarry et al. (1996) mentioned that internal damage is diverse depending on genotype, fruit maturity, environment conditions and physical, physiological and biochemical properties. Baritelle and Hyde (2003) noted that potatoes with higher specific gravity tubers were more sensitive to impacts.

Impact sensitivity has two components: bruise threshold and bruise resistance (Bajema and Hyde, 1998). Bruise threshold is the drop height onto a given impact surface at which a fruit of a given mass and radius of curvature will just begin to bruise. Bruise resistance is the ratio of bruising energy to the resulting bruise volume, which has correlation with tissue-failure stress. Mechanical injury, a bruise, results when impact-induced stress exceeds bruise threshold or tissue failure stress (Pitt, 1982). To reduce impact bruising requires reducing impact-induced stress and/or increasing tissue failure stress. Modifications to some transfer points resulted in over 50% reduction in impact levels (Sargent et al., 1992a). Most impact damage produced in packing lines is usually at transfer points where fruit is conducted from one unit operation to the next (Ortiz-Cañavate et al., 2001). Once the damaging transfer points were identified, several solutions have been proposed (Marshall et al., 1989), such as reduction of the drop heights, use of padding materials (García-Ramos et al., 2002), use of decelerator elements (García-Ramos et al., 2003b; Ortiz-Cañavate et al., 2001), and elimination of structural elements in the receiving belts (Ortiz-Cañavate et al., 2001). Cushioned rollers, powered

brushes, the presence of padding material on the lateral plate of the transporting belts can reduce the impact values (García-Ramos et al., 2003a). Since there are limitations to the improvement of handling equipment, the alternative of improving the commodity is worth investigating. The influence of temperature, relative turgor, and strain-strain rate on the tissue impact failure properties were reported (Bajema et al., 1998a,b; Baritelle and Hyde, 2000). Also, bruising can be controlled to some degree by management of hydration. The packinghouse compromise is between loss in fruit weight and gain in bruise-free fruit (Hyde et al., 2001).

Reducing mechanical damage can also increase food safety by decreasing the potential for microbial infection, and disposal problems associated with these defects are also reduced. For example, increases in total glycoalkaloids (TGA) levels in response to damage were observed in potato (Dale et al., 1998). TGAs at high concentrations impart a bitter taste (Siden et al., 1984). At level above 20 mg glycoalkaloids 100 g⁻¹ fresh weight, potato tubers are considered unsuitable for human consumption. Consumption of high levels of TGAs may result in a number of symptoms typically associated with food poisoning (Van Gelder, 1990).

Several factors that influence bruising susceptibility are mentioned above. However, researchers frequently have reported conflicting results. Klein (1987) and Johnson and Dover (1990) showed that bruising increased from early to late harvest time in apple. However, Diener et al. (1979) reported that bruising decreased as apples matured. With respect to storage, Klein (1987) concluded that bruise volume decreased with storage time. On the other hand, Brusewitz and Bartsch(1989) reported that the change in bruise volume per unit change in total impact energy increased with storage

time. Saltveit (1984) reported that bruise susceptibility increased with increasing fruit temperature; however, Schoorl and Holt (1978) reported the opposite effect.

To improve handling systems, reduce losses, and protect human health, more research efforts to better understand the physical and physiological factors influencing the mechanical injury are required.

2.3 Impact Simulation

2.3.1 Methods of Impact Simulation

In the study of impact injury, simulating the impact during handling is the most important and difficult part. Most common ways to simulate impact were dropping fruits from different heights, dropping certain mass onto the fruits, or using a pendulum impactor.

Dropping individual fruit from different heights onto a solid surface is a method modifying actual fruit falling during harvest, dumping, and packing procedures (Brusewitz et al., 1991; Fluck and Halsey, 1973; Kunze et al., 1975; Maness et al., 1992). Hand-held samples are dropped from a specified height onto a hard surface (Fluck and Ahmed, 1973; McRae, 1978; Vergano et al., 1991) or on other fruits (Kunze et al., 1975). Sargent et al. (1992b) used a vacuum pump to hold fruits, which allowed fruits to fall without rotation. Since incidence of mechanical injury was influenced by the kind of tissue damaged (Moretti et al., 1998), this method is a useful way to research response of each tissue to the mechanical stress. Lichtensteiger et al. (1988) used an impact force transducer to record impact characteristics of fruit falling onto it. Although the falling specimen simulated more closely the kind of impacts occurring in real handling systems, problems were found in drop test compared to the other method. Kunze et al. (1975) reported that the height of the drop may be uniform, but the weight of the specimen fruit

varies, causing the impact energy to vary from fruit to fruit. Similarly, Bajema and Hyde (1998c) mentioned the disadvantage of the falling system is that it must be weighed and its mass must be considered, since heavier specimens impact with greater energy when dropped.

A modified method of the drop method was to drop a known mass from predetermined heights onto the fruits (Bajema et al., 1998a; Chen et al, 1987; Garcia et al., 1988; Kunze et al., 1975; Weaver and Steen, 1966). Dale et al. (1998) built standard bruising equipment, consisting of a 100 mg bolt dropped from a height 100 cm onto a hand-held tuber placed immediately below the constraining tuber. This method is one of appropriate simulation ways because of defining inflicting impacts, and excluded factors affecting the kinetic energy of impact. Furthermore, this method is repeatable.

The pendulum impactor is the most commonly used system in the testing mechanical injury (Bajema and Hyde, 1995; Desmet et al., 2004; Hung and Prussia, 1989; Marshall and Burgess, 1991; Molema et al., 1997; Noble, 1985; Sober et al., 1990; Topping and Luton, 1986; Zeebroeck et al., 2003). Finney and Massie (1975) used an instrumented pendulum to improve the understanding of impact characteristics and the damages to fruit. Topping and Luton (1986) constructed a model similar to that of Finney and Massie (1975). The apparatus consisted of a jig to hold the fruit, and an impactor head was mounted on the end of a single pendulum. A single two-channel incremental optical encoder was mounted on the pivot axis to measure either drop or rebound height via a digital pulse counter. In practice, the impact energy was kept constant by drawing the pendulum back to an adjustable stop. The indentation of fruit during impact was measured using a photoelectric system. In their test, it was found that about 90% of the

impact energy was absorbed and the rebound energy was small. Therefore, that result showed there was very little difference between the impact and absorbed energy. Another method of Bittner et al. (1967) and Nelson and Mohsenin (1968) used a single pendulum technique to swing an apple into a solid steel block or a cushion, while Hyde and Ingle (1968) used a double pendulum apparatus to swing a spherical impact object into a stationary apple. More advanced techniques with pendulum were also used to measure bruise susceptibility of fruits. The Constant-Height Multiple-Impact technique was used to measure bruise resistance and the Increasing-Height Multiple-Impact technique was used to measure the bruise threshold of individual specimens.

Hemmat (1987) reported that with the pendulum impactor method, impact energies are not dependent on the mass of a fruit. Thus, this method is more appropriate for answering questions about the susceptibility of fruit to internal damage and is more useful in making comparisons between fruits of different mass, density or shape. Fluck and Ahmed (1973) and Molema et al. (1999) also mentioned that pendulum impactor has the advantage that impacts can be quantified in terms of impact energy. Another advantage of using the pendulum is repeatability, similar to the known mass drop impact method. Bajema and Hyde (1998) cited that using an impact device has the disadvantage that the impacting energy is dissipated in more than one place, while the impact energy is predictably absorbed in one area of the specimen in the method of dropping a specimen onto a rigid surface. In addition to the energy-partitioning problem with impacting devices, if the pendulum has a significantly smaller mass than the specimen, then it must be dropped from a significantly higher height to achieve the same impact energy. Bajema and Hyde (1998) cited that a high drop height would result in an inappropriately higher

impact-loading rate than a real commodity impact rate. This is not an ignorable problem. Since fruits and vegetables are viscoelastic, their mechanical properties vary significantly with impact rate.

Questions about correlations between laboratory studies and commercial practices have been solved by studies focusing on the incidence of bruises produced by moving through postharvest operations (Grant et al., 1986; O'Brien et al., 1978; Sargent et al., 1987) and by mathematical modeling (Holt and Schoorl, 1985).

2.3.1 Impact Data Loggers

Researchers have spent a lot of time and effort to reduce horticultural losses due to mechanically caused damage. They have tried to locate the critical points in fruit and vegetable handling chains. At the end of the 1980's, pseudo-fruits were introduced to measure handling impacts in packing lines in the U.S. (Siyami et al., 1988; Tennes et al., 1990; Zapp et al., 1990). A self-contained spherical impact data-acquisition system, an Impact Recording Device (IRD) was developed by Tennes et al. (1989) as a result of collaborative research by the Agricultural Research Service of the U.S. Department of Agriculture (USDA), the Michigan Agricultural Experimental Station and Michigan State University. The instrumented sphere (IS) (Model IS100, Techmark, Lansing, Mich.) measures accelerations in three directions. It has a 300.6-g mass and is in 8.8-cm diameter, consisting of a spherical shell containing a tri-axial accelerometer, co-processor, clock, memory, and battery. The IS records acceleration and velocity change for each impact over elapsed time. It has a port to communicate with a PC for set-up and data transfer of records in internal memory.

After the introduction of the IS100, other portable data loggers become available such as the PTR100 (Bioteknisk Institute, Denmark) in 1990 and the PMS60 (Institute of

Agricultural Engineering, Germany) in 1992. An advanced electronic sphere measuring pressure first was described by Herold et al. (1996) from the Institute of Agricultural Engineering (ATB Germany). This device registered static and dynamic forces acting on the fruit and vegetables. Recently, the range of models increased with the development of the PTR 200 (SM Engineering, Denmark) in 1999 and the ‘Smart Spud’ (Sensor Wireless, Canada) in 2000. Most impact data loggers measure impact acceleration (IS100, PTR100, PTR200, Smart Spud). Impact data loggers are now used worldwide to evaluate harvesting and handling operations to locate bruising high risk zones (Molema, 1999).

Research using the IS100 was carried out on packing lines for apple (Brown et al., 1987; Bollen and Dela Rue, 1990; Garcia et al., 1994; Jourdain et al., 1993; Ragni and Berardinelli, 2001; Schulte-Pason et al., 1989, 1990; Sober et al., 1990), citrus fruit (Miller and Wagner, 1990), kiwifruit, pear (Bollen and Dela Rue, 1990), potato (Molema, 1999), onion (Herold et al., 1998), tomato (Desmet et al., 2004; Sargent et al., 1996a) and peach (Berardinelli et al., 2001; Schulte-Pason et al., 1990; Crisosto et al., cited by Hansen, 1999). A study on the risk that fruits can get damaged during transportation was carried out using IS100 (Schulte-Pason et al., 1989).

Another way of using impact recording devices is to analyze impacts during handling by dropping onto various surfaces. The relationship between the impact and the size of the bruise was verified using apples dropped from the same distance onto the same surface (Sober et al., 1990). Also, Marshall and Burgess (1991) used the IS100 as a pendulum, which has been proven to be a valuable tool in evaluating bruise damage

criteria. There was a high correlation coefficient and low standard error between bruise diameter and impact energy.

The following research answered the question about the efficiency and accuracy of one impact data logger. Leicher(1992) and Nerinckx and Verschoore (1993) used the PTR 100 and found a predictive capability of 53%. Molema (1999) stated that the IS100 cannot be used to predict potato discoloration and the sensor output should rather be quantified as the equivalent height of a drop onto a defined surface. Zapp et al. (1990) stated that IS100 impact data are of very limited value unless bruise threshold and bruise resistance of the commodity are known. Mathew and Hyde (1997) developed a damage predicting model on the basis of peak acceleration for the IS 100.

2.4 Reduction of Tissue Sensitivity

2.4.1 Ethylene Action Inhibitors

Ethylene is a natural plant hormone that coordinates ripening processes in fruits such as softening, color, firmness, soluble solids, loss of acidity, flavor, etc. Tomato is a climacteric fruit and its ripening is highly dependent on ethylene action (Nagata et al., 1995; Rothan and Nicolas, 1989; Theologis et al., 1992; Yang and Hoffman, 1984). Ethylene plays a central role in initiating and acceleration of ripening-related processes that we perceive as changes in quality in tomato. To maintain quality and delay over-ripening and senescence, many studies have focused on the inhibition of ethylene action. As a result, effective ethylene antagonists were identified.

Compounds such as carbon dioxide, silver thiosulfate (STS), aminoethoxyvinylglycine (AVG), 2, 5-norbornadiene (2, 5-NBD) and diazocyclopentadiene (DACP) were reported as effective antagonists of ethylene. Hobson et al. (1984) reported silver ions inhibit ethylene action and prevent ripening, and DACP

and STS increased the display life of potted rose (*Rosa*) ‘Victory Parade’ (Serek et al., 1994). DACP binds permanently to the ethylene binding site, so it inhibits ethylene action (Serek et al., 1994). However, its usage is limited because of explosiveness in high concentrations (Sisler and Serek, 1997). NBD, the first widely applied cyclic olefin, competes with ethylene at the receptor level (Sisler et al., 1986). However, NBD usage is limited to use under continuous gas flow because of its toxic and offensive-smell (Sisler and Serek, 1999). 1-ethylcyclopropene (1-ECP) and 1-propylcyclopropene (1-PCP), two structural analogues of 1-methylcyclopropene (1-MCP), were also found to inhibit ethylene action (Goren et al., 2001). 1-ECP was found in all cases more potent than 1-PCP but less potent than 1-MCP. Feng et al. (2004) cited 1-ECP and 1-PCP might be used as practical antagonists of ethylene, since these compounds are volatile, non-corrosive, non-toxic, odorless, and effective at low concentrations.

1-methycyclopropene (1-MCP) is the most commercially used ethylene inhibitors, having a molecular weight of 54 and a formula of C₄H₆ at standard temperature and pressure. Johnson (1987) mentioned that 1-MCP is nontoxic, odorless, and less volatile than cyclopropene which is breakdown products of diazocyclopentadiene (DACP). Sisler and Serek (1997) showed that low concentration (0.5 nl·l⁻¹) of 1-MCP could inhibit ethylene action. Synthesis of 1-MCP can be accomplished using the methods of Magid et al. (1971) or Fisher and Applequist (1965). 3-MCP is another effective inhibitor, but a higher concentration is required compared with 1-MCP (Sisler et al., 1999).

Commercialization of 1-MCP was first undertaken by Floralife, Inc. (Walterboro, SC) for use on ornamental crops. The product was approved by U.S. Environmental Protection Agency (EPA) in 1999 for ornamentals and is sold under the trade name

EthylBloc®. Commercial application of 1-MCP to edible crops was undertaken by AgroFresh, Inc., a subsidiary of Rohm and Haas (Spring House, PA), under the commercial name SmartFresh®. Both EthylBloc® and SmartFresh® are approved for use in United States and many other countries.

2.4.2 Effect of 1-MCP

Sisler and Serek (1997) proposed a model of how 1-MCP reacts with the ethylene receptor, and determined that the affinity of 1-MCP for the receptor is approximately 10 times greater than that of ethylene. Thus, 1-MCP can be active at much lower concentrations than other ethylene antagonists. 1-MCP binds permanently to receptors present at the time of treatment. Sisler et al. (1996a) showed 1-MCP permanently binds in carnation tissue. If ethylene binding is prevented, ethylene no longer promotes ripening and senescence. Commodities can be ripen and soften more slowly, so their quality and edible condition is maintained for longer periods of time. 1-MCP also influences ethylene biosynthesis in some species through feedback inhibition.

1-MCP has been shown to have an effect on the physiology of fruits and vegetables. Less ethylene production and less respiration rate by 1-MCP has been reported in apple (Fan and Mattheis, 1999), apricot (Botondi et al. 2003; Dong et al., 2002), avocado (Jeong et al., 2002), pear (Trinchero et al., 2004), plum (Abdi et al, 1998; Dong et al., 2002) and chinese jujube (Jiang et al., 2004). 1-MCP also delayed softening in banana (Jiang and Joyce, 2003), persimmon (Xu et al., 2004), plum (Menniti et al., 2004) and strawberry (Jiang et al., 2001). Another effect of 1-MCP is reduction of color change in broccoli (Gong and Mattheis, 2003; Ku and Wills, 1999), orange (Porat et al., 1999), and pear (Trinchero et al., 2004). In biochemical level, 1-MCP reduced levels of phenolics (Jiang et al., 2001), dimethyl trisulfide production, which contributes to off-

odor development in broccoli florets(Forney et al., 2003), α -Farnesene accumulation and oxidation (Shaham et al., 2003), increase of (alpha)-L-arabinofuranosidase (AF-ase) activity (Xu et al., 2004) and cell-wall degradation (Botondi et al., 2003; Jeong et al., 2002; Mao et al., 2004).

In the addition, 1-MCP exposure prevented crops from quality loss. In watermelon, 1-MCP prior to ethylene exposure reduced ethylene-induced increases in the activities of lipid-degrading enzymes and completely prevented water-soaking, major cause in loss of watermelon (Mao et al., 2004). Interestingly, development of lettuce russet spotting (Fan and Mattheis, 2000) and accelerating senescence of damaged pollinia (Heyes and Johnston, 1998) were also inhibited by 1-MCP.

On the other hand, 1-MCP exposure had no significant effects on soluble solids content in apple (DeEll et al., 2002), nectarine (Liguori et al., 2004) and plum (Menniti et al., 2004). Also, respiration rate, color and stem browning in sweet cherry (Gong et al., 2002) and the loss of fruit weight and firmness in orange (Porat et al., 1999) were not altered by 1-MCP treatment.

The effectiveness of 1-MCP treatment is affected by the concentration, treatment time, and treatment temperature of 1-MCP. The accelerated degradation of protein and chlorophyll in parsley treated with $0.01\mu\text{l}\cdot\text{l}^{-1}$ 1-MCP was reported by Ella et al. (2003) who mentioned “such a low 1-MCP concentration might have been too low to block the ethylene receptors efficiently, while being enough partially to relieve the ethylene auto-inhibitory effect.” Mir et al. (2004) also found that the continuous 1-MCP application completely inhibited color development of tomato at breaker ripeness stage. Ethylene production in peaches was not inhibited by 1-MCP at 20°C but was inhibited after

application at 0 °C (Liguori et al., 2004). In banana, the effectiveness of 1-MCP varied with banana fruit maturity (Harris et al., 2000). Another factor altering the efficacy of 1-MCP is the air composition. 1-MCP was more effective in the control atmosphere (CA) storage than in air for apple (Watkins et al., 2000). Dauny et al. (2003) reported that avocado fruit having high oil concentration absorbed 1-MCP more and faster than apple and water, and mentioned that the ability of crops to absorb 1-MCP may influence the efficacy of 1-MCP as a ripening inhibitor.

Maturity of fruit treated with 1-MCP also can affect 1-MCP effectiveness. Watkins et al. (2000) mentioned that high ethylene-producing cultivars, especially entering the climacteric period at harvest time, might show less responsiveness to 1-MCP. Receptor regeneration may provide explanation of this. Although 1-MCP binding to the ethylene receptor sites is irreversible, the formation of new receptor sites during the climacteric period causes ethylene sensitivity to return (Sisler et al., 1996a; Yen et al., 1995).

More care on the 1-MCP treatment is required. Given the reduced pathogen resistance found for 1-MCP-treated plants (Diaz et al., 2002; Hofman et al., 2001; Jiang et al., 2001), the possibility of decay enhanced by 1-MCP treatment should be evaluated. Harris et al. (2000) reported an unacceptable, uneven skin coloration in banana ripened with 1-MCP and Mir et al. (2004) reported the locular gel of 1-MCP-treated tomato was noticeably greener than that of untreated fruit ripened to the same external color. The increased ethylene biosynthesis in 1-MCP-treated parsley was also reported by Ella et al. (2003).

There are numerous reports about 1-MCP effects on tomato. Serek et al. (1995) reported that 1-MCP applied before the initiation of ripening can prevent tomatoes from responding to applied ethylene for a period of several days. Furthermore, 1-MCP can arrest tomato ripening at various stages of ripeness (Hoeberichts et al., 2002; Mir et al., 1999; Moretti et al., 2002a; Rohwer and Gladon, 2001; Wills and Ku, 2002). Inhibition of the accumulation of ACC synthase and ACC oxidase mRNA's by 1-MCP indicates that a strong negative feedback regulation exists for ethylene production in tomato. Negative feedback regulation of ethylene production is mediated through the repression of certain ethylene biosynthetic pathway genes expressed in the early stages of ripening (system I) while other genes are not affected by ethylene (Nakatsuka et al., 1998). This 1-MCP mediated decrease in transcript levels of ripening-related genes in tomato may account for its effect on ripening (Hoeberichts et al., 2002).

1-MCP can arrest fruit ripening at low concentration, so it is welcomed commercially. In addition, no death or clinical signs on humans, animals and the environment treated with 1-MCP was reported by Environmental Protection Agency (2002). In case of tomato, 1-MCP may make harvest of tomato at breaker stage or greater possible, which can eliminate the ethylene treatment used to initiate ripening and the harvest of immature fruit, improve uniformity of fruit color, avoid the need for repacking, and reduce the need for refrigeration detrimental to flavor (Maul et al., 2000).

In the future, it is necessary to learn how to effectively use 1-MCP to maximize the quality of products and profits for agricultural businesses and to use 1-MCP as a research tool to understand the role of ethylene in plant development.

2.5 Research Objectives

The objectives of this research are following:

- 1) To evaluate the effect of different impact forces on the quality of roma-type tomatoes at breaker ripeness stage.
- 2) To evaluate the effect of impact on the quality of roma-type tomatoes at each ripeness stage.
- 3) To evaluate the effect of pretreatment of 1-methylenecyclopropene (1-MCP) on reducing the expression of mechanical injury on roma-type tomatoes subjected to impact stress.

CHAPTER 3

DEVELOPMENT OF IMPACT PROCEDURES FOR ROMA-TYPE TOMATOES

3.1 Introduction

Mechanical injury is related to metabolic disorders and quality change. Tomato subjected to impact stress showed increased ethylene production (MacLeod et al., 1976) and changed aroma volatiles (Moretti et al., 2002). One of factors influencing the susceptibility to mechanical injury is the stage of maturity. For example, ripe tomato was more subject to injury than less ripened fruit (Olorunda and Tung, 1985). Bruising is also linearly related to impact energy (Chen and Sun, 1981; Hung and Prussia, 1989; Pang et al., 1992). McColloch (1962) said that the damage increased with the drop height and two drops caused more internal bruising than single drop. Hyde (1997) also showed that drop height influenced not only the amount but the type of bruise damage in whole potato tubers.

Dropping individual fruit from different heights onto a solid surface is a method modifying actual fruit falling during harvest, dumping, and packing procedures (Brusewitz et al., 1991; Maness et al., 1992). Sargent et al. (1992b) used a vacuum pump to hold fruits, which allowed fruits to fall without rotation. Since incidence of mechanical injury was influenced by the kind of tissue damaged (Moretti, 1998), this method is a useful way to research response of each tissue to the mechanical stress. As another method of impact simulation, the pendulum impactor is the most commonly used system in the testing mechanical injury (Bajema and Hyde, 1995; Desmet, 2004; Zeebroeck, 2003). Hemmat (1987) reported that with the pendulum impactor method, impact

energies are not dependent on the mass of a fruit. Thus, this method is more appropriate for answering questions about the susceptibility of fruit to internal damage and is more useful in making comparisons between fruits of different mass, density or shape. Fluck and Ahmed (1973) and Molema (1999) also mentioned that pendulum impactor has the advantage that impacts can be quantified in terms of impact energy.

The objective of this experiment was to develop the proper impact procedures for roma-type tomatoes.

3.2 Materials and Methods

3.2.1 Test1. Drop Impacts on Roma-Type Tomato at Different Ripeness Stages and Fruit Quality

3.2.1.1. Plant material

Roma-type tomato (variety unknown) was sampled at commercial packinghouse located in Palmetto, FL in May 2003. Fruits were transported to the Postharvest Horticulture Laboratory at the University of Florida, Gainesville. Tomatoes were chosen at mature green (no red coloration on surface), breaker (<10% red coloration), pink (30-60% red coloration), light-red (60-90% coloration) or red (>90% red coloration) ripeness stages (n=6 per ripeness stage).

3.2.1.2 Drop test

Roma-type tomato of uniform size and weight (about 100g) was dropped from two different heights, 15 or 60 cm. A vacuum pump was used to hold tomato prior to dropping; this prevented the fruit from rotating during the drop. Each fruit was held at the desired height and the vacuum released, allowing the fruit to drop directly into a hard plate. Each fruit was caught after one bounce. White chalk dust was scattered on the plate to indicate the location of the impact on the tomato surface. After impact treatment, two

roma tomatoes were placed in each of three clamshells (110*130*70 mm) and ripened at 20 °C.

3.2.1.3 Respiration rate and ethylene production

Two tomatoes were weighed and placed in a 850 ml plastic container, loosely capped with plastic lids fitted with a rubber septum, and were stored at 20 °C. Every other day, the containers were sealed for 1 h, followed by removal of a 0.5 ml sample (for CO₂) and 1.0 ml sample (for ethylene) of the headspace from each container. Carbon dioxide concentration was measured using a gas chromatograph (series 580; GOW MAC, Bridgewater, N.J.) fitted with a thermal conductivity detector and a 1/4 inch Carbo pack column, and ethylene production rate was measured using another gas chromatograph (HP 5890; Hewlett Packard, Avondale, Pa.) fitted with a flame ionization detector and alumina packed column.

3.2.1.4 Firmness

Firmness of whole fruit was evaluated using an Instron Universal Testing Instrument (Model 4411, Canton, MA, USA) equipped with a convex-tip probe (8.0 mm diameter) and 5-kg load cell using a crosshead speed of 50 mm·min⁻¹. Force at 2 mm deformation was recorded in Newtons (N) and there were 2 to 3 measurements per fruit at the equator. Internal firmness was determined on the pericarp slices of tomato using the Instron equipped with a convex-tip probe (4.0 mm diameter) and 5-kg load cell. Each roma tomato was cut equatorially with a sharp, stainless steel knife into 10 mm thick slice, and then this slice was immediately placed on a stationary steel plate. After establishing zero height between the probe and the pericarp tissue, the probe was driven with a crosshead speed of 10 mm·min⁻¹. The force was recorded at 2.5 mm deformation and was measured at 2-3 points per fruit at the junction of the pericarp wall and radial

wall. Whole fruit and pericarp firmness were evaluated on the initial day and 13 d after storage.

3.2.1.5 Compositional analyses

Upon reaching the full-ripe stage (3 d after light-red stage), ten individual fruits were homogenized, followed by centrifugation with 8060 x g, 5 °C for 20 min. The resulting supernatant was filtered using cheesecloth and then frozen at -20 °C for later analysis of the following parameters.

Soluble solids content (SSC). One to two drops of the supernatant as prepared above was placed on the prism of the digital refractometer (Model 10480, Reichert-Jung, Mark Abbe II Refractometer, Depew, NY) and SSC was reported as °Brix.

pH. The pH was determined from the same supernatant with a pH meter (pH meter 140, Corning Scientific Instruments, Medfield, MA) standardized with pH 4.0 and 7.0 buffers.

Total titratable acidity (TTA). Each sample supernatant (6 g) was weighed out and diluted in 50 ml of distilled water. The samples were analyzed by an automatic titrimeter (No. 9-313-10, Fisher Titrimeter II, Pittsburg, Pa.), titrated with 0.1 N NaOH to endpoint of pH 8.2. TTA was expressed as percentage of citric acid.

3.2.1.6 Statistical analysis

The experiment was conducted using a completely randomized design. Statistical analysis was performed using the PC-SAS software package (SAS-Institute, 1985). All data were subjected to analysis of variance and treatment means were compared using Duncan's Multiple Range Test ($P < 0.05$).

3.2.2 Test 2. Development of Pendulum Impact Device

3.2.2.1 Impact simulation – pendulum

During the previous test (drop test), a serious problem was observed. The vacuum used to hold fruit caused bruising on the surface of tomato contacting vacuum, which was not an intended mechanical injury. Thus, the impact method was changed from drop system to pendulum impacting system. Hemmat (1987) reported that impact energies are not dependent on the mass of a fruit in pendulum impactor method. Thus, this method is more appropriate for researching the susceptibility of fruit to internal damage and is more useful in making comparisons between fruits of different mass, density or shape. Fluck and Ahmed (1973) and Molema (1999) also mentioned that the pendulum impactor has advantages that impacts energy can be quantified.

To impact on the surface of tomatoes, a pendulum impactor was built as the following figure 3.1. A lead sinker (230 g) used as a pendulum was coated with rubber material to make even and smooth surface, and was suspended by nylon string. Two steel stands were connected by a steel cross-bar and a pocket (to suspend the tomato) was hung from the cross-bar. A sliding bar stand in the middle of the board was marked to indicate desired angles (equivalent to vertical drops of 20 cm, 40 cm, 60 cm or etc.). To make impacts from pendulum impactor same as the actual dropping from certain heights, the fixed weight of fruits and fixed pendulum angle were used on each experiment.

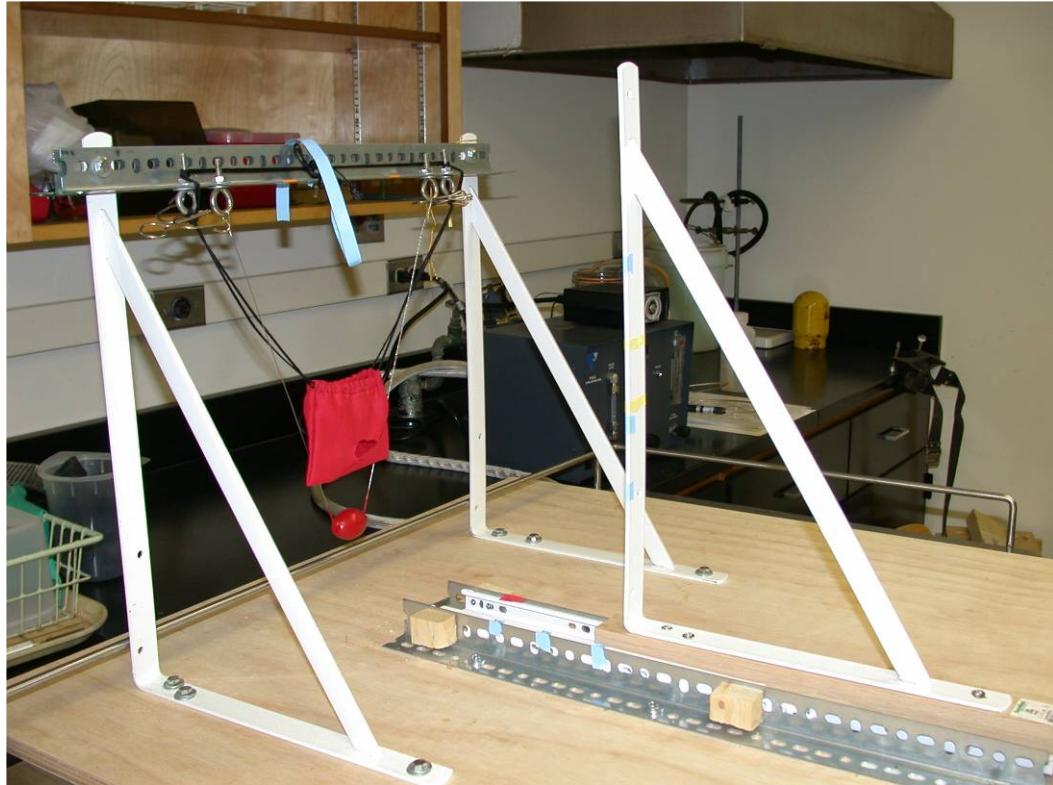
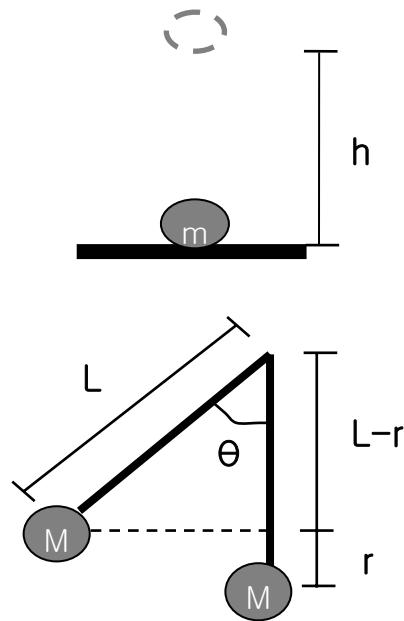


Figure 3-1. General view of the pendulum impactor

Drop force was converted into pendulum impact force by solving the following set of equations:



1) $Mrg = mhg$

where M = mass of pendulum, r = drop height of pendulum, g = gravitational acceleration (9.8 m/s^2), m = mass of tomato, h = drop height of tomato. Solving for r , $r = (m/M)h$

2) $\cos \theta = (L - r)/L$

where L = length of string of pendulum, θ = angle of pendulum.

3) substituting for r ,

$$\cos \theta = (L - (m/M)h)/L$$

Tomatoes were marked with a white paint marker on the locule surface suspended in the pocket, and impacted by the pendulum from desired angles.

3.2.2.2 Validation of the pendulum impactor

To test the consistency of impacts generated by pendulum impactor, an Impact Recording Device (IRD) was used (Model IS 100, Techmark, Lansing, Mich.). The IRD was placed in the pocket of the impact apparatus and impacted 30 times by the pendulum from two fixed angles (30° angle or 50° angle), which were equivalent to drops from about 15 cm or 45 cm. This test was repeated three times. Impacts were measured as maximum acceleration (MA) in G (1 Gravity = 9.8 m/s^2) and velocity change (VC) in m/s. VC indicates the hardness of the surface and is positively correlated with MA. After impact, the IRD was connected to a computer and the impact force data was downloaded. All data was subjected to analysis of variance (ANOVA) and each test mean was compared using F-Test ($P < 0.05$).

3.3 Results and Discussion

3.3.1 Test 1. Drop Impacts on Roma-Type Tomato at Different Ripeness Stages and Fruit Quality

3.3.1.1 Respiration rate

Respiration rate of roma-type tomato during storage changed differently depending on the impact magnitude and fruit maturity when fruit was subjected to impact (Figure 3-2). Roma tomato at mature-green ripeness stage produced the same amount of CO₂ during day 3 to 5 and showed climacteric peak at day 7 when fruit produced around 13.5 ml CO₂·kg⁻¹·h⁻¹. While a drop of tomato at mature-green stage from 15 cm resulted in a 30% increase in the respiration rate during day 3 to 5, fruit dropped from 60 cm height showed respiratory climacteric peak 2 d earlier and its respiration rate at that time was 15% higher than non-impacted fruit.

At day 3, roma tomato at breaker ripeness stage had the highest respiration rate and produced CO₂ less and less until day 8. Although tomato at breaker stage dropped from 15 cm had higher respiration rate than control, its pattern of CO₂ production was similar to non-impacted fruit. The 60 cm drop height induced a change in CO₂ production pattern during the entire storage and a 30% decrease in respiration rate at day 9.

Carbon dioxide production of tomato at pink ripeness stage continuously decreased during the entire storage. While there was no difference in CO₂ production pattern between dropped and non-dropped fruit, a drop from 60 cm height caused 20% increase in respiration rate of fruit at pink stage during day 3 to 7 (Figure 3-2).

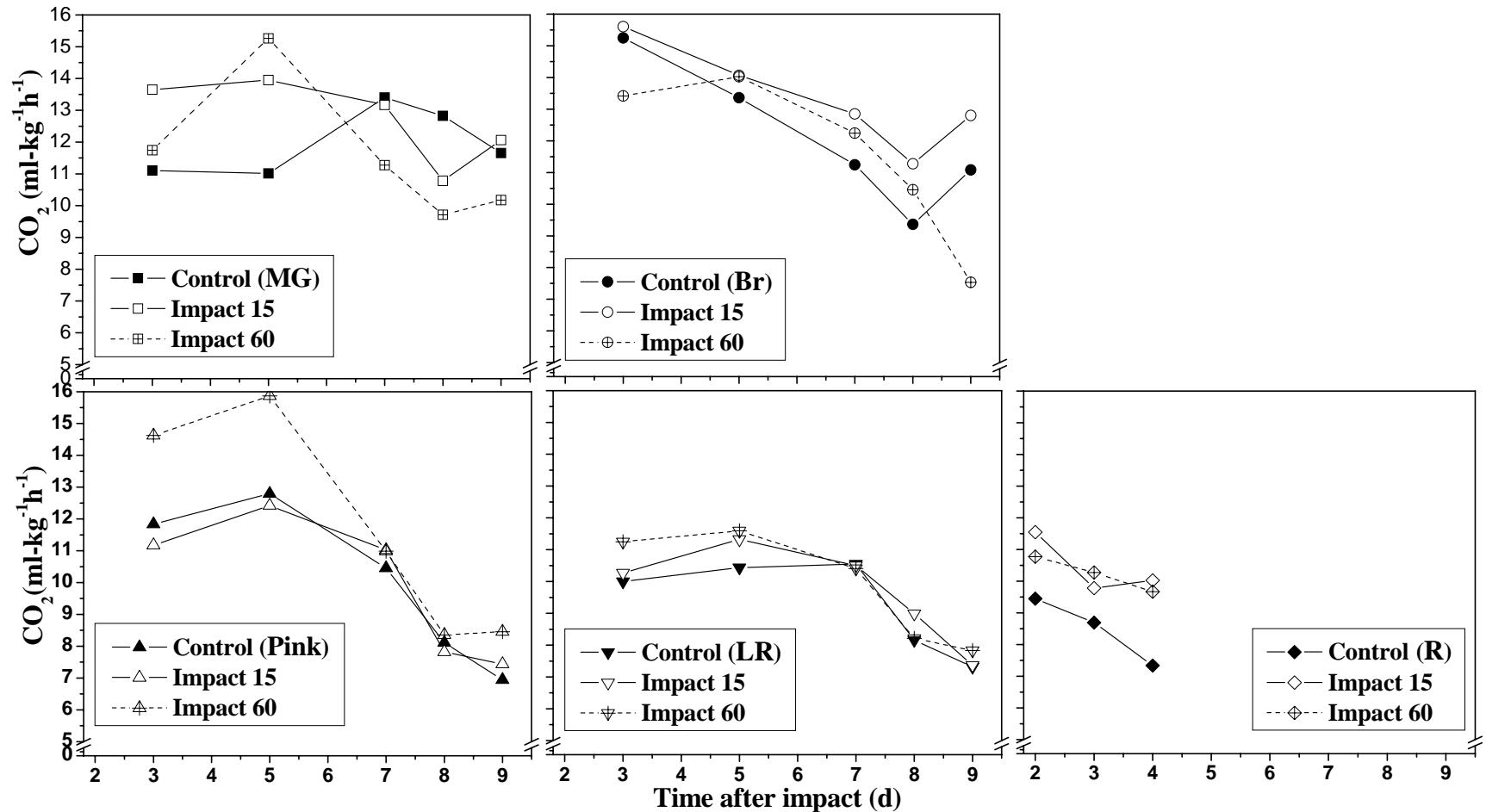


Figure 3-2. Respiration rate of roma-type tomato during ripening at 20 °C. Fruit at mature-green (■), breaker (●), pink (▲), light-red (▼) or red ripeness stage (◆) was dropped from 15 cm (each open symbol) or 60 cm height (each open symbol with cross).

Dropping roma tomato at light-red stage from 15 or 60 cm height drop did not affect the respiration rate during storage. The respiration rate of roma-type tomato at red ripeness stage also continuously decreased regardless of drop height. Roma tomato dropped at red color stage produced 20 to 40% more CO₂ than non-dropped fruit during the entire storage.

Impact affected differently the respiration of roma-tomato at each ripeness stage. Fruit dropped at mature-green stage showed accelerated climacteric peak and increased maximum CO₂ production while there was no significant change in respiration of tomato caused by a drop at light-red stage. Olorunda and Tung (1985) and Halsey and Showalter (1953) reported that the susceptibility to mechanical injury depended on fruit maturity. In the addition, a 60cm drop at pink ripeness stage seems to cause mechanical injury on roma tomato based on the significant increase in respiration. Damaged plant tissue had higher respiration rate because of oxidation of phenolic compounds (Knee and Miller, 2002). Massey et al. (1982) also suggested that respiration rate can be used as an indicator of mechanical injury in cranberries.

3.3.1.2 Ethylene production

Similar to respiration rate, ethylene production of roma-type tomato during ripening changed depending on the impact magnitude and fruit maturity when fruit was subjected to impact (Figure 3-3). Roma tomato at mature-green ripeness stage produced the same amount of ethylene during day 3 to 5 and showed climacteric peak at day 7 when fruit produced around 4 µl C₂H₄·kg⁻¹·h⁻¹. Otherwise, tomato dropped at mature-green stage exhibited a continuous increase in ethylene production after day 5 and no climacteric peak. At day 9, the dropped mature-green fruit produced 60% more ethylene than non-dropped one.

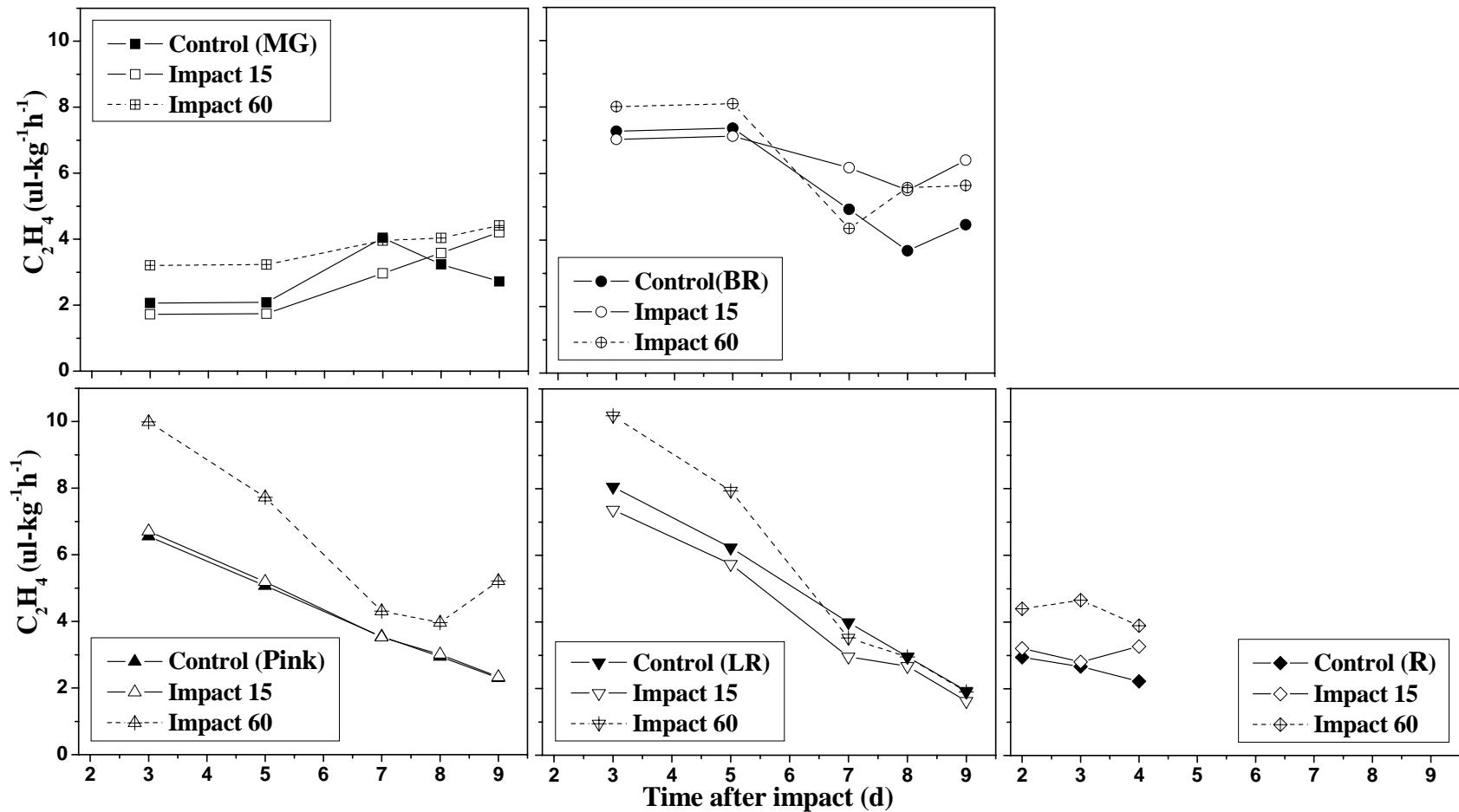


Figure 3-3. Ethylene production of roma-type tomato during ripening at 20 °C. Fruit at mature-green (■), breaker (●), pink (▲), light-red (▼) or red ripeness stage (◆) was dropped from 15 cm (each open symbol) or 60 cm height (each open symbol with cross).

Roma-type tomato at breaker ripeness stage had the same ethylene production during day 3 to 5 regardless of drop height; ethylene production steeply decreased during day 5 to 8 (Figure 3-3). Although a drop from 15 cm did not change the trend of ethylene production, breaker fruit dropped from 15 cm produced more ethylene after day 5 than control. Ethylene production of breaker fruit dropped from 60 cm height was not different from that of non-dropped fruit with exception of increased ethylene production during day 7 to 8.

Ethylene production of roma-type tomato at pink ripeness stage continuously decreased during the entire storage. While there was no change in ethylene production caused by a drop from 15 cm, a drop from 60 cm height caused a 23% increase in ethylene production of fruit at pink stage at day 3 (Figure 3-3). Ethylene production of pink stage fruit dropped from 60 cm sharply decreased during day 3 to 7.

At day 3, roma tomato at light-red stage had the highest ethylene production. Otherwise, respiration rate was the highest in breaker fruit at day 3. Ethylene production of light-red stage fruit decreased continuously during storage. Tomato dropped at light-red stage from 60 cm height produced 25 % more ethylene production at day 3 and its ethylene production decreased steeply during day 3 to 7, the same as pink-stage fruit. A drop from 15 cm did not affect ethylene production of fruit at light-red stage. The ethylene production of roma-type tomato at red ripeness stage, also, continuously decreased during the entire storage. Red fruit dropped from 15 cm produced 60% increased ethylene at day 4, while a drop from 60 cm induced 50 to 90% increase in ethylene production of red stage during storage (Figure 3-3).

The effect of a drop on the ethylene production of roma tomato was different by fruit ripeness stage based on the above-mentioned observations. Fruit at different ripeness stages had different susceptibility to mechanical stress (Olorunda and Tung, 1985). Similar to respiration rate, a 60cm drop at pink and light-red ripeness stage induced the significant increase in ethylene production. MacLeod et al. (1976) also reported that C₂H₄ production increased within 1h after drop impacts in tomatoes. Elevated ethylene production in bruised fruit is usually caused by the increased 1- aminocyclopropane-1-carboxylic acid (ACC) and its conversion to ethylene (Yang and Hoffman, 1984). Hyodo et al. (1993) also observed increased biosynthesis of ethylene in winter squash subjected to mechanical stress, and mentioned it was related to increases in ACC synthase and ACC oxidase. In addition, roma-type tomato dropped from 60 cm produced more ethylene regardless of fruit maturity when fruit was dropped oppositely to 15 cm height drop. McColloch (1962) reported that the damage in tomato increased with the increased drop height. In peach, the low level of impact energy did not cause any bruise damage for 21 d storage (Hung and Prussia, 1989).

3.3.1.3 Firmness

Roma-tomato softened during ripening (Table 3-1). External firmness of tomato at mature-green stage was 12.56 N, which was 2.5 times firmer than fruit at red ripeness stage. At 13 d after storage, fruit dropped at mature-green and breaker ripeness stage was still firmer externally than those treated at red stage. However, there was no significant change in external firmness caused by a drop except for breaker fruit dropped from 60 cm which was 33% softer than non-dropped fruit.

As with whole-fruit firmness, the internal tissue of tomato treated at mature-green ripeness stage was significantly firmer than that from the riper fruit. A significant

Table 3-1. Whole-fruit and pericarp firmness of roma-type tomato 13 d after storage at 20 °C.

Treatment	Whole-fruit firmness (N)		Pericarp firmness (N)
	Initial	13 d after storage	13 d after storage
MG ^z (Control)	12.56±0.67 a ^y	4.51±1.71 ab	10.85 ±3.14 a
MG dropped from 15 cm		5.09±0.59 a	9.51±1.63 ab
MG dropped from 60 cm		4.55±0.92 ab	8.28±1.72 b
Br (Control)	11.20±0.76 ab	5.09±0.59 a	5.77±0.56 c
Br dropped from 15 cm		4.38±0.82 ab	5.53±2.25 c
Br dropped from 60 cm		3.43±0.33 bcd	5.88±0.35 c
Pink (Control)	8.11±3.49 bc	3.97±1.11 abc	5.72±0.29 c
Pink dropped from 15 cm		3.59±0.52 bcd	5.50±1.92 c
Pink dropped from 60 cm		3.59±0.81 bcd	4.88±0.65 c
LR (Control)	7.09±0.29 c	3.71±0.87 bcd	4.13±0.33 c
LR dropped from 15 cm		2.93±0.49 cd	4.05±0.31 c
LR dropped from 60 cm		3.40±0.37 bcd	4.34±0.34 c
Red (Control)	5.29±0.21 c	3.06±0.54 cd	4.18±0.97 c
Red dropped from 15 cm		3.00±0.51 cd	4.15±0.57 c
Red dropped from 60 cm		2.54±0.73 d	3.61±0.40 c

^zMG, Br, Pink, LR or Red = roma-type tomato at mature-green, breaker, pink, light-red or red ripeness stages, respectively.

^yMean (n=12) following by standard deviation. Columns with different letters are significantly different at P< 0.05, according to Duncan's Multiple Range Test.

decrease in internal firmness due to dropping was observed only on mature-green fruit at 13 d after storage. The internal firmness of mature-green fruit dropped from 60 cm height was about 70% of non-dropped fruit.

A drop from 60 cm caused softening in roma tomato dropped at mature-green or breaker stage. Softening by mechanical stress was also reported in cucumber (Miller et al. 1987). Miller (1992) said that impact caused cell rupture and loss of tissue integrity, immediately inducing the decrease in firmness at the impacted site. Paralleled to this experimental results, Vergano et al. (1991) reported green peaches were more susceptible to bruising than ripe ones. This can be explained by findings of Garcia et al. (1995) who

noted that mechanical stresses in the tissues were higher in turgid fruit, so turgid fruits were more susceptible to mechanical injury.

3.3.1.4 Compositional analyses

To examine the possibility that a drop impact could affect the flavor of roma-type tomato, the values of soluble solids content (SSC), total titratable acidity (TTA) and pH were measured when fruit at each ripeness stage reached at full-red ripeness stage (Table 3-2).

SSC of fully ripe roma-type tomato was not significantly affected by drop height or by initial fruit maturity. SSC of roma tomato ranged from 1.78 to 2.65. At the evaluated day, fruits dropped during post-climacteric period (light-red and red ripeness stage) showed higher SSC (2.65) than the others (1.85 to 2.20).

Table 3-2. Compositional analyses at full-ripe stage.

Treatment	SSC ^z (°Brix)	TTA (%)	Sugar/acid ratio	pH
MG (Control)	2.20±1.27 a ^y	0.39±0.01 b	5.68±3.22 ab	4.41±0.03 abc
MG dropped from 15 cm	2.25±0.37 a	0.43±0.14 ab	5.62±1.88 ab	4.42±0.03 ab
MG dropped from 60 cm	2.60±0.62 a	0.52±0.11 ab	5.32±2.45 ab	4.41±0.06 abc
Br (Control)	1.98±0.45 a	0.59±0.26 a	3.83±1.73 b	4.29±0.01 c
Br dropped from 15 cm	2.18±0.79 a	0.49±0.09 ab	4.62±2.11 ab	4.33±0.10 bc
Br dropped from 60 cm	2.08±0.54 a	0.42±0.08 ab	5.06±1.55 ab	4.37±0.04 abc
Pink (Control)	1.85±0.34 a	0.36±0.02 b	5.19±0.90 ab	4.44±0.09 ab
Pink dropped from 15 cm	2.10±0.24 a	0.42±0.08 ab	5.14±1.21 ab	4.42±0.11 ab
Pink dropped from 60 cm	2.08±0.40 a	0.38±0.05 b	5.47±0.99 ab	4.41±0.07 abc
LR (Control)	2.65±0.47 a	0.50±0.15 ab	5.52±0.94 ab	4.45±0.08 ab
LR dropped from 15 cm	2.25±0.58 a	0.49±0.05 ab	4.59±1.20 ab	4.41±0.02 abc
LR dropped from 60 cm	1.78±0.58 a	0.48±0.15 ab	4.11±2.57 b	4.45±0.06 ab
Red (Control)	2.65±0.44 a	0.38±0.05 b	7.14±1.28 a	4.44±0.04 ab
Red dropped from 15 cm	1.98±0.72 a	0.59±0.11 a	3.35±1.19 b	4.36±0.12 abc
Red dropped from 60 cm	2.05±0.62 a	0.41±0.08 ab	5.36±2.48 ab	4.46±0.03 a

^zSSC = Soluble Solids Content; TTA = Total Titratable Acidity (citric acid equivalent); MG, Br, Pink, LR or Red = roma-type tomato at mature-green, breaker, pink, light-red or red ripeness stages, respectively.

^yMean (n=8) following by standard deviation. Columns with different letters are significantly different at P< 0.05, according to Duncan's Multiple Range Test.

Total titratable acidity (TTA) of roma tomato at full-red ripeness stage ranged from 0.38 to 0.59. Fruit had the different TTA depending on the maturity when fruit was treated and a drop impact. Fruit treated at breaker stage had the highest TTA (0.59) and tomato treated at mature-green or red ripeness stage had the lowest TTA (0.38 to 0.39).

The pH of fully ripe roma tomato ranged from 4.29 (breaker-stage fruit) to 4.44 (red-stage fruit). Roma-type tomato had the different pH depending on maturity when fruit was treated. Drop impact did not affect pH of fruit at full-red ripeness stage.

Sugar/acid ratio is used to indicate tomato flavor. This ratio was changed by the ripeness stage. The highest ratio (7.14) was observed on red-stage fruit, while breaker fruit had the lowest ratio (3.83) (Table 3-2). On red-stage roma tomato, the dropped fruit had a 25-50% lower ratio than control fruit. Impact at red stage induced an increase in TTA while there was no difference in SSC. Since sugar/acid ratio was calculated as dividing SSC by TTA, this decreased ratio was induced by increased TTA.

There was no significant difference in SSC, TTA, pH and sugar/acid ratio between impacted and non-impacted fruit except red-ripeness stage. Oppositely, the decrease in SSC of grape by mechanical injury was reported (Morris et al., 1979). This suggests that the effect of impact on SSC, TTA, pH and sugar/acid ratio may differ by the kind of commodities. So far, this observation indicates that the flavor of roma-type tomato harvested before red-stage may be maintained in spite of a 60-cm impact. Since sugar/acid ratio is an indicator of tomato flavor and Wills and Ku (2002) mentioned that tomato taste is related to TTA.

3.3.2 Test 2. Development of Pendulum Device

3.3.2.1 Validation of the pendulum impactor

Impact by the pendulum impactor with 30 degree angle or 50 degree angle was obtained using Impact Recording Device (IRD) and expressed as maximum acceleration (maxG) and velocity change (m/s) (Table 3-3).

Impact by the pendulum impactor with 30 degree angle or 50 degree angle was significantly consistent (Table 3-4 or Table 3-5, respectively). Thus, the pendulum impactor constructed for this experiment would provide reliable impacts to study on mechanical injury of roma-type tomato.

Table 3-3. Impact of pendulum impactor with 30 degree angle and 50 degree angle.

	Velocity change (m/s)			Maximum acceleration (maxG)		
	test 1	test 2	test 3	test 1	test 2	test 3
30 ° angle	0.72±0.12 ^z	0.74±0.15	0.75±0.19	72.78±6.22	76.43±6.18	75.94±6.77
50 ° angle	1.02±0.04	1.02±0.17	1.04±0.12	100.77±9.15	121.71±20.46	114.87±26.11

^zMean (n=30) following by standard deviation.

Table 3-4. Consistency of pendulum impactor with 30 degree angle.

	Velocity change (m/s)	Maximum acceleration (maxG)
Between test	** ^z	*
Within test	*	*

^z, ** Significant at P=0.05 or 0.01, respectively.

Table 3-5. Consistency of pendulum impactor with 50 degree angle.

	Velocity change (m/s)	Maximum acceleration (maxG)
Between test	* ^z	**
Within test	*	*

^z, ** Significant at P=0.05 or 0.01, respectively.

3.4 Summary

The effect of a 60-cm drop on the respiration rate and ethylene production of roma tomato differed by fruit ripeness stage. After impact, mature-green showed accelerated respiration rate (climacteric peak 2 d earlier) and pink stage fruit produced 20% increased CO₂. Although ethylene production increased in all fruit stages by a 60-cm drop, pink and light-red fruit had higher increased ethylene production than fruit at other stages. A drop from 60 cm also accelerated softening in roma tomato dropped at mature-green or breaker stages.

A drop from 60 cm caused no changes in SSC, TTA, pH and sugar/acid ratio, except red-stage fruit in which TTA increased. This observation suggests that roma tomato should be harvested before red-stage to prevent flavor change by impact.

Use of a vacuum to hold tomato in drop simulation caused bruising on the surface of the fruit, so the impact method was changed from drop system to pendulum impacting system. A pendulum impactor was proven to be an accurate method by analyzing impact data collected from an Impact Recording Device (IRD).

CHAPTER 4

EFFECT OF IMPACT ON THE QUALITY OF ROMA-TYPE TOMATO

4.1 Introduction

Mechanical injury has been related with metabolic disorders and quality change. Tomato subjected to impact stress showed increased ethylene production (MacLeod et al., 1976) and changed aroma volatiles (Moretti et al., 2002). Bruised locular tissue of tomato showed about 15% lower Vitamin C content than non-bruised tomatoes (Moretti et al., 1998). Impact on fruits also affected soluble solid content, firmness, and aroma volatiles. The panelist in sensory test described the internally bruised tomato as “watery” and “bland” flavor (Moretti et al., 1998).

Bruising is linearly related to impact energy in apple (Chen and Sun, 1981; Pang et al., 1992) and peach (Hung and Prussia, 1989). McColloch (1962) reported that the injury increased with the drop height and two drops caused more internal bruising than single drop. Hyde (1997) also has shown that drop height influenced not only the severity but also the type of bruise damage in whole potato tubers.

Incidence of mechanical injury is influenced by the stage of maturity. Olorunda and Tung (1985) noted that ripe tomatoes were more subject to injury. Turning stage developed four times as much as bruising injury as mature-green tomato, and eight times as much as immature-green tomato (Halsey and Showalter, 1953). Sargent et al. (1989) reported that internal bruising was more pronounced on breaker stage than mature-green stage tomatoes. Mature peaches had also larger bruise volumes and were more susceptible to bruising than less mature peaches (Hung and Prussia, 1989). Oppositely,

Vergano et al. (1991) reported green peaches were more susceptible to bruising than mature or ripe ones. As apples matured, bruising decreased (Diener et al., 1979).

The objective of the experiments in this chapter was to evaluate the effect of physical impacts on the quality of roma-type tomato depending on the impact forces and fruit ripeness stages.

4.2 Materials and Methods

4.2.1 Test 1. Effect of Impact Force on the Quality of Roma-Type Tomato at Breaker Stage

4.2.1.1 Plant materials

Roma tomatoes ('BHN 467') were hand-harvested at mature-green stage near Immokalee, FL in Feb 2004. Harvested fruits were transported to the Postharvest Horticulture Laboratory at the University of Florida, Gainesville. Green tomatoes were stored in sealed containers with flow-through air containing 100 ppm ethylene for 60 h at 20 °C, 90% R.H. After 3 d, breaker stage tomatoes (<10% red coloration) were sorted by weight (around 100 g), washed with chlorine solution (pH 6.8-6.9), rinsed and dried by air. After impact treatment, two roma tomatoes were placed in each vented clamshell (110*130*70 mm, n=8 per treatment) and ripened at 20 °C with 90% R.H.

4.2.1.2 Impact simulation – pendulum

A pendulum impactor was used as previously described (Chapter 3). The impact point was marked with a white paint marker over the locule of each tomato suspended in the pocket. The marked point of each fruit was impacted once by the pendulum from the calculated angle (equivalent to vertical drops of 20, 40, or 60 cm), and the fruit was caught after one impact.

4.2.1.3 Respiration rate and ethylene production

Two tomatoes were weighed and placed in a 1,025 ml plastic container, loosely capped with plastic lids fitted with a rubber septum and stored at 20 °C. Every day, the containers were sealed for 1 h, followed by removal of a 0.5 ml sample (for CO₂) and 1.0 ml sample (for ethylene) of the headspace from each container. Immediately after impact treatment, measurements were conducted every 2 to 3 h for 18 h. Carbon dioxide concentration and ethylene production rate were measured using a gas chromatograph as previously described.

4.2.1.4 Days to full-ripe stage

Ripening of each roma tomato fruit was determined subjectively using the percent of red color on the surface of fruits according to U.S. Dept. of Agric. Grade Standards. Fruits were considered to reach full-ripe ripeness stage 3 d after all surface of fruit reached the light-red stage (orange-red color). At that point, the hue angle ranges from 36 to 38°.

4.2.1.5 Electrolyte leakage

Electrolyte leakage was measured using a conductivity bridge (YSI 3100 conductivity instrument) equipped with a conductivity electrode at full-ripe ripeness stage (3 d after light red) and at 18 d after impact treatments. Pericarp discs (n=7) of 1.5 cm diameter, trimmed of locular gel tissue, were excised using No. 4 Cork borer from each fruit. Discs were rinsed with distilled water, briefly dried on Whatman #4 filter paper, and transferred into 10 ml of 300 mM mannitol solution in a 50 ml capped centrifuge tube. After each sample was shaken for 4 h, electrical conductivity was read. Samples then were frozen at -20 °C for 24 h. The next the day samples were thawed at room temperature, boiled in water for 15 min, and the final conductivity was taken to determine

total electrolyte conductivity. All leakage data were expressed as a percentage of the total electrolyte conductivity, where initial conductivity was divided by total conductivity, and multiplied by 100.

4.2.1.6 Compositional analyses

Upon reaching the full-ripe stage (3 d after light-red stage), ten individual fruits were homogenized, followed by centrifugation with 8060 x g, 5 °C for 20 min. The resulting supernatant was filtered using cheesecloth and then frozen for later analysis of the following parameters.

Soluble solids content (SSC). One to two drops of the supernatant as prepared above was placed on the prism of the digital refractometer (Model 10480, Reichert-Jung, Mark Abbe II Refractometer, Depew, NY) and the soluble solids content (SSC) was reported as °Brix.

pH. The pH was determined from the same supernatant with a pH meter (pH meter 140, Corning Scientific Instruments, Medfield, MA) standardized with pH 4.0 and 7.0 buffers.

Total titratable acidity (TTA). Each sample supernatant (6 g) was weighed out and diluted in 50 ml of distilled water. The samples were analyzed by an automatic titrimeter (No. 9-313-10, Fisher Titrimeter II, Pittsburg, Pa.), titrated with 0.1 N NaOH to endpoint of pH 8.2. TTA was expressed as percentage of citric acid.

4.2.2 Test 2. Effect of Impact on the Quality of Roma-Type Tomato at Different Ripeness Stages

4.2.2.1 Plant materials

Roma tomatoes ('BHN 467') were hand-harvested at mature-green color stage near Immokalee, FL in March 2004. Harvested fruits were transported to the Postharvest

Horticulture Laboratory at the University of Florida, Gainesville. Green tomatoes were stored in sealed containers with flow through air containing 100 ppm ethylene at 20 °C, 90% R.H. Tomatoes at breaker (<10% red coloration), pink (30-60% red coloration) , or light-red ripeness stage (60-90% red color) were sorted, washed with chlorine solution (pH 6.8-6.9), rinsed and dried by air. After impact treatment, two roma-type tomatoes were placed in each vented clamshell (110*130*70, n=25 per treatment) and ripened at 20 °C with 90% R.H.

4.2.2.2 Impact simulation – pendulum

The pendulum impactor was used as previously described. Each fruit was impacted twice in the same location by the pendulum from the desired angle (equivalent to vertical drop of 40 cm) and caught after each impact. Each tomato was marked with white paint marker on the locule surface suspended in the pocket.

4.2.2.3 Respiration rate and ethylene production

Carbon dioxide concentration and ethylene production rate were measured using a same gas chromatograph as previously described. Every other day, the containers were sealed for 1 h, followed by removal of a 0.5 ml sample (for CO₂) and 1.0ml sample (for ethylene) of the headspace from each container. Immediately after impact treatment, measurements were conducted everyday for 4 d.

4.2.2.4 Firmness

Pericarp firmness was determined using an Instron Universal Testing Instrument (Model 4411, Canton, MA, USA) equipped with a convex-tip probe (4 mm diameter) and 5-kg load cell. Roma tomato was cut equatorially with a sharp, stainless steel knife into 10 mm thick disc, and then this slice was immediately placed on a stationary steel plate. After establishing zero height between the probe and the pericarp tissue, the probe was

driven with a crosshead speed of 10 mm·min⁻¹. The force was recorded at 2.5 mm deformation and was measured at 2-3 points per fruit at the junction of the pericarp wall and radial wall. Five individual fruit per treatment were evaluated repeatedly every other day.

4.2.2.5 Electrolyte leakage

Every other day, electrolyte leakage was measured using a same conductivity bridge and method described in the previous experiment. All leakage data were expressed as a percentage of the total electrolyte conductivity, where initial conductivity was divided by total conductivity, and multiplied by 100.

4.2.2.6 Compositional analyses

The supernatant was prepared at the full-ripe stage (3 d after light-red stage) as the previous test to determine Soluble Solids Content (SSC), Total Titratable Acidity (TTA), pH and Sugar/acid ratio. Ten fruit of each treatment were evaluated.

4.2.2.7 Enzyme activity

Endo-polygalacturonase (PG, E.C. 3.2.1.15) activity was assayed by incubating a 100 µl aliquot of the cell-free protein extract with 500 µl (2 mg) of polygalacturonic acid (from orange peel, Sigma Chemical Co., St. Louis, MO, USA) dissolved in buffer solution (pH 4.5) containing 30 mM NaOAc and 30 mM KCl. After incubation for 30 min at 34 °C, the method of Milner and Avigad (1967) was used to measure uronic acid (UA) reducing groups. PG activity was expressed as molecular D-galacturonic acid equivalents produced per kilogram protein per minute. Protein content was measured using the bicinchoninic acid protein assay (Smith et al., 1985) with bovine serum albumin as a standard. Five fruits of each treatment were evaluated every other day.

4.2.3 Statistical Analysis

The experiment was conducted using a completely randomized design. Statistical analysis was performed using the PC-SAS software package (SAS-Institute, 1985). All data were subjected to analysis of variance and treatment means were compared using Duncan's Multiple Range Test ($P < 0.05$).

4.3 Results and Discussion

4.3.1 Test 1. Effect of Impact Forces on the Quality of Roma-type Tomato at Breaker Stage

4.3.1.1 Respiration rate

Both impacted and non-impacted breaker tomato showed peak CO_2 production after about 48 h (2 d) after impact treatment (Figure 4-1). During the first 48 h (2 d), fruits at breaker stage ripened to turning ripeness stage (10 to 30% red coloration). The initial CO_2 production of roma-type tomato was from 35 to 38 $\text{ml CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Respiration rate of control fruit decreased steeply by 13 h, and then increased until reaching the climacteric peak at 40 h, $28.79 \text{ ml CO}_2 \text{ kg}^{-1} \cdot \text{h}^{-1}$, which was lower than the initial respiration rate.

The impacted fruit showed similar respiration trend as control fruit. There was no significant difference in time to reach climacteric peak and in maximum respiration rate between impacted and non-impacted fruit except tomato subjected to the impact equivalent to 60 cm drop. In this case the impact produced $32.65 \text{ ml CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, 15% more CO_2 than control fruit at climacteric peak. In addition, roma-type tomato impacted with the force equivalent to 40 or 60 cm drop height exhibited a steep increase in respiration rate at 5 h, while the respiration rate of control fruit decreased from 3 to 5 h. This difference may have been caused by impact on the fruit. In other words, impact on

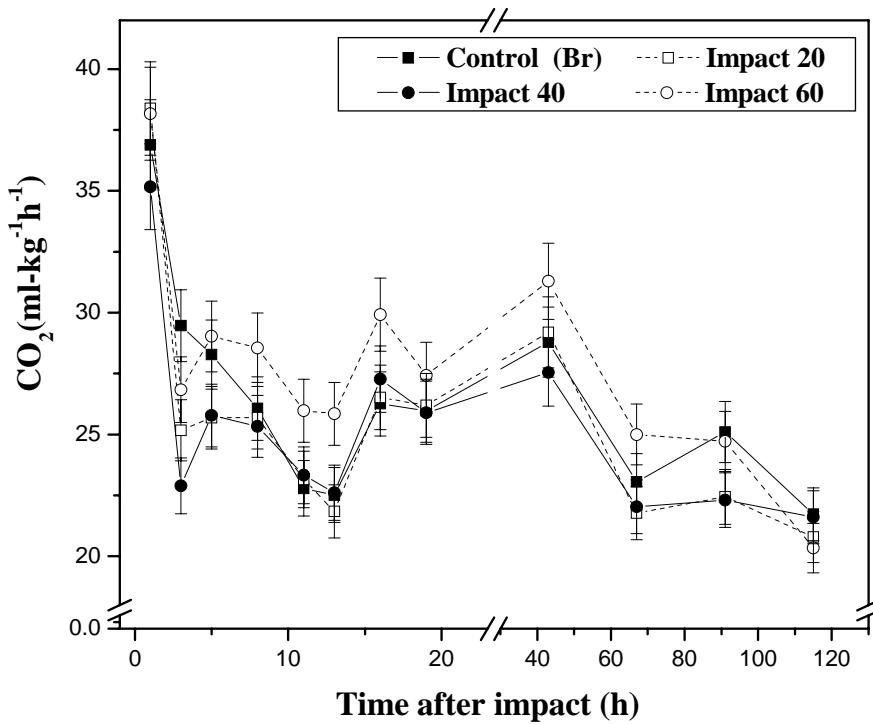


Figure 4-1. Respiration rate of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was impacted by the pendulum from desired angles (equivalent to vertical drops of 20 (□), 40 (●), or 60 (○) cm) and non-impacted (control, ■). Each point indicates the mean of 5 fruits. Vertical bar represents standard error.

roma-type tomato induced an increase in respiration rate during the early storage period after impact. Tomatoes impacted with the force of 60 cm drop height consistently produced more CO₂ during 5 to 90 h after the impact treatment than tomato from the other treatments. Increased respiration rate by impact was reported in apple (Dewey et al., 1981), cherry (Mitchell et al., 1980), and sweetpotato (Saltveit and Locy, 1982). Knee and Miller (2002) noted that mechanical injury induces the oxidation of phenolic compounds by catechol oxidase, so damaged fruits uptake more oxygen.

Roma-type tomato produced maximum CO₂ immediately after storage regardless of impact, and then respiration rate sharply decreased within 3 h of storage (Figure 4-1). An interesting observation was that all impacted fruits produced less CO₂ than non-impacted ones at that time, which may indicate that some self-defense reactions would be activated in Roma-type tomatoes subjected to the impact stress. Miller (1992) mentioned that plants synthesize the secondary compounds for defensive or healing purposes against mechanical stress.

4.3.1.2 Ethylene production

Initial ethylene production of control breaker fruit was about $2 \mu\text{l}\cdot\text{C}_2\text{H}_4\text{ kg}^{-1}\cdot\text{h}^{-1}$ and ethylene production decreased 3 h after impact treatment (Figure 4-2). A small peak was observed at 11 h and ethylene production of control fruit reached climacteric peak at day 48 h (2 d) when fruit produced $5 \mu\text{l C}_2\text{H}_4\text{ kg}^{-1}\cdot\text{h}^{-1}$.

Roma-type tomato impacted with the force of 20-cm drop height produced ethylene with the same pattern as control fruit during the entire storage (Figure 4-2). Initial ethylene production was 50% more than control and the time to reach climacteric peak was day 2 (48 h), same as control fruit. Maximum production, however, was 30% higher than control. Tomato impacted with the force of 40 or 60-cm drop height produced ethylene in a similar trend (Figure 4-2). Impact on fruit caused the increase in ethylene production immediately after impact treatment. Initial ethylene production was twice as much as control. This observation is supported by findings of MacLeod et al. (1976) who reported ethylene increase within 1 h after dropping in tomatoes. In addition, fruits impacted by the force of 40 or 60-cm drop height showed the peak of ethylene production at 15 h and produced 25 or 40% more than control fruit, respectively. Control fruit produced the maximum ethylene during 48 h (day 2). Tomato impacted with the force

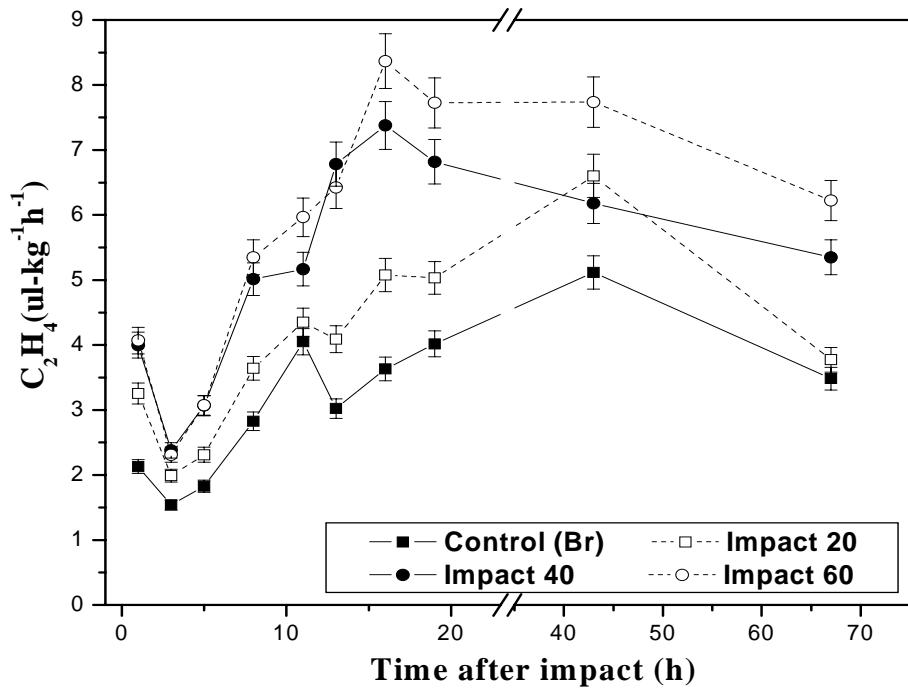


Figure 4-2. Ethylene production of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was impacted by the pendulum from desired angles (equivalent to vertical drops of 20 (□), 40 (●), or 60 (○) cm) and non-impacted (control, ■). Each point indicates the mean of 5 fruits. Vertical bar represents standard error.

equivalent to 60 cm height drop had the highest ethylene production during the entire ripening period, and the impact force of 20, 40 or 60 cm drop height induced a 13, 40 or 64% increase in ethylene production at climacteric peak than control fruit, respectively. Based on these observations, impact on roma-type tomato can be expected to promote ethylene production. Increase in ethylene biosynthesis by mechanical stress was also reported in winter squash, which was related to increases in 1- aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase (Hyodo et al., 1993). In addition, higher impact force induced higher ethylene production on roma tomato.

Compared with respiration rate (Figure 4-1), the acceleration in ethylene production by impact had higher correlation with the impact force. This means that ethylene production is a more useful parameter to examine the impact stress on roma tomato than respiration rate.

4.3.1.3 Days to full-ripe stage

Ripening rate was inversely related to impact force. Roma-type tomato reached full-ripe ripeness stage (3d after light-red ripeness stage) around 10-11 d. Tomatoes impacted with the force equivalent to 60 cm height drop reached full red-ripeness stage about 1 d earlier than control fruit (Table 4-1). There was no significant difference in time required to reach full-ripe stage between control fruit and fruit impacted with the force of 20 cm or 40 cm drop height. This observation means that the impact on the roma-type tomato can promote fruit ripening. Enhanced ethylene production by impact observed in Figure 4-2 may affect the ripening of roma-type tomato, since ethylene is a major hormone coordinating ripening processes in fruits and vegetables.

Table 4-1. Days to full-ripe stage after impact treatments.

Treatment	Days
Control	10.13±2.17 a ^y
I-20 ^z	9.58±1.79 ab
I-40	9.33±1.52 ab
I-60	9.08±1.56 b

^zI-20, I-40 or I-60 means impact treatment with the force equivalent to 20, 40, or 60 cm height drop, respectively.

^yMean (n=5) following by standard deviation. Columns with different letters are significantly different at P< 0.05, according to Duncan's Multiple Range Test.

4.3.1.4 Electrolyte leakage

The initial response of impact-bruised fruit is cell rupture and/or a loss of tissue integrity (Miller, 1992). Wilson and McMurdo (1981) said that electrolyte leakage could

be used as the indicator of cellular membrane integrity. According to Table 4-2, the electrolyte conductivities of roma-type tomato from all treatments were similar (about 16%) at full-ripe stage, and there was no significant difference in conductivity 18 d after treatment (about 20 to 23%). This observation can be explained as roma tomato has a high impact threshold over 60 cm drop height, or the electrolyte leakage is not usable parameter to indicate mechanical injury on roma tomato.

As reported in other fruits, electrolyte leakage in roma tomato gradually increased during ripening. This may be related to the deterioration of cell membranes in pericarp tissue, since electrolyte leakage is the indicator of cellular membrane integrity (Wilson and McMurdo, 1981). In addition, the deviation within each treatment was higher at later ripeness stage, which indicates individual fruit has different resistance against disruption of cell wall tissue.

Table 4-2. Electrolyte leakage measurement 3 d after light red ripeness stage and 18 d after treatments.

Treatment	Electrolyte Conductivity (%)	
	3 d after light red	18 d after treatments
Control	15.86±1.38 a ^y	20.45±3.55 a
I-20 ^z	15.99±1.44 a	20.64±3.84 a
I-40	16.29±1.91 a	23.60±4.86 a
I-60	16.19±1.55 a	20.22±3.82 a

^zI-20 means tomato subjected to the impact equivalent to the force of 20 cm height drop.

^yMean (n=5) following by standard deviation. Columns with different letters are significantly different at P< 0.05, according to Duncan's Multiple Range Test.

4.3.1.4 Compositional analyses

The values for soluble solids content (SSC), total titratable acidity (TTA) and pH were similar for non-impacted fruit (control) and impacted fruit (Table 4-3). SSC and

TTA of tomato ranged from 2.18 to 2.55 ($^{\circ}$ Brix) and from 0.451 to 0.515, respectively. Roma-tomato is an acidic fruits (around pH 4.4).

Sugar/acid ratio was calculated to estimate tomato flavor. The sugar/acid ratio of tomatoes impacted with the force of 60 cm height drop (5.41) was about 23.5% higher than control fruit. To examine how this magnitude of change in sugar/acid ratio affects tomato flavor, a sensory panel test is needed in the future.

Impact did not cause any significant change in SSC, TTA, pH and sugar/acid ratio of roma tomato at breaker ripeness stage. This result indicates that flavor of breaker fruit could be maintained even though fruit dropped form 60 cm or lower height.

Table 4-3. Compositional analyses at full-ripe stage.

Treatment	SSC ^z ($^{\circ}$ Brix)	TTA (%)	Sugar/acid ratio	pH
Control	2.18±0.69 a	0.515±0.156 a	4.38±2.86 a	4.42±0.09 a ^y
I-20	2.20±1.07 a	0.500±0.098 a	5.06±2.88 a	4.39±0.08 a
I-40	2.55±0.65 a	0.451±0.213 a	5.06±2.05 a	4.41±0.04 a
I-60	2.50±0.35 a	0.488±0.110 a	5.41±1.59 a	4.40±0.05 a

^zSSC = Soluble Solids Content; TTA = Total Titratable Acidity (citric acid equivalent)

^yMean (n=10) following by standard deviation. Columns with different letters are significantly different at P< 0.05, according to Duncan's Multiple Range Test.

4.3.2 Test 2. Effect of Impact on the Quality of Roma-Type Tomato at Different Ripeness Stages

4.3.2.1 Respiration rate

In every ripeness stage, impacted tomato produced more CO₂ during the entire ripening period (Figure 4-3). The differences in respiration rate between non-impacted fruit and impacted fruit were greatest immediately after impact treatment regardless of ripeness stage.

Roma-type tomato at breaker ripeness stage produced initially 33.2 ml CO₂ kg⁻¹·h⁻¹ and its respiration rate decreased continuously to 15 ml CO₂·kg⁻¹·h⁻¹ within 11 days

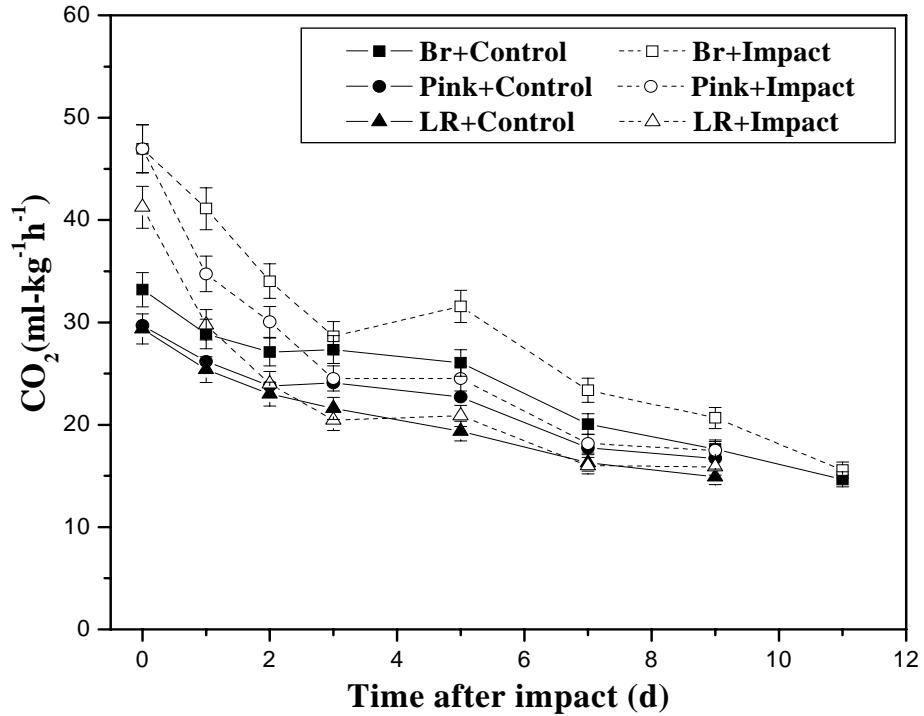


Figure 4-3. Respiration rate of roma-type tomato during ripening at 20 °C. Tomato at each ripeness stage—breaker (■), pink (●), or light-red (▲)—was subjected to double impacts of 40 cm (open symbol for each ripeness stage). Each point indicates the mean of 5 fruits. Vertical bar represents standard error.

of storage. A steep decline in respiration rate was observed during the first 3 d and days 5 to 11. Fruit impacted at breaker stage had 40% higher respiration rate immediately after the impact treatment. The sharp decrease in respiration rate of impacted-breaker fruit was observed within 3 d, followed by a rise at day 5, and then by a continuous decrease. At day 5, the impacted fruit had 20% higher respiration rate than non-impacted fruit.

The initial respiration rate of tomato at pink ripeness stage was 30 ml $\text{CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, which was about 10% less than that of fruit at breaker stage. Roma-type tomato at pink stage exhibited the decreasing respiration rate same as fruit at breaker stage. Carbon dioxide production of pink-stage tomato was less than that of breaker until day 9. Impact

on tomato at pink ripeness stage induced a 60% increase in the initial respiration rate (Figure 4-3). The respiration rate of impacted fruit steeply decreased within the first 3 d after impact treatment and there was no significant difference in respiration rate in non-impacted and impacted fruit at pink ripeness stage after day 3.

Roma-type tomato at light-red ripeness stage had the same initial respiration rate as fruit at pink stage and its respiration decreased continuously. Impact on tomato at light-red stage immediately induced about 40% higher respiration rate than non-impacted fruit. The respiration rate of impacted fruit steeply decreased within the first 2 d after impact treatment, and then there was no significant difference in respiration rate between non-impacted and impacted fruit.

Sturm and Chrispeels (1990) hypothesized that stressed cells demand high amounts of hexoses to generate energy and to supply carbon skeletons needed to synthesize response compounds. Since respiration is one of hexose metabolisms converting storage sugars to hexoses and producing energy, increased respiration rate might be observed in the impacted tomato. Roma-type tomato showed the highest respiration rate when it was impacted at breaker stage. Considering the correlation between ripening and respiration rate, careful handling to avoid impacts is required especially for roma-type tomato at breaker ripeness stage. The impacted roma tomato also showed higher respiration rate than non-impacted fruit immediately after impact regardless of ripeness (Figure 4-3). Many fold increase of ethylene production in bruised fruits and vegetables in a very short time (5 to 60 min) were reported by Miller (1992).

During the ripening period, fruit treated with advanced color produced less CO₂ in both impacted and non-impacted group (Figure 4-3). Breaker-stage fruit exhibited higher

respiration rate than fruit at light-red ripeness stage, since breaker stage fruit is at or near the climacteric peak in respiration rate in contrast to the light-red stage fruit which is already past the respiratory peak.

4.3.2.2 Ethylene production

Roma-type tomato at breaker ripeness stage initially produced $2 \mu\text{l C}_2\text{H}_4\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ and ethylene production increased until reaching climacteric peak at day 5 (Figure 4-4). Maximum ethylene production was $3.5 \mu\text{l C}_2\text{H}_4\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. A steep increase in ethylene production (3 times as initial rate) was seen within 1 d after impact, and fruit impacted at breaker stage reached the climacteric peak at day 5, the same as non-impacted fruit. Impact on fruit at breaker stage increased ethylene production during the entire storage and induced a 50% increase in maximum ethylene production. At day 11, there was no difference in ethylene production between fruit impacted at breaker stage and non-impacted fruit.

The initial ethylene production rate of roma tomato at pink ripeness stage was $3 \mu\text{l C}_2\text{H}_4\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, which was 40% higher than that of breaker fruit (Figure 4-4). Ethylene production of pink tomato increased within 1 d of storage and decreased after day 3. There was no significant climacteric peak of ethylene production. Impact on the tomato at pink ripeness stage induced 30% increase in initial ethylene production rate and 100% increase in ethylene production at day 1. Ethylene production of fruit impacted at pink stage steeply increased within 1 d after impact treatment and sharply decreased during days 1 to 3 and days 5 to 7. Impacted fruit produced much more ethylene than control fruit by day 7.

Tomato at light-red ripeness stage, already passing climacteric period, showed continuously decreasing ethylene production after day 1 (Figure 4-4). Its initial rate of

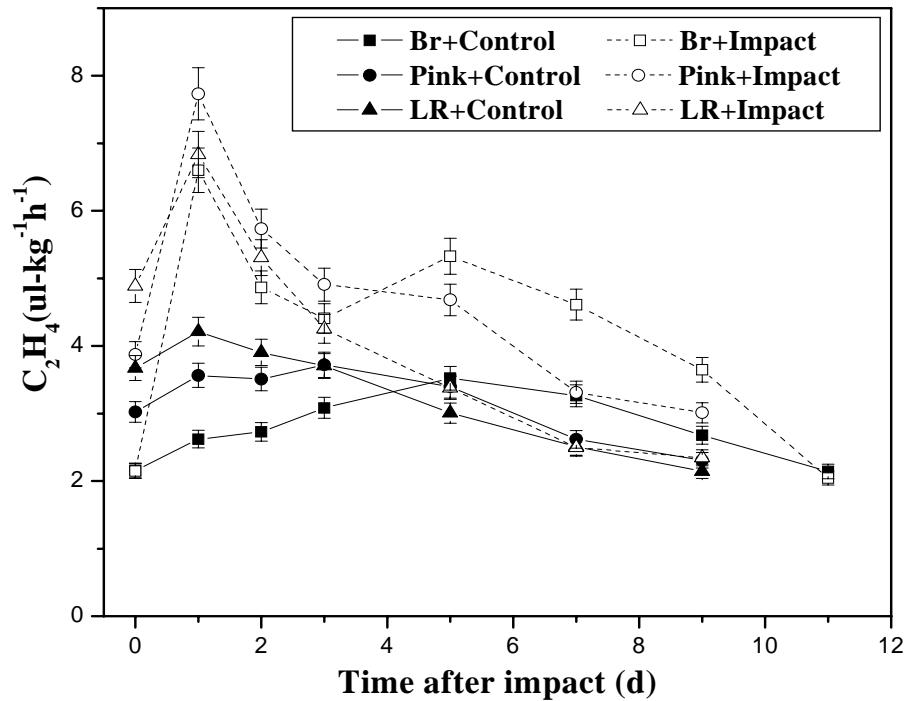


Figure 4-4. Ethylene production of roma-type tomato during ripening at 20 °C. Tomato at each ripeness stage—breaker (■), pink (●), or light-red (▲)—was subjected to double impacts of 40 cm (open symbol for each ripeness stage). Each point indicates the mean of 5 fruits. Vertical bar represents standard error.

ethylene production was $3.7 \mu\text{l C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Round-type tomato showed a climacteric peak around pink stage. Since light-red fruit has just passed climacteric peak, light-red fruit showed higher ethylene production than breaker fruits. Ethylene production of fruit at light-red ripeness stage increased within 1 d of storage, and then continuously decreased. Roma-type tomato impacted at light-red stage had 40% higher initial rate of ethylene production and 60% increased ethylene production at day 1. The ethylene production decreased steeply after day 1.

Ethylene production increased within 1 d of storage regardless of fruit maturity and impact application (Figure 4-4). Importantly, the impacted fruit showed a steep increase

in ethylene production within 1 d, consistent with the report of Miller (1992) who mentioned many bruised fruits and vegetables produced many fold ethylene in a very short time (5to 60 min.). Yang and Hoffman (1984) reported that the elevated ethylene production in bruised fruit is induced by increases in 1- aminocyclopropane-1-carboxylic acid (ACC) and its conversion to ethylene. In addition, an impact at breaker stage induced higher ethylene production than other stages. Klee and Estelle (1991) mentioned that growth regulators such as ethylene and zeatin might regulate the expression of genes related to particular organ's stress response. Therefore, this indicates that breaker fruit is more susceptible to impact stress than other stage fruit.

As with the previous test (Test1), the ethylene production was more affected by impact than the respiration rate (Figure 4-3).

4.3.2.3 Firmness

Roma-type tomato softened during ripening at 20 °C. Initial pericarp firmness of roma-type tomato at breaker, pink, or light-red ripeness stage was around 14, 9.5 or 6.8 N, respectively (Figure 4-5).

Roma-type tomato at breaker stage maintained the initial firmness during the first 2 d and firmness decreased steeply to half of initial firmness within 2 to 4 d after storage. Firmness of fruit at breaker stage reached 4 N (full-ripe firmness) at day 9 to 10. However, impact on fruit at breaker stage initiated sharp softening within first 2 d of storage and its firmness was 4 N at day 8. Miller (1992) mentioned that the softening at the impacted site happens immediately after impact due to cell rupture and loss of tissue integrity. Roma- type tomato impacted at breaker ripeness stage showed decline in firmness continuously after impact application. This observation means that impact at breaker stage can promote softening and shorten shelf life.

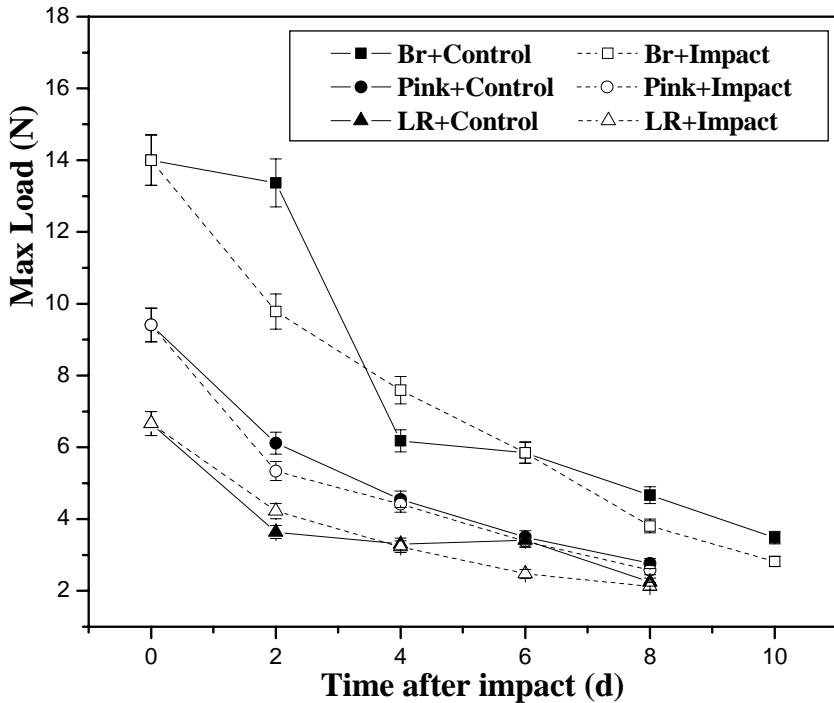


Figure 4-5. Firmness of roma-type tomato during ripening at 20 °C. Tomato at each ripeness stage—breaker (■), pink (●), or light-red (▲)—was subjected to double impacts of 40 cm (open symbol for each ripeness stage). Each point indicates the mean of 5 fruits. Vertical bar represents standard error.

Otherwise, there was no significant difference in pericarp firmness between non-impacted fruit and fruit impacted at pink or light-red ripeness stage (Figure 4-5). While double impacts of 40 cm at breaker ripeness stage initiated softening early, a firmness of tomatoes at pink or light-red ripeness stage was not affected by impact. Reports of Garcia et al. (1995) may give an explanation of this observation. He noted that mechanical stresses in the tissues were higher in turgid fruit. Initial firmness of pink or light-red fruit was about 63 or 50% of that of breaker fruit, respectively, so fruits at these stages were less susceptible to mechanical injury. Firmness of roma-type tomato at pink or light-red ripeness stage reached 4 N at day 2 or day 5 to 6, respectively.

In firmness, there was a big deviation among measurements and no significant difference between impacted fruit and non-impacted fruit. This may indicate that the firmness is not a usable indicator for mechanical injury on roma-type tomato stored at 20 °C. Esselen and Anderson (1956) found that the softening of whole cucumber was not detected by the firmness tester. Miller et al. (1987) also noted that firmness was dependent on temperature.

4.3.2.4 Electrolyte leakage

Electrolyte leakage was traced during storage as the indicator of cellular membrane integrity of bruise fruit (Wilson and McMurdo, 1981). Although a steep decline in electrolyte leakage was observed during the first 2 d, the electrolyte leakage was increased as roma-type tomato was ripening (Figure 4-6). That means the deterioration of cell membranes in pericarp tissue during fruit ripening.

Impact on tomato at later ripeness stages induced more electrolyte leakage (Figure 4-6). There was more difference in electrolyte leakage between non-impacted fruit and fruit impacted at light-red stage than at breaker or pink ripeness stage. Electrolyte leakage of fruit impacted at light-red ripeness stage was 27% higher than that of non-impacted fruit at day 6. It can be explained that impacts on light-red fruit accelerated ripening, which induced the increased electrolyte leakage. In addition, fruits impacted at light-red ripeness stage showed higher electrolyte leakage than control fruit during the entire storage, while tomato impacted at pink or breaker ripeness stage exhibited higher electrolyte leakage than control after day 4 or day 6, respectively. This observation supports the hypothesis that the increase in electrolyte leakage caused by impact can appear when tomato reaches the certain ripeness stage, around light-red ripeness stage.

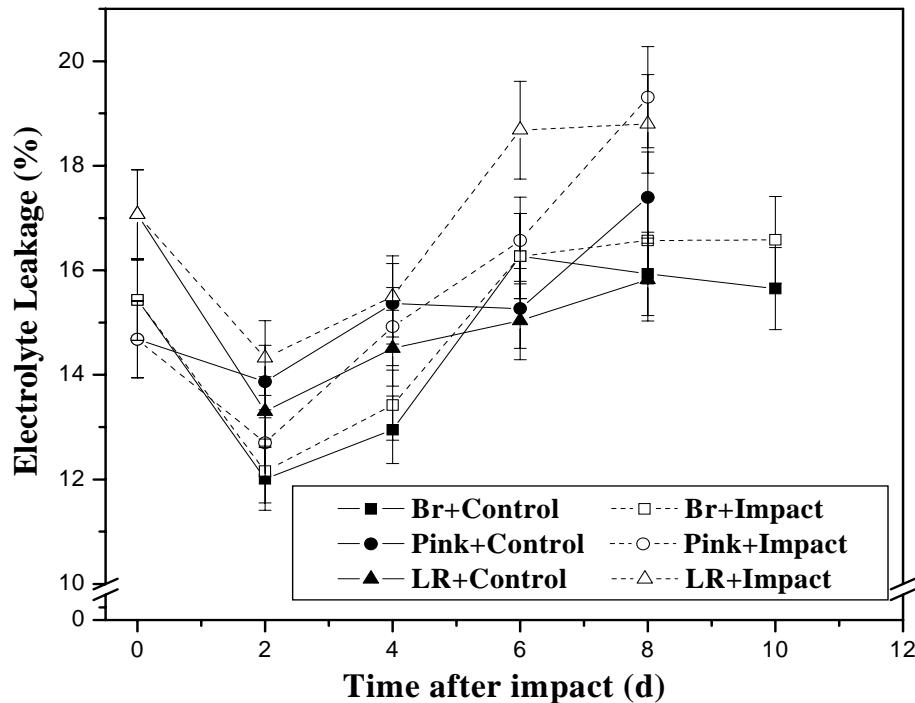


Figure 4-6. Electrolyte leakage of roma-type tomato during ripening at 20 °C. Tomato at each ripeness stage—breaker (■), pink (●), or light-red (▲)—was subjected to double impacts of 40 cm (open symbol for each ripeness stage). Each point indicates the mean of 5 fruits. Vertical bar represents standard error.

Fruit had higher initial electrolyte leakage than at day 2, which indicates that fruit underwent many stresses during several experiment procedures such as sorting, washing, and impact treatment. In addition, tomato fruit seems to have recovery ability based on the decreased electrolyte leakage at day 2.

4.3.2.5 Compositional analyses

To examine the possibility that impact can affect the flavor of roma-type tomato, the values of soluble solids content (SSC), total titratable acidity (TTA) and pH were measured at full-ripe stage (Table 4-4). Roma-type tomato showed decreasing SSC (from 4.04 to 3.53) and TTA (from 0.42 to 0.38) and increasing pH (from 4.49 to 4.55) as fruits

at later ripeness stage were used. It may have been caused by ethylene treated to get fruit at desired ripeness stages. Light-red fruit was treated with ethylene about 1-2 d longer than breaker fruit. Roma-tomato at breaker stage was significantly more acidic than a light-red color fruit when fruits reached the full-red ripeness stage. This observation means that the longer ethylene treatment on roma-type tomato may be caused less acidic flavor when fruit reaches the full-ripe stage.

There was no difference in SSC, TTA and sugar/acid ratio caused by the double impact of 40 cm in all ripeness stages. Although fruit impacted at light-red stage had significantly lower SSC and sugar/acid ratio than the impacted-breaker fruit, there was no significant difference in these values between control fruits. This result indicates that the combination of impact and fruit maturity may induce a significant change in tomato flavor.

Table 4-4. Compositional analyses at full-ripe stage.

Treatment	SSC ^z (°Brix)	TTA (%)	Sugar/acid ratio	pH
Br+Control	4.04±0.63 ab	0.42±0.08 a ^y	9.83±2.36 ab	4.49±0.04 b
Br+Impact	4.62±0.46 a	0.42±0.05 a	11.23±1.75 a	4.52±0.05 ab
Pink+Control	3.95±0.47 b	0.41±0.08 a	9.88±1.59 ab	4.52±0.06 ab
Pink+Impact	4.19±0.29 ab	0.38±0.03 a	10.94±0.54 a	4.55±0.04 a
LR+Control	3.53±0.87 bc	0.38±0.09 a	9.33±1.96 b	4.55±0.05 a
LR+Impact	3.18±0.85 c	0.37±0.06 a	8.65±1.89 b	4.52±0.05 ab

^zSSC = Soluble Solids Content; TTA = Total Titratable Acidity (citric acid equivalent); Br, Pink, or LR = roma-type tomato at breaker, pink, or light-red ripeness stages, respectively.

^yMean (n=10) following by standard deviation. Columns with different letters are significantly different at P< 0.05, according to Duncan's Multiple Range Test.

4.3.2.6 Enzyme activity

Endo-polygalacturonase (PG) hydrolyzes the linear α-1, 4-D-galacturonan backbone of pectin polysaccharides (Redgwell and Fischer, 2002). PG activity was

traced during storage since it has been related to loss of firmness during ripening (Grierson and Kader, 1986). PG activity increased from 3, 43, or 60 to 80-100 mole·kg⁻¹·min⁻¹*10⁵ (D-galacturonic acid) on the fruit at breaker, pink or light-red ripeness stage, respectively (Figure 4-7). Impacted fruit had higher PG activity than control fruits regardless of ripeness stage.

PG activity of roma-type tomato at breaker ripeness stage increased during day 2 to day 6 when PG activity was same as initial PG activity of tomato at light-red ripeness

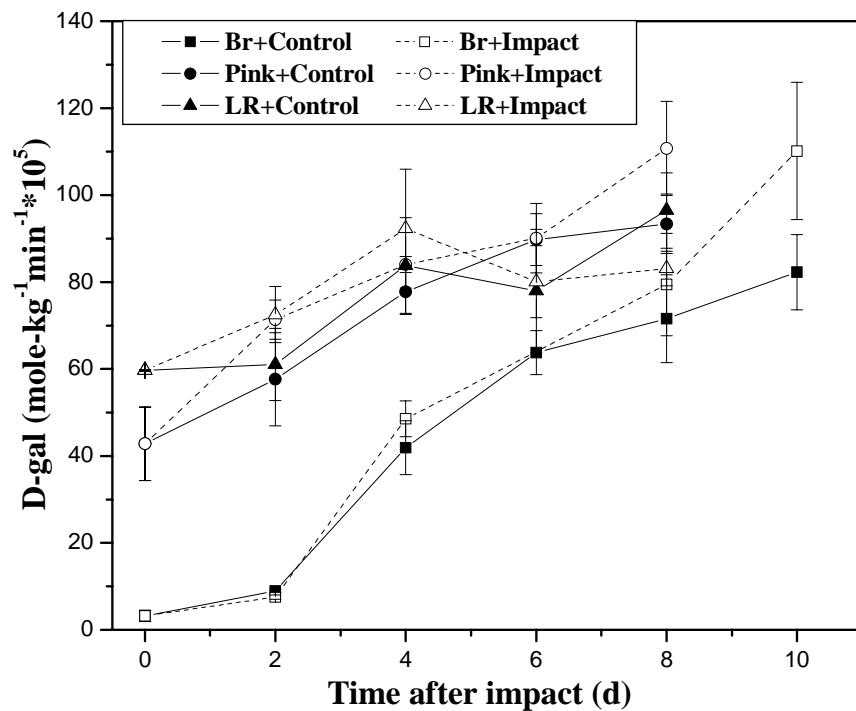


Figure 4-7. Change of polygalacturonase (PG) activity in pericarp tissue of roma-type tomato during ripening at 20 °C. Tomato at each ripeness stage—breaker (■), pink (●) or light-red (▲)—was subjected to double impacts of 40 cm (open symbol for each ripeness stage). Each point indicates the mean of 5 fruits, 3 measurements per fruit. PG activity is expressed as molecular D-galacturonic acid equivalents produced per kilogram protein per minute. Vertical bar represents standard error.

stage. Impact on fruit at breaker stage induced a higher increase in PG activity after day 6 while the initial increase in PG activity during the first 2 d was seen at pink and light-red fruit. At day 10, Tomato impacted at breaker stage showed 35% higher PG activity than non-impacted one. This observation can be explained by the report of Hadfield and Bennett (1998) that “PG mediated pectin assembly does not contribute to early fruit ripening but contributes significantly to tissue deterioration in the late stages ripening.” Roma-type tomato at pink ripeness stage exhibited increasing PG activity for the first 6 d of storage (Figure 4-7). PG activity at day 4 was same as that of breaker-fruit at day 10.

Impacted fruit at pink stage showed higher PG activity than non-impacted fruit during the entire storage period. Impact on pink stage-tomato caused a steep increase in PG activity within the first 2 d and during day 6 to day 8.

Initial PG activity of light-red tomato maintained during first 2 d, and PG activity increased during days 2 to 4 and days 6 to 8. Impact on fruit at light-red ripeness stage induced an increase in PG activity within the first 4 d of storage and higher PG activity than non-impacted fruit during the first 6 d after impact.

Double impacts of 40 cm promoted an increase in PG activity of the roma-type tomato regardless of fruit maturity (Figure 4-7). PG activity of fruits impacted at breaker or pink ripeness stage reached 80 or 90 mole-kg⁻¹min⁻¹*10⁵ (D-galacturonic acid) 2 d later than non-impacted fruits, respectively. Observations described above support that double impact of 40 cm drop height induced more and earlier cellular membrane breakdown or softening. Brady et al. (1983) reported the increase in endo-PG activity during softening. Another assumption also can be generated by this observation. In this experiment, only extractable PG was analyzed. PG tightly bound to cell wall might be

easily extracted on the impacted-bruised tissue, so higher concentrations of PG could be obtained from same weight of tissue in impacted fruit than non-impacted fruit resulting high concentration of D-galacturonic acid.

4.4. Summary

Immediately after a single impact equivalent to 60 cm, roma-type tomato showed significant increases in respiration rate, ethylene production and ripening rate, greater than those induced by single impacts equivalent to 20 or 40 cm drop height. Otherwise, there was no significant change in electrolyte leakage, TTA, SSC, pH and sugar/acid ratio. This observation indicates that a single drop from 20, 40 or 60 cm may not cause a significant change in tomato texture and/or flavor.

A double impact equivalent to the force of 40 cm drop height induced significant increases in the respiration rate and ethylene production immediately after impact, and showed higher electrolyte leakage and PG activity on roma-type tomato at breaker, pink or light-red stage. Among fruit at these stages, increases in the respiration rate and ethylene production were the greatest when fruit was impacted at breaker stage; softening occurred 2 d earlier than control fruit. Based on these results, it can be concluded that breaker fruit is more susceptible to impact stress than fruits at pink or light-red stage. Notably, the impacted breaker fruit had increased electrolyte leakage and PG activity after day 8, which was when the tomatoes reached light-red ripeness stage. Double impacts of 40 cm at breaker, pink or light-red stage also did not significantly affect SSC, TTA, pH or sugar/acid ratio.

CHAPTER 5

EFFECT OF 1-MCP TREATMENT ON IMPACTED ROMA-TYPE TOMATO

5.1 Introduction

Mechanical injury has been associated with metabolic disorders and quality change. MacLeod et al. (1976) reported ethylene production increased within 1 h after impact in tomato. Increased respiration rate was reported in pea and bean (Tewfik & Scott, 1954), apple (Dewey et al., 1981), cherry (Mitchell et al., 1980), sweetpotato (Saltveit and Locy, 1982) and tomato (MacLeod et al., 1976). Impact on tomato also caused changes in soluble solid content (Morris et al., 1979), firmness (Miller et al. 1987), and aroma volatiles (Moretti et al., 2002).

To extend the shelf life of tomatoes suffering mechanical stress, 1-methylenecyclopropene (1-MCP) has been tested. Heyes and Johnston (1998) reported that 1-MCP effectively prevented damaged pollinia from accelerated senescence. 1-MCP inhibits ethylene action, which has been shown to act at a very low concentration to block ethylene sensitivity for long periods (Serek et al. 1994; Sisler and Serek 1997). Ethylene action and tissue responses to ethylene can have detrimental effects on postharvest life and quality of fruit. Since 1-MCP is non-toxic at active concentrations, it may be commercially applicable to alleviate stressed tomatoes.

The objective of the experiments in this chapter was to evaluate the effect of pretreatment of 1-MCP to alleviate the mechanical injury on roma tomatoes subjected to impact stress.

5.2 Materials and Methods

5.2.1 Test 1. 1-MCP Pretreatment of Roma-Type Tomato Subjected to Double Impact Equivalent to 40 cm Drop Height

5.2.1.1 Plant materials

Roma tomatoes ('Sunoma') were hand-harvested at mature-green color stage near Palmetto, FL in May, 2004. Harvested fruits were transported to the Postharvest Horticulture Laboratory at the University of Florida, Gainesville. Green tomatoes were stored in sealed containers with flow through air containing 100 ppm ethylene for 60 h at 20 °C, 90% R.H. Roma-type tomato at breaker ripeness stage (<10% red coloration) was sorted by weight (about 100 g), washed with chlorine solution (pH 6.8-6.9), rinsed and dried by air. After 1-MCP and impact treatments, two roma-type tomatoes were placed in each vented and polystyrene clamshell package (n=35 per treatment) and ripened at 20 °C with 90% R.H.

5.2.1.2 Impact simulation – pendulum

A pendulum impactor previously described in Chapter 3 was used. Each fruit was impacted twice in the same location (over the locule) by the pendulum from the desired angle (equivalent to vertical drop of 40 cm) and caught after each impact. The impact point was marked with a white paint marker on the surface as the fruit was suspended in the pocket.

5.2.1.3 1-MCP treatment

170 fruits at breaker stage were placed in 174-l container and exposed to 1 $\mu\text{l}\cdot\text{l}^{-1}$ 1-MCP for 24 h at 22 °C. 1-MCP gas was generated from a commercial formulation of powder (SmartFresh®, Agro-Fresh Inc., a division of Rohm and Haas Co., Philadelphia, PA). The concentration of 1-MCP was achieved by dissolving the calculated mass of

powder in 25 ml water in a beaker and by opening this beaker inside the sealed container. To maintain the desired concentration, the same procedure was repeated after 12 h. Following 1-MCP treatment, the fruits were immediately subjected to the impact treatment.

5.2.1.4 Respiration rate and ethylene production

Two tomatoes were weighed and placed in a 1,025 ml plastic container, loosely capped with plastic lids fitted with a rubber septum and stored at 20 °C. Every 2 d, the containers were sealed for 1 h, followed by removal of a 0.5 ml sample (for CO₂) and 1.0 ml sample (for ethylene) of the headspace from each container. Immediately after impact treatment, measurements were conducted every 3 h for 15 h. Carbon dioxide concentration was measured using a gas chromatograph (series 580; GOW MAC, Bridgewater, N.J.) fitted with a thermal conductivity detector and a 1/4 inch Carbo pack column, and ethylene production rate was measured using a gas chromatograph (HP 5890; Hewlett Packard, Avondale, Pa) fitted with a flame ionization detector and alumina packed column.

5.2.1.5 Firmness

Pericarp firmness was determined using an Instron Universal Testing Instrument (Model 4411, Canton, MA, USA) equipped with a convex-tip probe (4 mm diameter) and 5-kg load cell. Roma tomato was cut equatorially with a sharp, stainless steel knife into 10 mm thick slice, and then this slice was immediately placed on a stationary steel plate. After establishing zero height between the probe and the pericarp tissue, the probe was driven with a crosshead speed of 10 mm·min⁻¹. The force was recorded at 2.5 mm deformation and was measured at 2 to 3 points on each fruit, the junction of the pericarp wall and radial wall. Five individual fruits per treatment were evaluated every 2 d on the

group without 1-MCP treatment and every 4 d on 1-MCP treated group until they reached the full-ripe stage.

5.2.1.6 Color

Surface color measurements were obtained from the equatorial regions (three per fruit) with a Minolta Chroma Meter CR-2000 (Minolta Camera Co Ltd, Japan) which has an 8 mm-diameter aperture and illuminant C lighting condition. The chromameter was calibrated with a white calibration plate. The color was reported as lightness (L^*), hue angle (h°), and chroma value (C). Lightness (L^*) represents the general illumination of the color, where 0 = black, 100 = white. A change in hue indicates fruit ripening from green to yellow or red, where 0 = red, 90 = yellow, 180 = green. Chroma (C) is the purity of hue or color saturation regardless of how light or dark it is. A highly chromatic color looks very luminous or concentrated, while a color with low chroma looks dull, gray, or diluted. Five fruits of each treatment were evaluated every 2 d on non 1-MCP treated group and every 4 d on 1-MCP treated group.

5.2.1.7 Compositional analyses

Upon reaching the full-ripe stage (3 d after light-red stage), ten individual fruits were homogenized, followed by centrifugation with 8060 x g, 5 °C for 20 min. The resulting supernatant was filtered using cheesecloth and then frozen for later analysis of the following parameters.

Soluble solids content (SSC). One to two drops of the supernatant as prepared above was placed on the prism of the digital refractometer (Model 10480, Reichert-Jung, Mark Abbe II Refractometer, Depew, NY) and SSC was reported as °Brix.

pH. The pH was determined from the same supernatant with a pH meter (pH meter 140, Corning Scientific Instruments, Medfield, MA) standardized with pH 4.0 and 7.0 buffer solutions.

Total titratable acidity (TTA). Each sample supernatant (6 g) was weighed out and diluted in 50 ml of distilled water. The samples were analyzed by an automatic titrimeter (No. 9-313-10, Fisher Titrimeter II, Pittsburg, Pa.), titrated with 0.1 N NaOH to endpoint of pH 8.2. TTA was expressed as percentage of citric acid.

5.2.1.8 Enzyme activity

Endo-polygalacturonase (PG, E.C. 3.2.1.15) activity was assayed by incubating a 100 µl aliquot of the cell-free protein extract with 500 µl (2 mg) of polygalacturonic acid (from orange peel, Sigma Chemical Co., St. Louis, MO, USA) dissolved in buffer solution (pH 4.5) containing 30 mM NaOAc and 30 mM KCl. After incubation for 30 min at 34 °C, uronic acid (UA) reducing groups were measured using the method of Milner and Avigad (1967). PG activity was expressed as molecular D-galacturonic acid equivalents produced per kilogram protein per minute ($\text{mole}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Protein content was measured using the bicinchoninic acid method (Smith et al., 1985) with bovine serum albumin as a standard. Five fruits of each treatment were evaluated every other day on non 1-MCP treated group and every 4 d on 1-MCP treated group.

5.2.2 Test 2. 1-MCP Pretreatment of Roma-Type Tomato Subjected to Double Impact Equivalent to 80 cm Drop Height

5.2.2.1 Plant materials

Roma tomatoes ('Sunoma') were hand-harvested at mature-green color stage near Quincy, FL in June 2004. Harvested fruits were transported to the Postharvest Horticulture Laboratory at the University of Florida, Gainesville. Green tomatoes were

stored in sealed containers with flow through air containing 100 ppm ethylene for 60 h at 20 °C, 90% R.H. To define roma-type tomato at breaker ripeness stage, tomatoes were sorted by weight (about 100 g), washed with chlorine solution (pH 6.8-6.9), rinsed and dried by air. After 1-MCP and impact treatment, two roma-type tomatoes were placed in each vented clamshell (n=30 per treatment) and ripened at 20 °C with 90% R.H.

5.2.2.2 Impact simulation – pendulum

The pendulum impactor was used as previously described. Each fruit was impacted by the pendulum from the desired angle (equivalent to vertical drops of 80 cm) and caught after each impact. Tomato was subjected twice to the same impact, and then marked with white paint marker on the locule surface suspended in the pocket.

5.2.2.3 1-MCP treatment

1-MCP was applied as in Test 1. Following 1-MCP treatment, the fruits were subjected to the impact treatment as previously described.

5.2.2.4 Respiration rate and ethylene production

Carbon dioxide concentration and ethylene production rate were measured using a same gas chromatograph as previously described. Everyday, the containers were sealed for 1 h, followed by removal of a 0.5 ml sample (for CO₂) and 1.0 ml sample (for ethylene) of the head space from each container. Immediately after impact treatment, measurements were conducted every 6 h for 18 h.

5.2.2.5 Firmness

Pericarp firmness was determined on the junction of the pericarp wall and radial wall using an Instron as previously described.

5.2.2.6 Color

Surface color measurements obtained with a Minolta Chroma Meter CR-2000 were presented as lightness (L^*), hue angle (h°) and chroma (C). Five fruits of each treatment (3 measurements per fruit; n=15) were evaluated every other day on non 1-MCP treated group and every 4 d on 1-MCP treated group.

5.2.2.7 Compositional analyses

The supernatant prepared at the full-ripe stage (3 d after light-red stage) through same way in previous test was used to get the values of Soluble Solids Content (SSC), Total Titratable Acidity (TTA), pH and Sugar/acid ratio. Ten fruits of each treatment were evaluated.

5.2.2.8 Enzyme activity

Endo-polygalacturonase (PG, E.C. 3.2.1.15) activity was assayed reductometrically by following the previously described procedure. Five fruits of each treatment were evaluated every other day on non 1-MCP treated group and every 4 d on 1-MCP treated group.

5.2.3 Statistical Analysis

The experiment was conducted using a completely randomized design. Statistical analysis was performed using the PC-SAS software package (SAS-Institute, 1985). All data were subjected to analysis of variance and treatment means were compared using Duncan's Multiple Range Test ($P < 0.05$).

5.3 Results and Discussion

5.2.1 Test 1. 1-MCP Pretreatment of Roma-Type Tomato Subjected to Double Impact Equivalent to 40 cm Drop Height

5.3.1.1 Respiration rate

Control fruit produced $31 \text{ ml CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ initially, and then decreased 18% CO_2 at 7 h after impact (Figure 5-1). The respiration rate of control fruit increased, and peaked at 15 h when fruit produced $30 \text{ ml CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ similar to the initial respiration rate (Table 5-1).

Impacted fruit, meanwhile, had 23% higher initial respiration rate than control fruit (Figure 5-1). Although there was no difference in tomato climacteric peak, the impact on roma-type tomato increased in peak amount of CO_2 production by 30%. Impacted fruit had higher respiration rate during the entire ripening period. After the climacteric peak, the difference in respiration rate between impacted and non-impacted fruit rapidly decreased. Increased respiration due to impact was reported in round-type tomato (MacLeod et al., 1976). Damaged fruits consume more oxygen because of the oxidation of phenolic compounds by catechol oxidase (Knee and Miller, 2002).

Roma-type tomato treated with 1-MCP produced initially about $15 \text{ ml CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, half the respiration rate of control fruit (Figure 5-1). Similar to control fruit, respiration rate of 1-MCP treated-fruit decreased at 7 h and increased after that. However, there was no dramatic respiratory peak during storage oppositely to control Avocado treated with 1-MCP also did not exhibit the respiratory peak during storage at 20°C (Jeong et al., 2002). This observation supported the hypothesis that 1-MCP application on roma-type tomato can suppress CO_2 production such as apricot (Fan et al., 2000), avocado (Jeong et al., 2002), and broccoli (Forney et al., 2003) and attenuate the respiratory climacteric

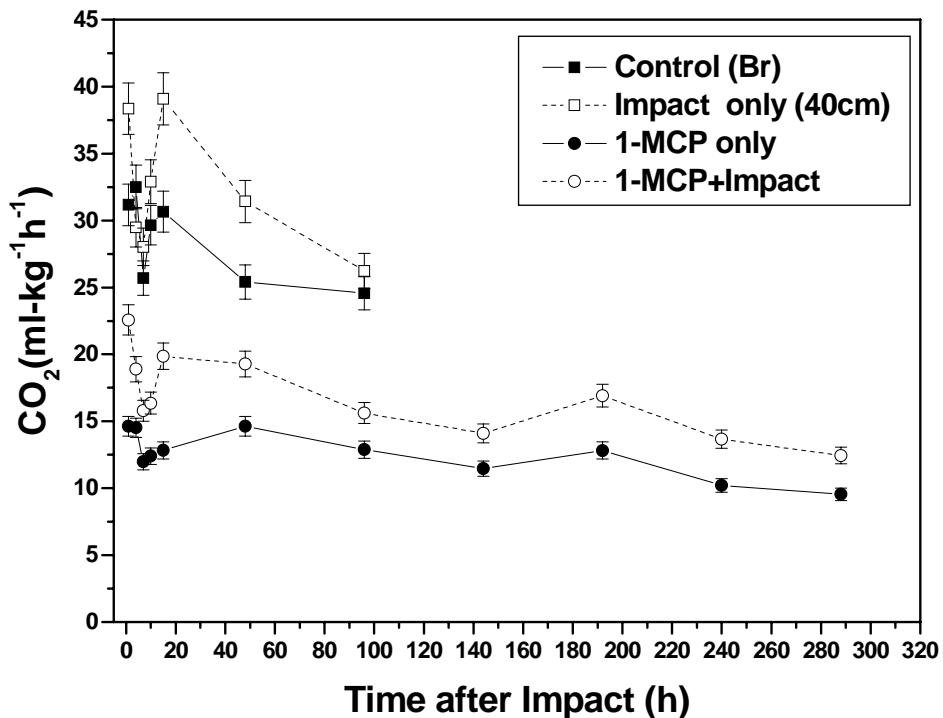


Figure 5-1. Respiration rate of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was subjected to double impacts (40 cm) (□), 1-MCP treatment (●), impact after 1-MCP treatment (○), or no treatment (control, ■). Each point indicates the mean of 5 fruits. Vertical bar represents standard error.

Table 5-1. Days to peak and maximum CO₂ production of 'breaker' roma-type tomato during ripening at 20 °C.

Treatments	Time to peak (h)	Maximum respiration rate (ml·kg ⁻¹ ·h ⁻¹)
Control	15	30.66 ± 3.18 b
Impact only	15	39.09 ± 7.71 a ^y
1-MCP only ^z	40	14.62 ± 2.08 c
1-MCP + Impact ^z	15	19.86 ± 3.46 c

^z Respiratory climacteric peak were not seen during storage, so days to exhibit maximum rate was expressed.

^yMean (n=5) following by standard deviation. Columns with different letters are significantly different at P< 0.05, according to Duncan's Multiple Range Test.

pattern.

Impacted tomato after 1-MCP application produced about $22.5 \text{ ml CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, which was about 154% of 1-MCP-only fruit and 60% of the impacted fruit without 1-MCP treatment (Figure 5-1). Minimum respiration rate was observed at 7 h same as the other treatments, and maximum CO_2 production was seen at 15 h. Tomato subjected to both impact and 1-MCP produced maximum of $19.86 \text{ ml CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, which was not significantly different from maximal respiration rate of 1-MCP treated fruit (Table 5-1). Impact only fruit significantly increased maxima of respiration rate. In addition, non-1-MCP treated tomato exhibited a greater difference in respiration rate between control fruit and the impacted fruit than the 1-MCP treated fruit (Figure 5-1). These observations support the hypothesis that 1-MCP can suppress the increased respiration rate caused by impact on the roma-type tomato.

5.3.1.2 Ethylene production

Ethylene production of control fruit increased until 48 h, and then decreased (Figure 5-2). Control fruit produced $3.45 \mu\text{l} \cdot \text{C}_2\text{H}_4 \text{ kg}^{-1} \cdot \text{h}^{-1}$ 12 h after storage, and maximum ethylene production at 48 h was $4.73 \mu\text{l} \text{ C}_2\text{H}_4 \text{ kg}^{-1} \cdot \text{h}^{-1}$.

During the entire ripening period, the ethylene production of impacted fruit was higher than that of control. Impacted fruit produced about 40% more ethylene than control at 12 h and maximum ethylene production was $6.09 \mu\text{l} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, which was 28% higher than that of control fruits. Also, impact induced earlier and higher ethylene peak. Increasing ethylene production caused by impact was reported on the round-type tomato (MacLeod et al., 1976). More ethylene is produced, as more the precursor 1-aminocyclopropene-1-carboxylic acid (ACC) is synthesized and converted into ethylene (Yang and Hoffman, 1984). Olson et al. (1991) reported that mechanical stress elevated

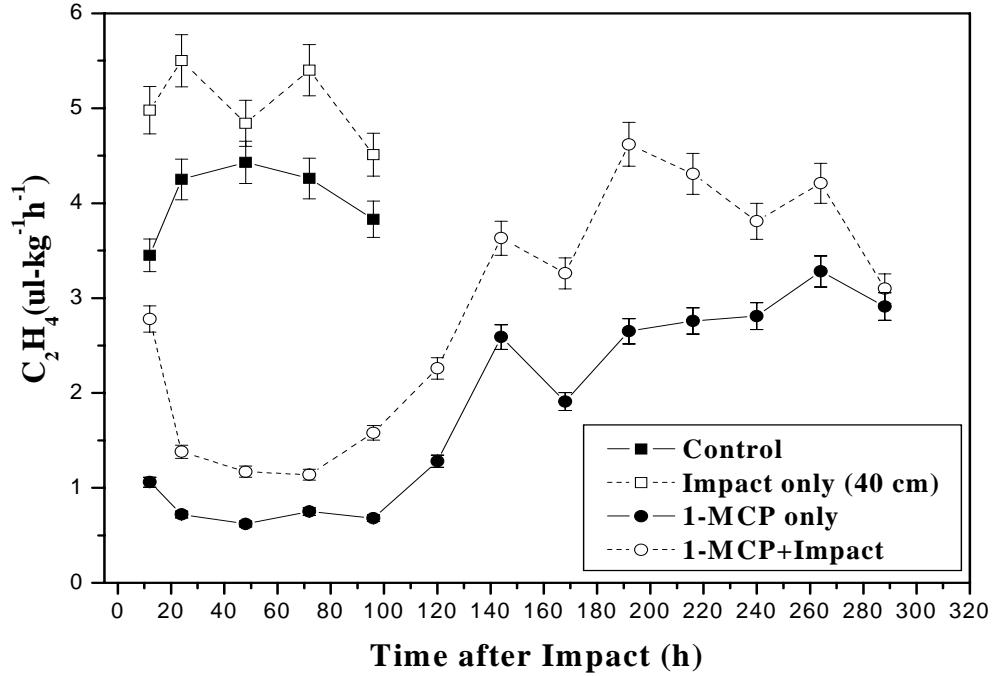


Figure 5-2. Ethylene production of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was subjected to double impacts (40 cm) (□), 1-MCP treatment (●), impact after 1-MCP treatment (○), or no treatment (control, ■). Each point indicates the mean of 5 fruits. Vertical bar represents standard error.

the expression of mRNA for ACC synthase 1. Meanwhile, impacted tomato exhibited a decrease in ethylene production at 48 h when control fruit produced maximum ethylene. The finding of Ketsa and Koolpluksee (1993) that “climacteric fruit produced a large amount of ethylene as ripening and wounding at this time may not increase or even decrease ethylene production” was parallel to this observation.

Roma-type tomato treated with 1-MCP exhibited a steep increase in ethylene production day 4 to day 6, and produced maximum ethylene at 260 h (day 11) (Figure 5-2). Maximum ethylene production was 4.12 $\mu\text{l} \text{C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, 87 % of control fruit's maximal ethylene production. The inhibition in ethylene production by 1-MCP was

reported in apricot (Botondi et al., 2003) and pear (Trinchero et al., 2004). In this experiment, 1-MCP-treated tomato reached climacteric peak about 8 to 9 d later than control fruit. Since climacteric fruits such as tomato become ripe after the climacteric peak in ethylene production, 1-MCP can be used to delay ripening in roma-type tomato.

Impacted fruit after 1-MCP application produced about $2.78 \mu\text{l C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ at 12h, which was 2.5-fold higher than 1-MCP-only treated fruit (Figure 5-2). An increase within 15 h after impact was followed by a return to the basal level in 24 h, and then by a climacteric rise in 192 h (day 8). Impact on the 1-MCP-treated tomato accelerated the climacteric rise 3 d earlier and induced 40% increase in peak ethylene production compared to only-1-MCP-treated fruit. Impact on the 1-MCP-treated fruit promoted ethylene production the same as on control fruit, although the former fruit ripened about 3 times slower than the latter. This observation means 1-MCP treatment did not alleviate the effect of impact on ethylene production; it only delayed it. Ripening in the climacteric fruit is accompanied by ethylene biosynthesis. Thus, mechanical injury can be expected to promote ripening of roma-type tomato based on the increase in ethylene production.

While ethylene production in control and impacted fruit increased continuously until reaching the climacteric peak period, impacted tomatoes after 1-MCP treatment produced less ethylene at 24 h (1 d) after impact than immediately after impact. Ethylene production in non-1-MCP-fruit increased not only by impact but also by natural ripening. As considering the delayed ripening by 1-MCP, the trend of ethylene production in 1-MCP-treated fruit during storage showed how mechanical injury affected on the fruit. Based this observation, a hypothesis that roma-type tomato tries to recover immediately from the impact stress and maintain normal metabolism can be formed.

5.3.1.3. Firmness

Roma-type tomato from all treatments softened during ripening at 20 °C. Initial internal firmness of roma-type tomato at breaker ripeness stage was approximately 11 to 12 N (Figure 5-3). Control fruit reached full-ripe firmness (4 N) at 7 days of storage. Impacted fruit exhibited a steep decrease in firmness during the first 2 d and again from days 4 to 6. This second steep decrease in firmness happened 2 d earlier on the impacted fruit than control. In addition, firmness of the impacted fruit reached 4 N at day 6, which was 1 d earlier than control. Following mechanical stress, the firmness decreased in cucumbers (Miller et al. 1987). Jiang et al. (1999) and Moretti et al. (2002) also reported

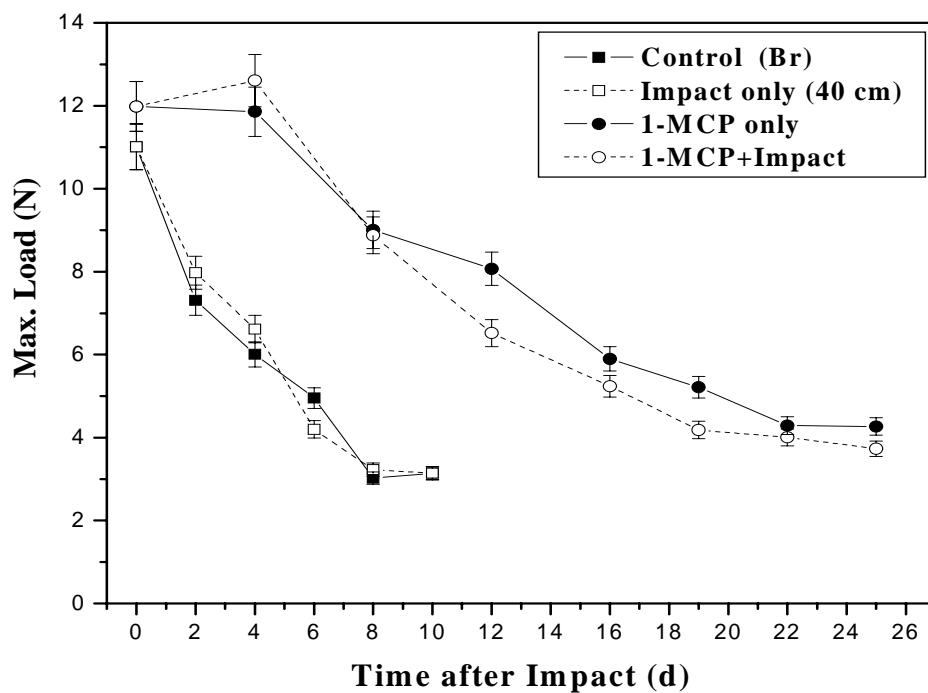


Figure 5-3. Pericarp firmness of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was subjected to double impacts of 40 cm (□), 1-MCP treatment (●), impact after 1-MCP treatment (○), or no treatment (control, ■). Each point indicates the mean of 5 fruits, 3 measurements per fruit. Vertical bar represents standard error.

accelerated softening of impacted banana and ‘Santa Clara’ tomato, respectively. These observations support the hypothesis that double impact of 40 cm at breaker stage could promote fruit ripening and shorten the shelf life.

1-MCP-treated fruit maintained initial firmness during the first 4 d (Figure 5-3). Firmness of 1-MCP-treated tomato decreased steeply during day 4 to day 8 and day 12 to day 16, and then slowly decreased to 4 N at 22 days of storage. Based on the firmness, 1-MCP application on roma-type tomato extended the shelf life 3 times as long as control. Delayed softening by 1-MCP was reported by Jeong et al. (2002) observing that avocado treated with 1-MCP at $0.45 \mu\text{l}\cdot\text{l}^{-1}$ for 24 h required twice the time to reach full-ripe firmness over untreated control fruit.

Firmness of tomato subjected to both impact and 1-MCP was lower than that of 1-MCP-only treated fruit after day 8. Impacted fruit after 1-MCP treatment also maintained initial firmness during the first 4 d; and rapid softening occurred from day 4 to day 19. Notably, at day 12 the impacted fruit after 1-MCP application was 20% softer than 1-MCP-treated fruit. In addition, tomato subjected to both impact and 1-MCP reached the full-ripe firmness stage (4N) at day 19, which was 3 d earlier than impact-free fruit. Observations previously described indicate that the 1-MCP treatment did not alleviate the softening caused by double impacts of 40-cm drop height.

5.3.1.4. Color

The external color of roma-type tomato was reported as lightness (L^*), hue angle (h°), and chroma value (C). Initial fruit color at breaker ripeness stage was 59 (L^*), 103 (h°) and 35 (C) (Figure 5-4). As tomatoes ripened, lightness and hue angle decreased and chroma value was increased.

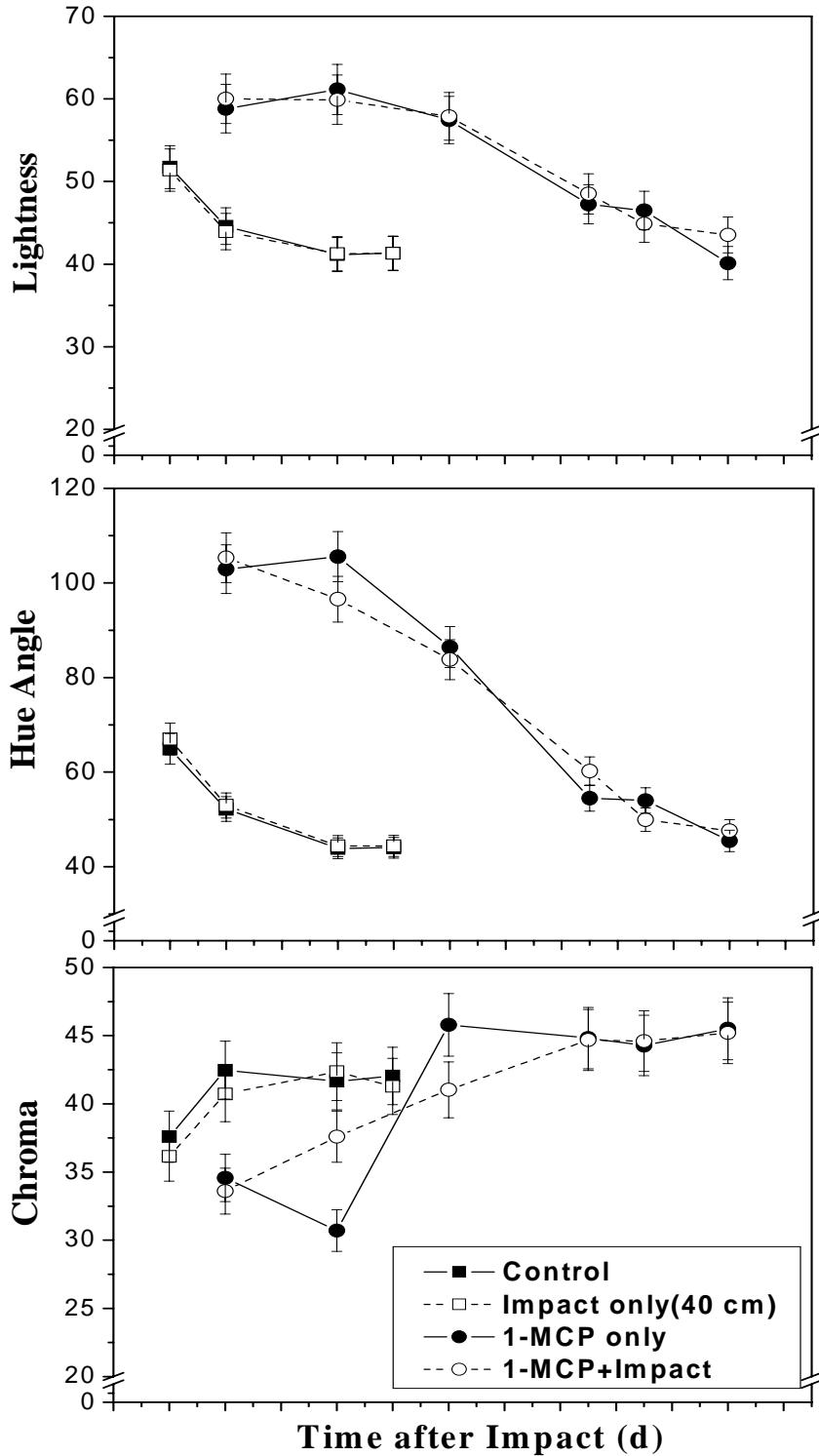


Figure 5-4. Color change of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was subjected to double impacts of 40 cm (□), 1-MCP treatment (●), impact after 1-MCP treatment (○), or no treatment (control, ■). Each point indicates the mean of 5 fruits, 3 measurements per fruit. Vertical bar represents standard error.

L^* of non 1-MCP-treated tomato decreased sharply immediately after storage by day 4, and reached the lowest value (42) at day 8, whereas 1-MCP delayed the decrease in lightness by day 12. Impact did not affect the lightness in both 1-MCP-treated fruit and non 1-MCP-treated fruit (Figure 5-4). DeMartino et al. (2002) observed that the difference in L^* between impacted and non-impacted apricot diverse depending on the temperature.

A change in hue angle indicates fruit ripening. The sharpest decrease in hue angle occurred within 4 days of storage in non 1-MCP treated tomatoes, while it did from day 4 to day 17 in 1-MCP treated fruits. Hue angle declined to the lowest value by 8 d for control fruit (no 1-MCP treatment), while tomato treated with 1-MCP at $1\mu\text{l l}^{-1}$ for 24 h reached the lowest value by day 22 (Figure 5-4). Comparing the impacted fruit and non-impacted fruit in 1-MCP treatment group, impact initiated the color change 4 d earlier. While the effect of impact on color development was not significantly clear, 1-MCP delayed color development on roma-type tomato similar to banana (Jiang et al., 1999) and avocado (Jeong et al., 2003). For example, avocado treated with 1-MCP at $0.9\ \mu\text{l l}^{-1}$ for 12 h showed the full-color development (hue angle=around 120) 4 d later than control fruit (Jeong et al., 2003).

The sharpest change in chroma happened during the first 4 d in non 1-MCP treated tomatoes, while it did from day 4 to day 17 in 1-MCP treated fruits. Impacted fruit had lower chroma value than control fruit, and roma tomato treated with only 1-MCP showed decreased then increased chroma during storage. This observation means that chroma in itself is not as useful a parameter in research to determine the effect of 1-MCP or impact treatment on the roma-type tomato.

5.3.1.5 Compositional analyses

To examine the possibility that 1-MCP and impact can affect the flavor of roma-type tomato, the values of soluble solids content (SSC), total titratable acidity (TTA) and pH were measured (Table 5-2). Neither impact nor 1-MCP application induced significant difference in the SSC range from 3.6 to 3.8 %. Moretti et al. (2002) and Mir et al. (2004) reported that 1-MCP did not change SSC on tomato. Tomato applied with 1-MCP showed 0.2 less pH and 0.15 to 0.20 more TTA value than control (non 1-MCP treatment group). Increase in TTA by 1-MCP was supported by the reports of Fan et al. (1999) and Wills and Ku (2002), while the opposite result, no difference by 1-MCP, was reported (Moretti et al., 2002; Mir et al., 2004, and Porat et al., 1999). On the other hand, impact on the fruits resulted in the increase of TTA only in 1-MCP treated roma tomatoes (Table 5-2).

The sugar/acid ratio is used to estimate tomato flavor. 1-MCP treatment induced a decrease in this ratio for non-impacted and impacted fruit over non-1-MCP treated. Baldwin et al. (2000) previously noted that the flavor might be similar for treated tomato and non-treated fruit because of no significant effect of $1 \mu\text{l l}^{-1}$ 1-MCP on SSC and acid concentrations. On the other hand, Wills and Ku (2002) noted 1-MCP inhibits the loss

Table 5-2. Compositional analyses at full-ripe stage.

Treatment	SSC ^z ($^{\circ}\text{Brix}$)	TTA (%)	Sugar/acid ratio	pH
Control	$3.72 \pm 0.28 \text{ a}^y$	$0.42 \pm 0.03 \text{ c}$	$8.92 \pm 0.61 \text{ a}$	$4.34 \pm 0.03 \text{ a}$
Impact only	$3.64 \pm 0.22 \text{ a}$	$0.42 \pm 0.05 \text{ c}$	$8.86 \pm 1.10 \text{ ab}$	$4.34 \pm 0.05 \text{ a}$
1-MCP only	$3.76 \pm 0.15 \text{ a}$	$0.58 \pm 0.06 \text{ b}$	$6.58 \pm 0.76 \text{ bc}$	$4.16 \pm 0.04 \text{ b}$
1-MCP + Impact	$3.70 \pm 0.23 \text{ a}$	$0.63 \pm 0.02 \text{ a}$	$5.84 \pm 0.30 \text{ c}$	$4.13 \pm 0.05 \text{ b}$

^zSSC = Soluble Solids Content; TTA = Total Titratable Acidity (citric acid equivalent).

^yMean (n=10) following by standard deviation. Columns with different letters are significantly different at P< 0.05, according to Duncan's Multiple Range Test.

of TTA resulting a lower sugar/acid ratio. In this experiment, combination of 1-MCP and impact treatment induced the 35% lower sugar/acid ratio than control (non 1-MCP and non impact treatment). Although tomato with higher TTA might have a “more acceptable taste for consumers” (Wills and Ku, 2002), the effect of combination of 1-MCP and impact treatment on the flavor of roma-type tomato has to be studied in the future using a sensory panel or aroma analysis.

5.3.1.6 Enzyme activity

Endo-polygalacturonase (PG) activity was tracked as index of bruising. PG has been related to loss of firmness during ripening (Grierson and Kader, 1986). PG activity of roma- tomato at breaker ripeness stage was very low (4 to 10 mole D-gal kg⁻¹·min⁻¹ *10⁵) and increased steeply after day 2, and continuously until full-ripe stage at day 10 (Figure 5-5).

Impacted fruits had higher PG activity than control during the entire storage period, whether tomatoes were treated with 1-MCP or not (Figure 5-5). At day 6, PG activity of the impacted fruit was twice as that of control. Steep increase in PG activity was observed during the first 6 d, which was initiated 2 d earlier than control. These observations mentioned above mean that double impacts of 40cm drop height on roma-type tomato promotes an increase in PG activity and accelerates cell wall degradation and softening over non-impacted fruit. Since, endo-PG activity increased during softening (Brady et al., 1983). In the addition, extractability of PG from cell wall might affect on the PG activity. Since PG binds to cell wall tenaciously, high concentrations of ionic strength are needed for extraction of PG (Pressey, 1986). Impact could damage the cell wall of fruit, which made PG extraction easy. The higher concentrations of PG from same weight of tissue in impacted fruit than non-impacted fruit might result in high

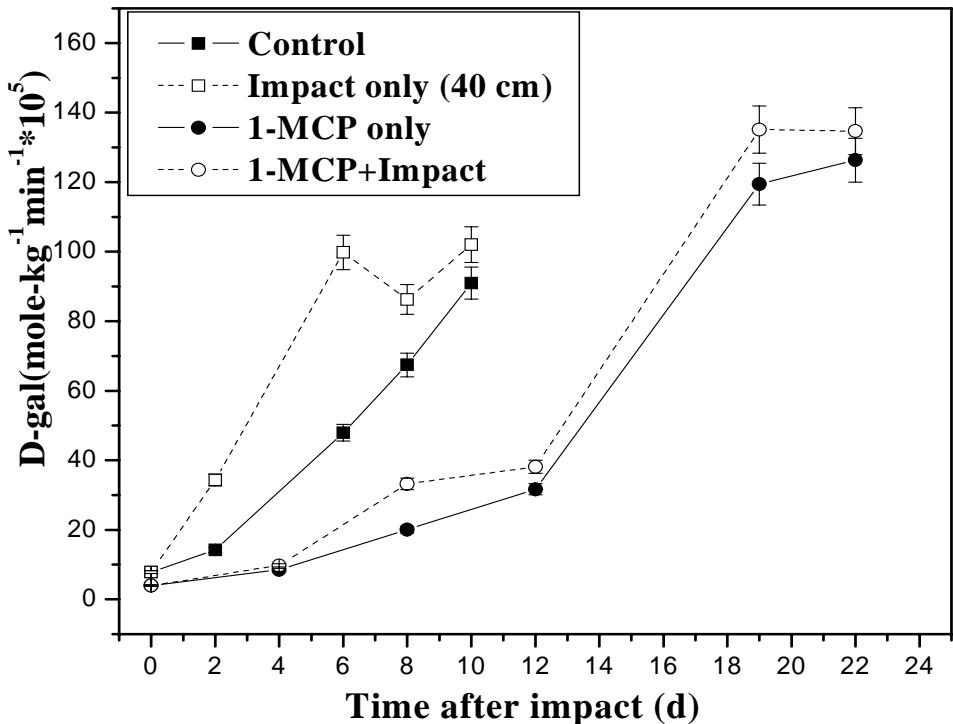


Figure 5-5. Change of polygalacturonase (PG) activity in pericarp tissue of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was subjected to double impacts of 40 cm (□), 1-MCP treatment (●), impact after 1-MCP treatment (○), or no treatment (control, ■). Each point indicates the mean of 5 fruits, 3 measurements per fruit. PG activity is expressed as molecular D-galacturonic acid equivalents produced per kilogram protein per minute. Vertical bar represents standard error.

concentration of D-galacturonic acid. Another explanation for this increased PG activity on impacted fruit can be induced by the finding of Rushing and Huber (1987) that the PG activity was affected by pH of the apoplast. PG reacted actively at acidic condition (pH 4.5), while no activation was observed at pH 11 (Rushing and Huber, 1990). Thus, cell wall damage by double impacts of 40 cm induced PG movement into vacuole of which acidic condition might increase PG activity.

There was a delay in increase of PG activity by 1-MCP application (Figure 5-5). PG activity of 1-MCP treated fruit steeply increased during day 12 to day 19 and 1-MCP-treated tomato reached the final PG activity at day 20 while control fruit did at day 10. In the addition, the final PG activity of 1-MCP-treated roma tomato was 38% higher than that of control. This observation was not paralleled to the result in firmness that the final firmness of 1-MCP-treated fruit was slightly higher than that of control. Thus, it can be assumed that PG activity of roma-type is not correlated linearly with firmness, and other enzymes such as rhamnogalacturonase A (Gross et al., 1995), pectin methylesterase (Hobson, 1963) might participate in softening of roma-type tomato.

PG activity of the impacted fruit after 1-MCP treatment increased during day 12 to day 19 same as fruit treated with only 1-MCP. Although the impacted fruit had higher activity of PG than non-impacted one in non-1-MCP treated group, there was no significant difference oppositely to the observation in non-1-MCP treated group. 1-MCP can be expected to reduce the increase in PG activity caused by impact. Suppressed increase in PG activity by 1-MCP treatment was also observed in avocado (Jeong et al., 2002).

On the other hand, PG activity of control or 1-MCP treated fruit increased most steeply during day 2 to 6 or day 12 to 20, respectively, which were immediately after the climacteric period. The findings of Jeong et al. (2003) on avocado supported this result.

5.3.2 Test 2. 1-MCP Pretreatment of Roma-Type Tomato Subjected to Double Impact Equivalent to 80 cm Drop Height

5.3.2.1 Respiration rate

Control fruit produced about $27 \text{ ml CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ initially, and then 38% less CO_2 at 12 h after impact (Figure 5-6). As the respiration rate of control fruits increased, the climacteric peak occurred at day 2 to 3 (Table 5-3).

Impacted fruit, meanwhile, produced initially same amount of CO_2 as control fruit. Although there was no difference in time to climacteric peak, impact on roma-type tomato increased 11% in peak amount of CO_2 production. Impacted fruit had 10 to 20% higher respiration rate during the entire storage (Figure 5-6). Increasing respiration rate by impact was reported in round-type tomato (MacLeod et al., 1976). Damage on fruits increases oxygen consumption because of the oxidation of phenolic compounds by catechol oxidase (Knee and Miller, 2002). Sturm and Chrispeels (1990) also hypothesized that a stress might induce a high demand for hexoses, activating hexose metabolism such as respiration.

Roma-type tomato treated with 1-MCP produced initially about $21 \text{ ml CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, that was 80% of a respiration rate of control fruit. Similar to control fruit, respiration rate of 1-MCP treated-fruit decreased by 12 h and increased after that. Respiration rate of 1-MCP treated fruit was lower than that of control fruit during the entire ripening period. This observation supported the hypothesis that 1-MCP application on roma-type tomato can suppress CO_2 production such as apricot (Fan et al., 2000), avocado (Jeong et al., 2002), and broccoli (Forney et al., 2003). Climacteric peak was seen at 240 h (day 10) and maximum respiration rate was $19 \text{ ml CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, which was 15% lower than that of control fruit (Table 5-3). Oppositely, avocado treated with 1-MCP did not exhibit the

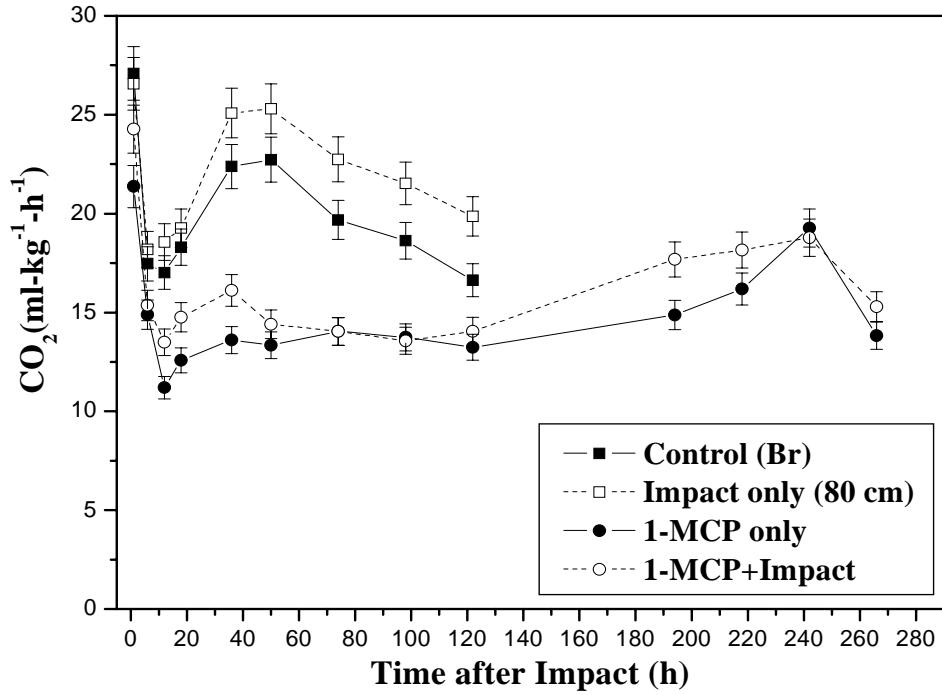


Figure 5-6. Respiration rate of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was subjected to double impacts of 80 cm (□), 1-MCP treatment (●), impact after 1-MCP treatment (○), or no treatment (control, ■). Each point indicates the mean of 5 fruits. Vertical bar represents standard error.

Table 5-3. Time to peak and maximum CO₂ production of 'Breaker' roma-type tomato stored at 20 °C.

Treatments	Time to peak (d)	Maximum CO ₂ production (ml·kg ⁻¹ ·h ⁻¹)
Control	2.8 b ^z	22.94 ± 1.76 a
Impact only	2.5 b	25.49 ± 2.03 a
1-MCP only	10 a	19.27 ± 1.98 b
1-MCP + Impact	9.6 a	18.87 ± 0.93 b

^zMean (n=5) following by standard deviation. Columns with different letters are significantly different at P< 0.05, according to Duncan's Multiple Range Test.

respiratory peak during storage at 20 °C (Jeong et al., 2002).

Impacted tomato after 1-MCP application produced initially about 24 ml CO₂·kg⁻¹·h⁻¹, which was about 115% of the respiration rate of fruit treated with only 1-MCP (Figure 5-6). Minimum respiration rate was observed at 12 h, the same as the other treatments, and a small peak of CO₂ production was seen at 36 h when the impacted fruit had 20% higher respiration rate than control fruit. A slight peak was seen also in previous test (Figure 5-1). While the size of peaks was similar at Test 1, a peak at 240 h (day 10) was larger than that at 36 h in this experiment. Thus, the second peak can be considered as climacteric peak in respiration rate and 1-MCP seems to cause another little peak at the early period. Respiration rate of the impacted fruit after 1-MCP treatment increased after 120 h (day 5) while fruit treated with only 1-MCP exhibited an increase in CO₂ production after 192 h (day 8). These observations means that impacts on roma-tomato treated with 1-MCP can increase the respiration rate. Maximum CO₂ production was seen at day 10. Tomato subjected to both impact and 1-MCP produced maximal 18.87 ml CO₂·kg⁻¹·h⁻¹, which was not significantly different from maximal respiration rate of 1-MCP treated fruit (Table 5.3). Impact on control fruit induced increased maxima of respiration rate. In addition, non-1-MCP treated tomato exhibited a greater difference in respiration rate between control fruit and impacted fruit than the 1-MCP treated fruit (Figure 5-6). These observations in this experiment support the hypothesis that 1-MCP can control increased respiration rate caused by impact on the roma-type tomato, the same as Test 1.

Roma-type tomato from all treatment produced the most CO₂ immediately after the impact treatment, meaning the fruit was under many stresses from several experimental

procedures such as sorting, washing, drying, 1-MCP application and impact treatment. However, tomato seems to have recovery ability based on the occurrence of a sharp decrease in respiration rate for the first 12 h.

5.3.2.2 Ethylene production

Figure 5-7 showed that initial ethylene production of control tomato at breaker ripeness stage was $2 \mu\text{l}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ and the peak was seen at 72 h (day 3). The maximum ethylene production was about $5.8 \mu\text{l}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. During the entire ripening period, the ethylene production of impacted fruit was higher than that of control. Impacted fruit produced about 23% more ethylene than control at 48 h (day 2), and maxima of ethylene was produced at 48-72 h (day 2-3). Impact on non-1-MCP fruit induced earlier and higher ethylene peak, consistent with finding of MacLeod et al. (1976) that respiration rate of the round-type tomato increased by impact. Olson et al. (1991) reported that mechanical stress elevated the expression of mRNA for ACC synthase 1. As more the precursor 1-aminocyclopropene-1-carboxylic acid (ACC) is synthesized and converted into ethylene, ethylene production increased (Yang and Hoffman, 1984).

Roma-type tomato treated with 1-MCP exhibited a steep increase in ethylene production during 120 h (day 5) to 240 h (day 10), and produced maximum ethylene at 240 h (day 10) (Figure 5-7). Maximum ethylene production was $6.6 \mu\text{l}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, 125% of that of control fruit. Otherwise, a decrease in maximum ethylene production by 1-MCP was observed in Test 1. Effect of 1-MCP on commodity can vary depending on variety, plant maturity, or environmental condition. The differences in Test 1 and Test 2 were the harvest time, grown place and impact height. Since 1-MCP-only treated fruit produced more ethylene than control fruit, the harvest time and/or grown place seems to affect the

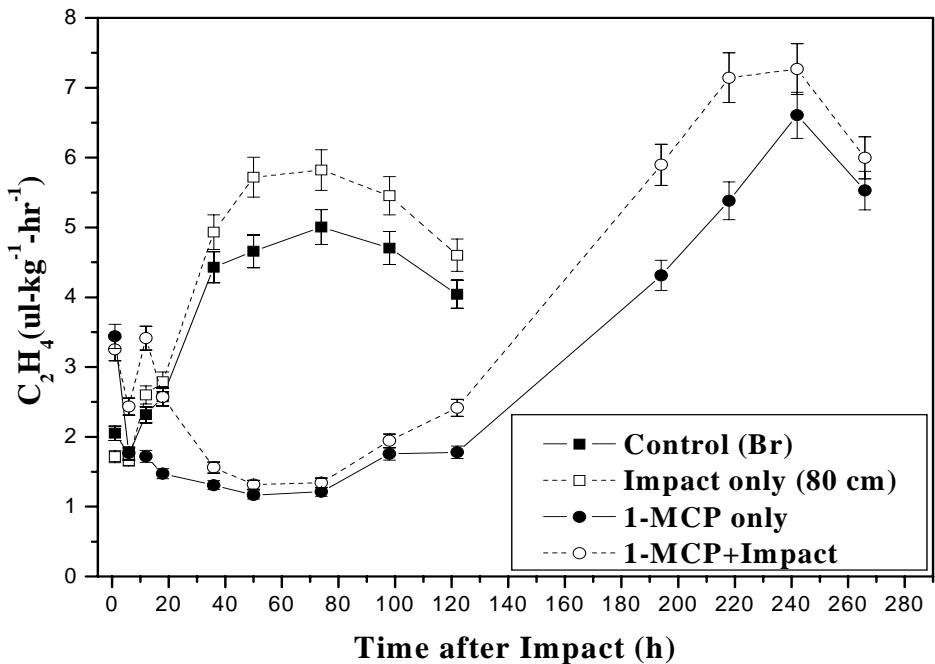


Figure 5-7. Ethylene production of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was subjected to double impacts of 80 cm (□), 1-MCP treatment (●), impact after 1-MCP treatment (○), or no treatment (control, ■). Each point indicates the mean of 5 fruits. Vertical bar represents standard error.

1-MCP effectiveness. In this experiment, 1-MCP-treated tomato reached climacteric peak about 7 d later than control fruit. This means that 1-MCP treatment on roma tomato can increase the shelf-life because climacteric fruits such as tomato become ripe after climacteric peak in ethylene production.

Impacted fruit after 1-MCP application initially produced about $3.25 \mu\text{l C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and showed a small peak at 12 h (Figure 5-7). At 12 h, tomato treated with both impact and 1-MCP produced twice the amount of ethylene than control. The rise and fall in ethylene production 12 h after impact was followed by a return to the basal level at 48 h (day 2) and by a climacteric rise at 216 to 240 h (days 9 to 10). Impact on the 1-MCP-treated tomato resulted in the climacteric peak occurring 1 d early and inducing a 10%

increase in peak ethylene production. Double impacts equivalent to 80-cm drop height on the 1-MCP-treated fruit promoted ethylene production the same as control fruit. Roma-type tomato subjected to both impact and 1-MCP produced 33 to 37% more ethylene than fruits treated with only 1-MCP during 100 h (day 5) to 216 h (day 9). This observation means 1-MCP treatment did not alleviate the effect of double impact of 80 cm on ethylene production. Ripening in the climacteric fruit is accompanied by ethylene biosynthesis. Thus, mechanical stress can also be expected to accelerate ripening of roma-type tomato treated with $1 \mu\text{l}\cdot\text{l}^{-1}$ 1-MCP based on the increase in ethylene production.

5.3.2.3 Firmness

Roma-type tomato softened during ripening at 20 °C. Initial pericarp firmness of roma-type tomato at breaker ripeness stage was approximately 11 to 13 N (Figure 5-8). Firmness of control fruit sharply decreased during the first 4 d and from day 6 to day 8; and control fruit reached full-ripe firmness (4 N) within 7 d of storage. Impacted fruit exhibited a steep decrease in firmness during the first 2 d, and firmness continuously decreased by day 8. In addition, firmness of the impacted fruit reached 4 N at day 6, which was 1 to 2 d earlier than control, the same as banana (Jiang et al., 1999) Those observations support the hypothesis that an impact on roma-type tomato at breaker stage could promote fruit ripening and shorten the shelf life.

1-MCP-treated fruit maintained initial firmness during the first 4 d (Figure 5-8). Firmness of 1-MCP-treated tomato decreased sharply from day 4 to day 16. Firmness decreased to 4 N within 14 d of storage. Based on the firmness, 1-MCP application on

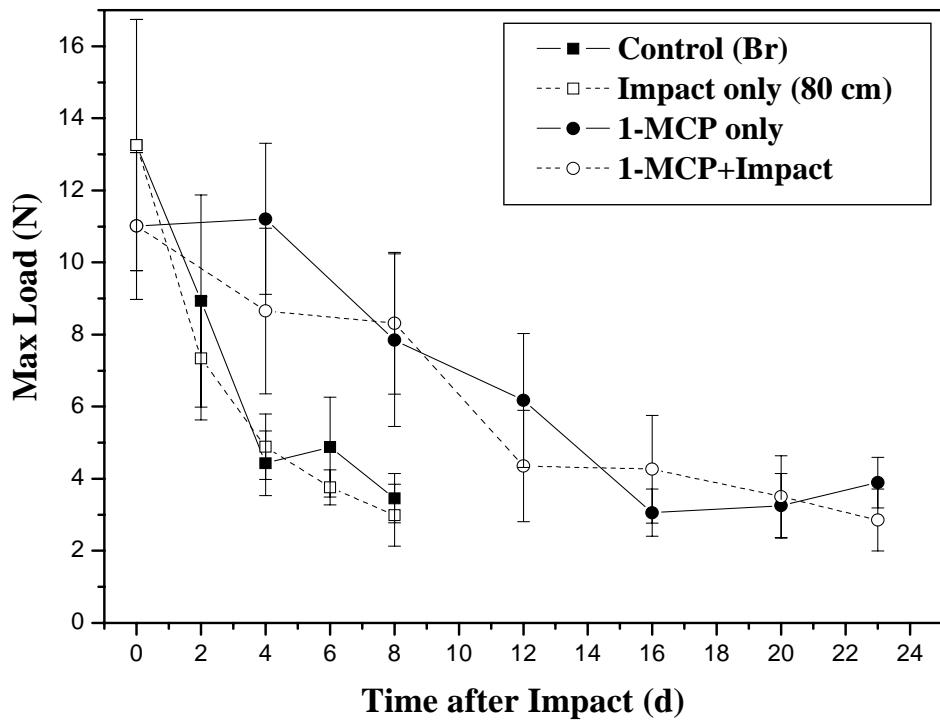


Figure 5-8. Pericarp firmness of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was subjected to double impacts of 80 cm (□), 1-MCP treatment (●), impact after 1-MCP treatment (○), or no treatment (control, ■). Each point indicates the mean of 5 fruits, 3 measurements per fruit. Vertical bar represents standard error.

roma-type tomato extended the shelf life 2 times as long as control, the same as avocado (Jeong et al., 2002).

Firmness of tomato subjected to both impact and 1-MCP decreased steeply during the first 4 d and day 8 to day 12. Otherwise, 1-MCP-treated fruit subjected to the impact equivalent to 40-cm height drop maintained an initial firmness during the first 4 d as in previous test (Test 1). The stronger impact in Test 2 might promote softening in roma-type tomato faster. Especially, at day 12 the impacted fruit after 1-MCP application was 30% softer than 1-MCP-treated fruit. In addition, tomato subjected to both impact and 1-

MCP reached the full-ripe firmness stage (4 N) at day 10, which was 3 d earlier than impact-free fruit. Observations previously described supported the hypothesis that double impact of 80 cm at breaker stage could promote fruit ripening regardless of 1-MCP treatment.

5.3.2.4 Color

The external color of roma-type tomato was reported as lightness (L^*), hue angle (h°), and chroma (C). Initial fruit color at breaker ripeness stage was 65 (L^*), 110 (h°) and 25 (C) (Figure 5-9). As tomatoes ripened, lightness and hue angle decreased and chroma increased.

Lightness of non 1-MCP-treated tomato decreased sharply immediately after storage by day 4 and reached the lowest value (47) at day 8, while 1-MCP delayed the decrease in lightness by days 8 to 12. Roma-type tomato treated with both impact of 80 cm and 1-MCP showed the decline in lightness 4 d earlier than fruit treated with only 1-MCP, while the impact did not affect the lightness change in non-1-MCP treated fruits. DeMartino et al. (2002) observed that the difference in L^* between impacted and non-impacted apricot diverse depending on the temperature at the impact time and after impact.

A change in hue angle indicates fruit ripening. Hue angle declined to the lowest value by 8 d for control fruit (no 1-MCP treatment), while tomato treated with 1 $\mu\text{l l}^{-1}$ 1-MCP reached the lowest value by day 20 (Figure 5-9). There was no difference in hue angle between impacted fruit and control fruit during the entire storage period. Impacted fruit had a sharp decline in hue angle 4 d earlier than control, and the hue angle of impacted fruit was 10% less than that of control at day 12. Delayed color development by

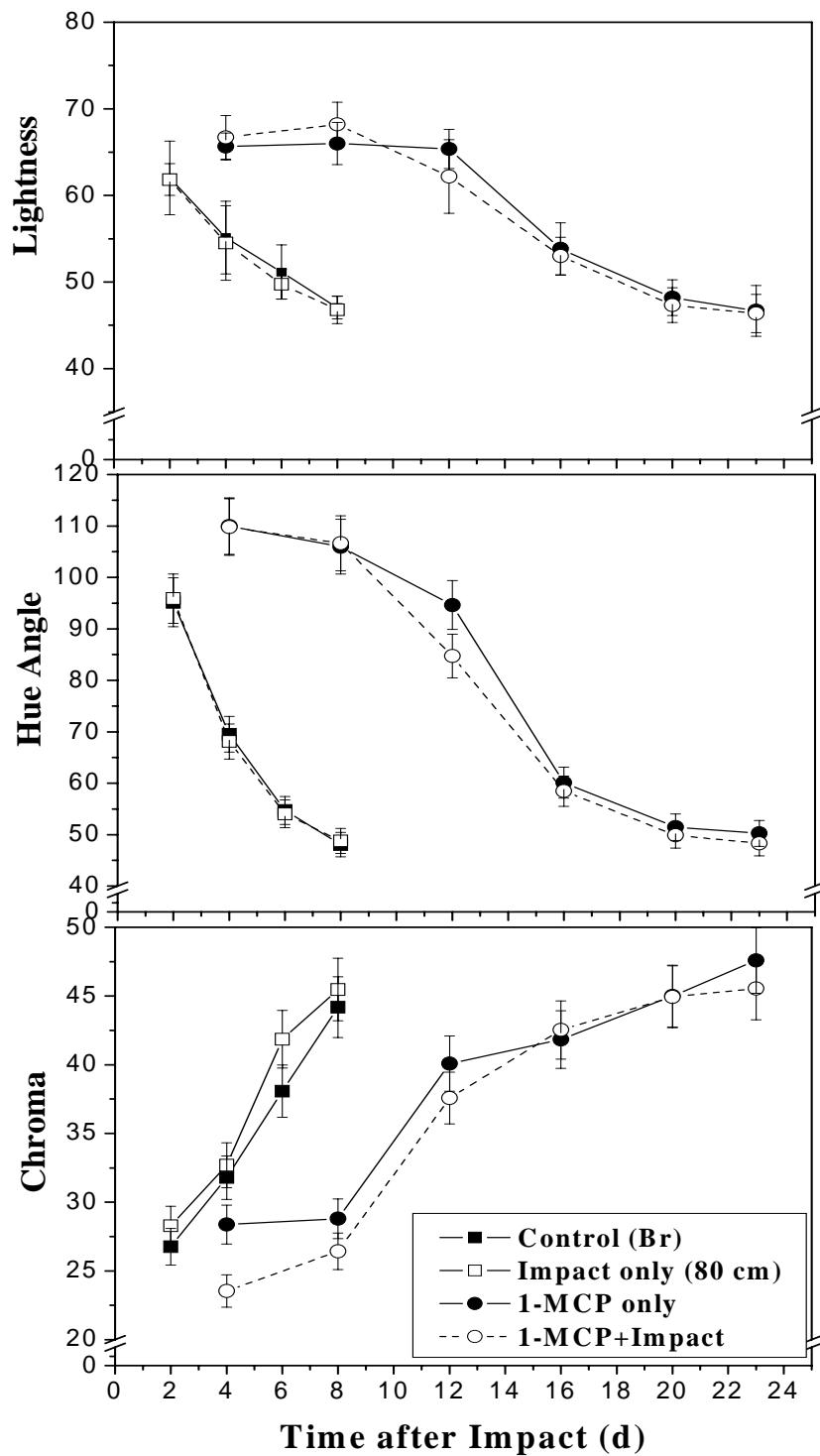


Figure 5-9. Color change of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was subjected to double impacts of 80 cm (□), 1-MCP treatment (●), impact after 1-MCP treatment (○), or no treatment (control, ■). Each point indicates the mean of 5 fruits, 3 measurements per fruit. Vertical bar represents standard error..

1-MCP was seen on roma-type tomato same as on banana (Jiang et al., 1999) and avocado (Jeong et al., 2003).

There was no significant difference in chroma between impacted and non-impacted tomato, whether fruit was treated with 1-MCP or not (Figure 5-9). The sharpest change in chroma happened during day 8 to day 12 in 1-MCP treated tomatoes. 1-MCP treated fruit and non-1-MCP treated fruit reached the final chroma value (45) at day 8 and day 23, respectively.

5.3.2.5 Compositional analyses

The values of soluble solids content (SSC), total titratable acidity (TTA) and pH were measured at full-red ripeness stage (Table 5-4). Control roma-type tomato had 2.95 °Brix, 0.31% TTA, pH 4.34 and 9.51 sugar/acid ratio.

Impact did not cause any difference in SSC, TTA, pH or sugar/acid ratio. However, 1-MCP induced an increase in SSC and TTA, and a decrease in pH. The increase in TTA by 1-MCP was reported by Fan et al. (1999) and Wills and Ku (2002) while Mir et al. (2004) noted no significant change in SSC on 1-MCP-treated tomato. The increase in TTA and the decrease in pH and sugar/acid ratio by 1-MCP were observed in both Test 1 and Test 2. But, 1-MCP-only treated fruit had the higher SSC than control fruit in Test 2 while there was no significant change in SSC due to 1-MCP in Test 1. This seems to be caused by the difference in fruit development, harvest time or grown environment.

Sugar/acid ratio is used as an indicator for tomato flavor. While there was no significant difference between impacted and non-impacted fruit regardless of 1-MCP treatment, the sugar/acid ratio of 1-MCP-treated tomato decreased into 83% of that of non-1-MCP treated fruit (Table 5-4). Since the increase in TTA by 1-MCP was larger than the increase in SSC by 1-MCP,

Table 5-4. Compositional analyses at full-ripe stage.

Treatment	SSC ^z (°Brix)	TTA (%)	Sugar/acid ratio	pH
Control	2.95±0.58 c	0.31±0.06 b	9.51±0.70 a	4.34±0.05 a ^y
Impact only	3.09±0.62 bc	0.33±0.06 b	9.39±0.95 a	4.37±0.05 a
1-MCP only	3.93±0.50 a	0.51±0.06 a	7.77±0.99 b	4.22±0.04 b
1-MCP + Impact	3.55±0.52 ab	0.46±0.08 a	7.86±0.79 b	4.21±0.03 b

^zSSC = Soluble Solids Content; TTA = Total Titratable Acidity (citric acid equivalent)

^yMean (n=10) following by standard deviation. Columns with different letters are significantly different at P< 0.05, according to Duncan's Multiple Range Test.

5.3.2.6 Enzyme activity

PG activity of roma-tomato at breaker ripeness stage was very low (4 to 10 mole D-gal·kg⁻¹·min⁻¹*10⁵) and increased continuously after day 4 until reaching full-ripe stage (Figure 5-10). Impacted fruit had the same PG activity as control fruit during the entire storage period.

There was a delay in increase of PG activity by 1-MCP application (Figure 5-10). PG activity of 1-MCP treated fruit steeply increased during day 12 to day 16, and then less dramatically until at day 23. Control fruit increased in PG until full-ripe stage at day 8. The final PG activity of 1-MCP-treated roma tomato was 15% lower than that of control.

PG activity of the impacted fruit after 1-MCP treatment increased immediately after storage and continuously (Figure 5-10). Impact on 1-MCP-treated tomato induced a fifth increase in PG activity from day 8 to day 12. This result was opposite to the observation in previous test (Test 1) showing no significant difference in PG activity between impacted and non-impacted fruit in 1-MCP treated group. The differences between previous test and present test were the impact magnitude (40 cm vs. 80 cm), harvest time (May vs. June) and growing location. Since there was no difference between

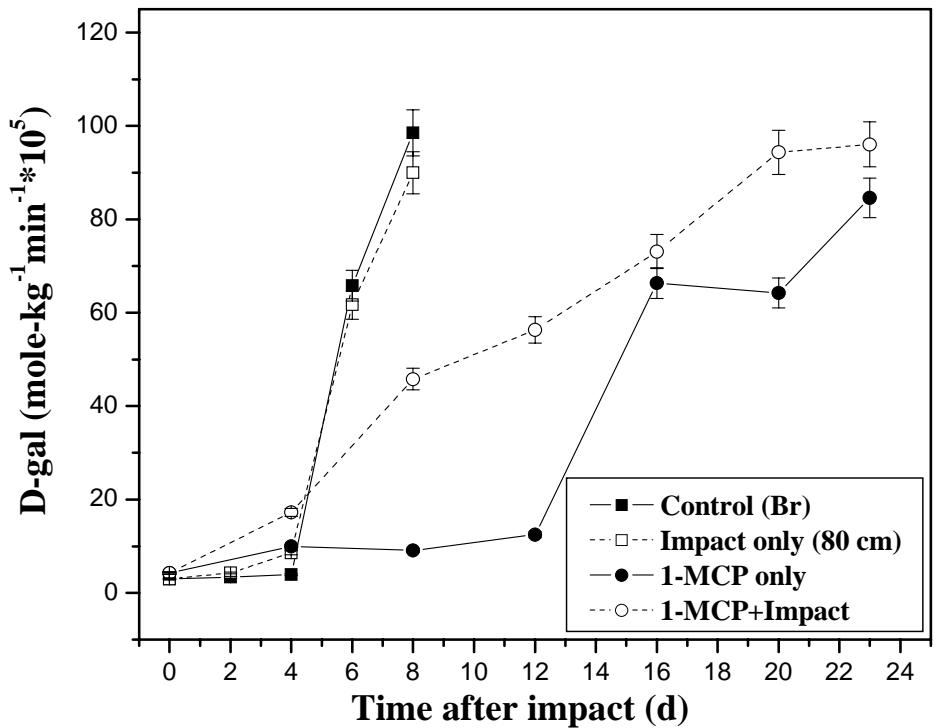


Figure 5-10. Change of polygalacturonase (PG) activity in pericarp tissue of roma-type tomato at breaker stage during ripening at 20 °C. Fruit was subjected to double impacts of 80 cm (□), 1-MCP treatment (●), impact after 1-MCP treatment (○), or no treatment (control, ■). Each point indicates the mean of 5 fruits, 3 measurements per fruit. Vertical bar represents standard error.

control and only-impacted fruit in this test oppositely to Test 1, harvested time or location could be a cause of this contradictory result. Otherwise, the impact force might effect on PG activity in 1-MCP-treated group. 1-MCP ($1 \mu\text{l}\cdot\text{l}^{-1}$ for 24 h) suppressed the increase in PG activity by impact of 40 cm drop height (Test 1) while the impact of 80 cm drop height caused the significant increase in PG activity in Test 2. Thus, it can be concluded that a strong impact promotes an increase in PG activity and that 1-MCP effectively

alleviates an increase in PG activity of roma-type tomato impacted with certain range of impact force.

On the other hand, PG activity of control or 1-MCP treated fruit increased most steeply during days 2 to 6 or days 12 to 20, respectively, which were immediately after the climacteric period. The findings of Jeong et al. (2003) on avocado supported this result.

5.3. Summary

Double impacts equivalent to 40-cm or 80-cm drop height increased the respiration rate and ethylene production during the entire ripening period. 1-MCP-treated tomato ($\mu\text{l}\cdot\text{l}^{-1}$ 1-MCP for 24 h at 22 °C) showed no or lower respiratory climacteric peak than control fruit, and impacts of 1-MCP-treated fruit did not increase CO_2 production. 1-MCP did not reduce the accelerated ethylene production caused by impacts.

Tomatoes treated with 1-MCP showed 2 to 2.5 times delayed softening, color development and increase in PG activity over untreated control fruit. However, 1-MCP did not delay the softening and color development accelerated by double 40-cm or 80-cm impacts. Roma tomato treated with 1-MCP had increased TTA and decreased pH and sugar/acid ratio. Impact did not affect these values.

Polygalacturonase (PG) activity, responsible for tomato ripening, increased during storage. The highest increase in PG activity was observed immediately after the climacteric period, regardless of treatment. 1-MCP delayed the lag of increased PG activity. In addition, 1-MCP controlled the PG activity depending on the impact magnitude applied on roma-type tomato. Impact equivalent to two drops from 40 cm did not induce any change in PG activity of 1-MCP-treated tomato, while PG activity of

tomato impacted with the force equivalent to two drops from 80 cm increased faster than that of non-impacted fruit.

CHAPTER 6

CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

The effect of impact on roma tomato was dependent on fruit maturity. After a single 60-cm impact, mature-green tomato showed accelerated respiration rate and softening; increased ethylene production was observed for pink and light-red fruit. Double impacts equivalent to the force of a 40-cm drop height induced the greatest increases in the respiration rate, ethylene production and softening in breaker stage fruit. Based on these results, it can be concluded that breaker stage fruit is more susceptible to impact stress than fruit at green, pink, light-red or red ripeness stage. In the addition, a single 60-cm drop caused no change in SSC, TTA, pH and sugar/acid ratio with the exception that fruit impacted at red stage had increased TTA. This observation suggests that harvest before red stage can prevent change in the flavor of roma tomato caused by impact during subsequent handling procedures.

Impact intensity affected the quality of roma tomato. Among three different impact forces tested (equivalent to a 20, 40 or 60-cm drop height), an impact of 60 cm induced greater increases in respiration rate, ethylene production and ripening rate of breaker tomato than the lower impact forces. Thus, roma-type tomato should not be more than 60 cm during handling operations.

Breaker-stage fruit were treated with 1-MCP ($1 \mu\text{l}\cdot\text{l}^{-1}$ for 24 h). Non-impacted fruit had delayed softening, color development and increased PG activity that were seen 2.5 times later than non-1-MCP control fruit; 1-MCP treatment also decreased the respiration rate over the control. Double impacts of 40 cm or 80 cm did not increase CO_2 production

in 1-MCP-treated fruit; however, 1-MCP did not suppress the acceleration in ethylene production, fruit softening and color development due to impacts. This means that 1-MCP may not alleviate every impact symptom caused by impacts. On the other hand, double impacts of 40 cm did not induce any change in PG activity of 1-MCP-treated tomato, while double impacts of 80 cm increased PG activity faster than that of non-impacted, 1-MCP-treated fruit. This observation indicated that the extent of 1-MCP control of PG activity in breaker-stage tomato depended on the magnitude of the impact. Although 1-MCP shows promise to alleviate stress due to mechanical injury, more studies are necessary to determine the relationship between concentration and treatment time over a range of impact forces. Furthermore, roma tomato treated with 1-MCP had higher TTA, lower pH and lower sugar/acid ratio for either impacted or non-impacted fruit. Therefore, the potential flavor changes in 1-MCP-treated fruit should be evaluated using sensory panel and/or aroma analyses.

APPENDIX

RAW OUTPUT OF STATISTICAL ANALYSIS ON TABLE 4-4

Output of SAS program used to analyze data of compositional analyses of roma-type tomato at full-ripe stage (Table 4-4) was placed as following.

The GLM Procedure

Duncan's Multiple Range Test for SSC

Alpha 0.05
 Error Degrees of Freedom 42
 Error Mean Square 0.406839
 Harmonic Mean of Cell Sizes 9.432314

Number of Means	2	3	4	5	6
Critical Range	.5927	.6233	.6433	.6577	.6688

Duncan Grouping	Mean	N	BLOCK
A	4.6200	10	BREAKER+IMPACT
A			
B A	4.1900	10	PINK+IMPACT
B A			
B A	4.0400	10	BREAKER+CONT
B			
B	3.8778	9	PINK+CONT
B			
B C	3.7250	8	LIGHT-RED+CONT
C			
C	3.1800	10	LIGHT-RED+IMPACT

The GLM Procedure

Duncan's Multiple Range Test for TTA

Alpha 0.05
 Error Degrees of Freedom 39
 Error Mean Square 0.003983
 Harmonic Mean of Cell Sizes 8.674699

Number of Means	2	3	4	5	6
Critical Range	.06129	.06444	.06650	.06799	.06912

Duncan Grouping	Mean	N	BLOCK
A	0.42400	10	BREAKER+CONT
A	0.41700	10	BIMPACT
A	0.39875	8	PINK+CONT
A	0.39833	6	LIGHT-RED+CONT
A	0.38400	10	PINK+IMPACT
A	0.37000	10	LIGHT-RED+IMPACT

The GLM Procedure

Duncan's Multiple Range Test for RATIO

Alpha 0.05
 Error Degrees of Freedom 42
 Error Mean Square 4.101334
 Harmonic Mean of Cell Sizes 9.432314

Number of Means	2	3	4	5	6
Critical Range	1.882	1.979	2.042	2.088	2.123

Duncan Grouping	Mean	N	BLOCK
A	11.2340	10	BREAKER+IMPACT
A	10.9350	10	PINK+IMPACT
A	9.8320	10	BREAKER+CONT
B A	9.4356	9	PINK+CONT
B	8.6510	10	LIGHT-RED+IMPACT
B	8.3000	8	LIGHT-RED+CONT

The GLM Procedure

Duncan's Multiple Range Test for PH

Alpha 0.05
 Error Degrees of Freedom 42
 Error Mean Square 0.002366
 Harmonic Mean of Cell Sizes 9.432314

Number of Means	2	3	4	5	6
Critical Range	.04520	.04753	.04905	.05015	.05100
Duncan Grouping	Mean	N	BLOCK		
A	4.54700	10	PINK+IMPACT		
A					
A	4.54500	8	LIGHT-RED+CONT		
A					
B A	4.52400	10	LIGHT-RED+IMPACT		
B A					
B A	4.52333	9	PINK+CONT		
B A					
B A	4.51700	10	BREAKER+IMPACT		
B					
B	4.49100	10	BREAKER+CONT		

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BIOGRAPHICAL SKETCH

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