EFFECT OF MULTIPLE 1-MCP TREATMENTS

TO EXTEND SHELF LIFE OF TOMATO

by

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ABSTRACT OF THE THESIS

Effect of Multiple 1-MCP Treatments to Extend Shelf Life of Tomato

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Ethylene is a plant growth hormone responsible for ripening and senescence of fresh produce. But production of excess ethylene leads to over ripening and facilitates microbial growth thereby reducing the shelf life. Therefore control over ethylene action is required. To increase shelf life of fresh produce 1-Methylenecyclopropene (1-MCP) ethylene antagonist shown promising result by inhibiting ethylene response at receptor level. The current 1-MCP treatment requires a storage room with controlled atmosphere overnight. However this treatment is effective only for the initial period of time as continuous formation of ethylene receptors triggers ripening. Controlled Release Packaging (CRP) technology can be used as an alternative to existing 1-MCP treatment by providing the ability to replenish 1-MCP and prolong is exposure to fresh fruits**.** The first objective of this study was to evaluate the effect of multiple 1-MCP treatments on quality attributes of fresh tomatoes. This study was conducted at turning stage of tomatoes using three treatments: 1) control (no 1-MCP), 2) single 1-MCP treatment (1200nl) and 3) multiple 1-MCP treatments (100nl/day for 12 days). The multiple 1-MCP treatments prolonged the shelf life of tomatoes by maintaining quality attributes including weight, color, firmness, pH and total soluble solids (TSS) at room temperature compared to the single 1-MCP treatment. Not all quality attributes were affected to the same extent, the strongest effect of multiple 1-MCP treatments was on firmness of the tomatoes and not much difference was noted on TSS and pH of tomato juice. The second objective was to quantify the release of 1-MCP through Tyvek, LDPE and PLA polymer sachets at room temperature under 90% RH to test their potential usage as CRP film material. The release of 1-MCP through Tyvek sachet is almost 90% in 10-12 hours whereas LDPE sachet has a slower a release around 10% in 10-12 hours. Both polymers have potential use as an active layer in CRP system. PLA sachet does not release 1-MCP, but it may be used as a barrier layer of the package of CRP system.

Key words: 1–Methylcyclopropene (1-MCP), Controlled Release Packaging (CRP), packaging polymers, shelf life of tomato.

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1. INTRODUCTION

1.1. Background

A large portion of fresh produce is lost worldwide after harvest. The main causes are physiological such as wilting, shriveling, chilling injury, decay due to fungi and bacteria, and physical like mechanical injury. Losses are estimated to be 20-40% in developing countries and 10-15% in developed countries. In order to reduce these losses fresh produce are harvested at green immature stage. However, early harvested fresh produce receives complaints about poor taste, green and hard fruit.

Fruits and vegetables are an important part of human diet due to their essential nutrients such as vitamins, minerals, fibers and antioxidants (Knee 2002). Regular consumption of fruits is associated with reduced risk of cancer, cardiovascular diseases, stroke and other chronic diseases (Kader 2002). The main impact of fruit and vegetable consumption on human health is due to the antioxidant activity. However, the factors influencing consumer to purchase fresh produce is appearance, shape, size, color, freshness, taste and flavor. It is suggested that postharvest life based on flavor and nutritional quality is shorter than postharvest life based on firmness and appearance of fresh fruits (Kader, 2003).

Fruit quality is affected by genetic makeup as well as environmental and cultural development of preharvest factors. Between harvest and consumption, fruits undergo many biological changes. Some of the changes are desired for consumption such as development of flavors, conversion of starch to sugar and reduction in organic acid content. Transportation and respiration may also lead to some inevitable changes which decrease fruit quality and causes postharvest and physical loss (Wills, McGlasson et al. 2007).

To reduce losses the main aim of postharvest technologies is to reduce metabolism such as respiration, transpiration and ethylene production of harvested produce. However, senescence and deterioration of harvested fruit cannot be stopped but can be delayed and slow down by using some technologies such as low temperature, high relative humidity and controlled atmosphere (reduced oxygen and elevated carbon dioxide). The fruit quality offered to consumer is determined by the level of quality achieved at harvest stage. Quality usually decreases during storage hence it is critically important to maintain consistently high fruit quality throughout the marketing period until it reaches the final consumer.

Ethylene, a plant growth hormone, plays a central role in initiation and acceleration of ripening process of fresh produce (Theologies, Zarembinski et al. 1992). The ethylene receptor sites located on plant cells are involved in ethylene action and trigger the ripening process.

Ethylene inhibitors have shown potential benefits for controlling ripening. Recently the synthetic gaseous compound 1-methylcyclopropene (1-MCP), found to be a good ethylene antagonist compound, is being used to extend the shelf life of fresh produce by controlling over-ripening and ripening related changes such as dramatic changes in color, texture, flavor, pH and aroma of the fruit flesh. It also provides insight into ethylene action and response in research programs. 1-MCP can inhibit the ethylene action by blocking the continuously forming receptor sites on the plant cell and would not allow ethylene to bind on to receptor site. 1-MCP has ten times greater affinity for the ethylene receptor sites than ethylene (Blankenship and Dole 2003). The efficacy of 1-MCP on the physiology of fruits and vegetables are proven such as less ethylene production, lower respiration rate and reduced color change. 1-MCP treatments may differ by the ripeness stage of the fruit, concentration, treatment time and temperature. The concentration can vary among different cultivars and exposure period. To avoid ethylene production and action, 1-MCP is applied before the initiation of ripening (Serek, E.C et al. 1995). The current treatment process involves continuous exposure of 1-MCP concentration in a controlled room environment for several hours, allowing the 1-MCP to bind to the ethylene receptors on the plant cell, which results in inhibiting ethylene action and delayed ripening (Serek, E.C et al. 1995; Hotchkiss, Watkins et al. 2007). This treatment however is effective in delaying the ripening only for a few days since the formation of receptor sites is a continuous process which triggers ripening. Hence there is a need for a system which provides continuous replenishment of 1-MCP to bind with the existing and continuously forming receptor sites to further delay the ripening.

Controlled Release Packaging (CRP) is a new packaging technology in which the package itself is used to deliver active compounds in a controlled manner to enhance food safety, quality and shelf life (Yam and Schaich 2005). CRP has impact on retarding lipid oxidation and inhibiting microbial growth by using antioxidant and antimicrobial as active compounds. CRP technology can be applied in the fresh produce industry to delay the ripening by providing a continuous replenishment of 1-MCP within the package of the produce.

Fresh produce are broadly classified under two categories:

1) Climacteric fruits (apple, pear, peach, banana, tomato).

2) Non-climacteric fruits (blueberry, strawberry, grapes).

Climacteric fruits can produce ethylene even after harvest whereas non-climacteric fruits stop producing ethylene postharvest. For this research study, tomato is taken as a real food model due to a combination of scientific and agricultural reasons. Tomatoes are typically climacteric fruit and they ripen most at the peak of respiration as well as increase ethylene production. Ripening of tomato is highly dependent on ethylene action (Yang and Hoffman 1984; Theologies, Zarembinski et al. 1992). For this reason tomatoes are harvested at full size before ripening and thus minimizing the damage during transportation and avoiding unwanted ethylene production. Early harvested (green stage) tomatoes have poor quality, taste and other attributes compared to tomatoes harvested at light red stage (Kader 1978) .

The investigation of this study helps to develop a base to design controlled release packaging of fresh produce for commercial application. It also investigates the effect of continuous replenishment or multiple 1-MCP treatment on respiration rate and quality attributes of tomato.

2. LITERATURE REVIEW

2.1. Tomatoes

Tomato belongs to the solanaceae family and is one of the most widely cultivated vegetable crops all over the world. It is the second largest exported fleshy fruit in the U.S. The origin of tomato is from western coastal plains of South America, between Ecuador and Chile. Apparently, the South American Indians did not know about the fruit, as there is no evidence of tomato in their tradition and archeological relics in the Andean region. There is also no name for tomato in their traditional language. Domestication of tomato happened in Mexico where weed tomatoes were common in the southern part of the country. The tomato was first introduced in the Europe countries during the sixteen century and was known by the name “pomodoro” (golden apple) in Italy. In mid 1800s commercial production began and has now become a widely marketed produce all over the world.

According to the National Agriculture Statistics Service (NASS), U.S. fresh-market tomato production in 2010 was more than 28.9 million cwt and valued at $1.4 billion, the highest ranked fresh-market vegetable. California harvested 38,000 acres, had average yields of 315 cwt per acre and produced nearly 12.0 million cwt, accounting for 41 percent of U.S. production. For tomato production California is the number one state, followed by Florida. The Florida produces tomatoes in the United States from December through May each year. Florida harvested 29,000 acres, had average yield of 300 cwt per acre and produced nearly 8.7 million cwt, accounting for 30 percent of U.S. production. These two states collectively encompass nearly two-thirds of total U.S. fresh-tomato acreage. NASS also reported 14 additional states producing a commercial fresh-tomato crop in 2010. Ohio, Tennessee and Virginia are the only three which produces with more than 4,000 acres in tomato production. Tennessee and Ohio are leading producers of fresh tomatoes (NASS 2011).

Tomato is a good source of lycopene, ascorbic acid, β-carotene and minerals. The low calorie content (24 cal/100g fresh wt) emphasizes the reason for its high consumption among the population. In recent years, tomato has attracted the attention due to the anticarcinogenic and antioxidant property of lycopene and ascorbic acid. Lycopene is very efficient quencher of singlet oxygen and free radicals that provides protection against prostate cancer. Additionally tomato helps in preventing platelet clots which cuts down deaths from heart diseases and strokes. As consumer's demand for fresh flavor tomato increased in the mid 1990's, several specialist tomatoes were introduced in the food market such as roma (plum), cherry, cluster, grape, ugly, yellow and mini pear type tomatoes. All these specialty tomatoes are unique in color, appearance, texture, flavor and taste. Roma or plum type tomatoes are also called Italian tomatoes and are most popular in the domestic market. Cherry and grape type tomatoes are small in shape and taste similar to the fruit by which they are named. Mini pear tomatoes are small, elongated pear shaped fruit having less water content and good flavor, which accounts for its use in the processing, canning, puree and paste manufacturing.

Consumer's preference of a good quality fresh tomato is based upon its appearance, color, texture, flavor and organoleptic property. Tomato also ranks number one among the vegetables for contributing vitamins and minerals in human diet, thus making it a popular choice among consumers. The increase in the demand for high quality nutritional foods by the consumers has necessitated the need for a longer market period within the retail as well as export market.

Figure 1: Plum tomatoes

According to USDA standard tomato color chart, tomato ripeness is divided in six stages (USDA 1997) as below:

1) Mature green stage (surface is completely green)
2) Breaker stage (break color from green to bruised fruit, <10% red color)
3) Turning stage (10% to 30% tomato surface shows red or pink color)
4) Pink stage (30% to 60% surface have red or pink color)
5) Light red stage (60% to 90% of tomato surface covered by red color)
6) Red stage (90% surface has red color).

Optimum quality of tomatoes is obtained during last red vine ripening stage. Ripe tomatoes are perishable, therefore they can get easily damaged during harvesting and shipping leading to loss of quality and exhibiting a short shelf life (Ratanachinakorn, Klieber et al. 1997). Hence, tomatoes are generally harvested at green mature stage, before ripening has begun.

2.1.1. Climacteric respiration pattern

All metabolic cells respire continuously and produce ethylene. Based on the postharvest respiratory behavior of all fruits, vegetables and flowers; fresh produce can be classified into two types 1) Climacteric and 2) Non-climacteric. Climacteric fruits can produce ethylene even after harvest and still respire to reach at the final stage of ripening and senescence. Non-climacteric fruits cannot produce ethylene by itself and do not respire like climacteric commodities after harvesting (Lelievre, Latche et al. 1997).

Respiration plays a key role in maintaining quality and shelf life of fresh produce. The basic mechanism of respiration is to take in oxygen and produce carbon dioxide, heat and moisture. This also helps in the oxidative breakdown of complex materials like starch, sugar and organic acids, that are present in the cells, into simple molecules such as carbon dioxide and water. Respiration also produces energy which can be used for other synthetic reactions.

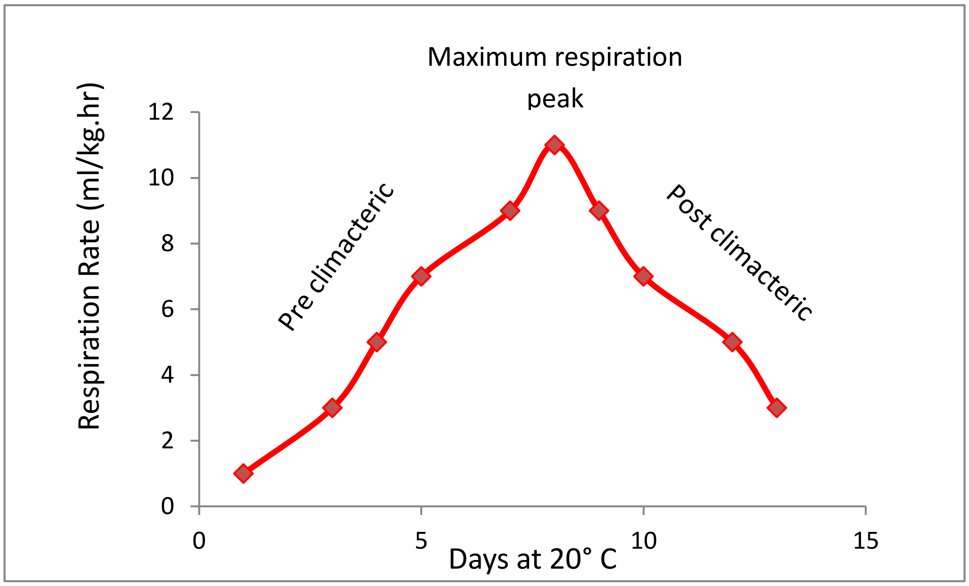

Figure 2: Normal respiration trend of climacteric fresh produce

Tomatoes are typically climacteric fruit. In climacteric fruit, respiration passes through the pre-climacteric stage followed by a rapid increase in respiration to reach a peak level. The visual changes, ripening and senescence would be noticed in this stage. Most favorable eating quality is also reached at this stage of tomato. The final stage of ripening is known as post-climacteric stage and characterizes an irreversible decline in the rate of respiration and the final degradation of the commodities. The picture above explains pre-climacteric, post-climacteric, peak of respiration. The storage life of the commodities can be judge by the ratio of climacteric maximum to pre climacteric minimum. The storage life of the commodities decreases with increase in ratio and decreases in the time required to reach the climacteric maximum. Therefore the goal of all postharvest treatments is to prolong storage life of fresh produce by suppressing the rate of respiration. At room temperature tomato shelf life at green stage is higher. As tomatoes respire and its color changes across the various stages, the shelf life decreases. When the red color stage is reached the shelf life is not more than 3 days at room temperature.

Ripening stage	12.5° C	15° C	17.5° C	20° C	22.5° C	25° C
Mature green	18	15	12	10	8	7
Breaker	16	13	10	8	6	5
Turning	13	10	8	6	4	3
Pink	10	8	6	4	3	2

Table 1: Days to full red color at room temperature and refrigerated temperature

2.2. Ethylene

Ethylene is a unique plant growth hormone, naturally produced in small quantities by fresh produce and accelerates respiration rate resulting in ripening and senescence. It is an endogenous plant hormone associated with various phenomena of plant growth and development. It plays a major role in this aspect especially in all climacteric plants (Biale, Young et al. 1954).

The production of ethylene in plant tissue is low in quantity and it is regulated by developmental and environmental factors, in spite of low production, even a small quantity of ethylene significantly affects plant physiology. Ethylene production depends on the commodity and maturity of fresh produce as well as the temperature of the environment. It enhances the fresh produce growth at specific stages of plant development, including seed germination, fruit ripening, leaf senescence and abscission(Yang and Hoffman 1984). It also plays an important role in tissue differentiation, root formation, root elongation, lateral bud development, flowering initiation, flower opening and flower senescence, with or without pollination (Grichko and Glick 2001). Ethylene‘s effect can be easily noticed during fruit ripening and degreening. Ethylene production can also be induced by a variety of external aspects such as mechanical wounding and environmental stresses. Excess ethylene gas production has adverse effect on plants such as causing decay, mold, discoloration, wilting, softening, scald and loss of crunch.

Climacteric commodities increases the synthesis of ethylene during the final stage and also accelerates ripening and senescence. Ethylene production may peak either before or after climacteric respiration rate peak (Lelievre, Latche et al. 1997). Ethylene production also steps up the onset respiration rate in climacteric plants and reduces shelf life.

2.2.1. Ethylene biosynthesis

Methionine is an amino acid used by plants in ethylene biosynthesis. The biosynthesis of the hormone starts with conversion of the amino acid methionine to S-adenosyl methionine (SAM) by the enzyme Met Adenosyltransferase. The SAM is further converted into 1-aminocyclopropene-1-carboxylate (ACC) using the enzyme ACC synthase (ACS), which determines the rate of ethylene production, thus the regulation of this enzyme is important during ethylene production. Finally, with the action of the enzyme ACC-oxidase (ACO) and in presence of oxygen and carbon dioxide, ethylene is produced. Ethylene biosynthesis can be induced by endogenous or exogenous ethylene.

Figure 3 below shows the ethylene biosynthesis pathway (Atwell, Kriedemann et al. 1999).

Methionine

$\downarrow$ SAM synthesis

S-adenosyl-methionine

$\downarrow$ ACC synthesis

ACC

$\downarrow$ ACC synthesis

Ethylene

Figure 3: Ethylene biosynthesis pathway

2.2.2. Ethylene mechanism

Ethylene (C_2H_4) is a simple alkenes type molecule consisting of two carbons linked by a double bond with two hydrogen atoms on each carbon. Ethylene is self-produced by the plant in the presence of oxygen and carbon dioxide. It is a key hormone that induces ripening, ripening related changes and senescence of fresh produce.

Ethylene receptors are embedded in plant cells, the presence of ethylene molecules in the air bind on the receptor sites and act like a “key” to unlock the receptor site, which sends a chemical signal to plant cells to perform a series of reactions (Blankenship 2001; Choi and Huber 2008). This chemical reaction results in ripening of fruits which is visually noticeable in certain fruits in the form of changes in color, texture and aroma. Some composition of fruit such as water, starch and sugar content as well as acidity of the fruit will also change.

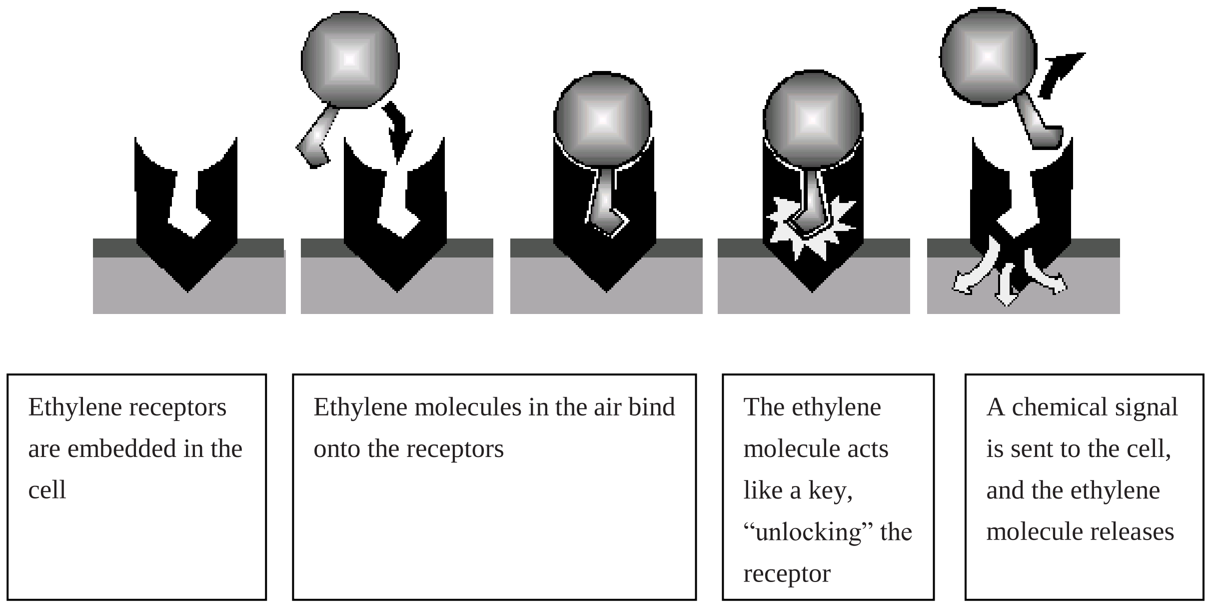

Figure 4: Ethylene binding mechanism, (Adapted from: (Blankenship 2001) diagram by Jenny Bower, Dept. of Pomology, UC Davis.

2.2.3. Ethylene action inhibitors

Ethylene that is present in the fresh produce has deleterious effect on the fruit such as softening and degreening, leading to reduction in the shelf life. Due to the presence of double bond in the chemical structure of ethylene, ethylene can degrade in many ways. It is well known that ethylene can cause impulsive death of plants and plant parts. Ethylene can promote leaf yellowing, accelerates ripening of harvested fruits and vegetables. Because of the numerous bad effects of ethylene on plants and fresh produce, there is a need to develop a technique to reduce these deleterious effects and help maintain quality, delay over-ripening and senescence of fresh produce. There is extensive research study performed to inhibit ethylene action by using ethylene antagonist compounds like CO2 and AG+, and packaging technologies such as MAP. A few other means used to inhibit ethylene action are listed below:

1) Presence of plant hormones such as gibberellins, cytokinins and polyamines can inhibit the ethylene action. Cytokinins limit the binding ability of ethylene to the receptors and helps to delay senescence (Whitehead 1994).
2) Chemicals like aminoethoxyvinyl glycine and aminooxy-acetic acid restrict ethylene biosynthesis by inhibiting activity of ACC-synthase and limits ethylene production (Buchanan, Gruissem et al. 2000).
3) CO_2, silver thiosulfate (STS), 2,5-nonbormadiene (2,5-NBD) and diazocyclopentadiene (DACP) are identified as an effective ethylene antagonist compound. (Beyer 1979) reported silver ions attach to ethylene binding sites in the cellular membrane and prevents ethylene action.
4) Ethylene production can also be controlled by using technologies such as low temperature and modified atmosphere (MA).
5) 1-methylenecyclopropene (1-MCP) is a novel ethylene antagonist compound that can inhibit the role of ethylene on fresh produce by blocking the binding site of ethylene production, thus prolonging storage life of fresh produce (Serek 1994; Ku 1999).

2.3. 1-MCP

1-Methylcyclopropene (1-MCP) is a highly volatile, non toxic gas and is widely used as an ethylene inhibitor. The molecular weight is 54 and chemical structure is C_2H_6 at standard temperature and pressure. One of the major disadvantages of 1-MCP is that it is unstable in nature and due to this it causes hazardous effects when it is compressed. Commercially this compound is incorporated into a molecular encapsulation agent in order to stabilize its reactivity and provide convenient safe storage and transportation of the compound. α-cyclodextrine is the preferable molecular encapsulation agent for 1-Methylcyclopropene and since α-cyclodextrine is in powder form, this makes 1-MCP CD to also be available in power or tablet form. The application or delivery of this compound to the fresh produce or plant is done by dissolving it in water or other solvents which then releases 1-MCP gas into the atmosphere where fresh produce or plant need to be treated. 1-MCP diffuses through the epidermis of the host tissue, binding with ethylene receptor sites of the host and consequently slows down the onset of ripening process and ethylene action. 1-MCP has been registered by Rohm and Haas Company (AgroFresh Inc) and commercially named by SmartFresh in 2002. In the same year it also got approved by FDA for apple and pear treatment. 1-MCP is an odorless and non-toxic volatile gas that binds irreversibly to the ethylene receptors and prevents ethylene action even with a low concentration of 1 ppm for 24 hours. 1-MCP responds positively on climacteric fruits but for non climacteric produce it does not have the same reaction. Applying 1-MCP treatment at early stage of tomato ripening immediately after its harvest will make the treatment ineffective and tomato would go rotten even before become viable for sale. The effectiveness of 1-MCP treatment is affected by the concentration, treatment time and treatment temperature of 1-MCP.

2.3.1. 1-MCP mechanism

1-MCP has 10 times greater affinity than ethylene to bind with receptor sites (Sisler and Serek 1997). At the time of the treatment 1-MCP permanently binds to the receptor sites present in the carnation tissue (Sisler, Dupille et al. 1996). It also influences ethylene biosynthesis. When ethylene production and action is delayed by preventing the binding due to 1-MCP application, the ripening and senescence of fresh produce can be delayed, hence their quality and edible condition is maintained for a longer period of time. The binding of 1-MCP to ethylene receptor sites is irreversible, but the formation of new receptor sites during the climacteric period is a continuous process and ethylene regains sensitivity to trigger ripening (Sisler, Dupille et al. 1996).

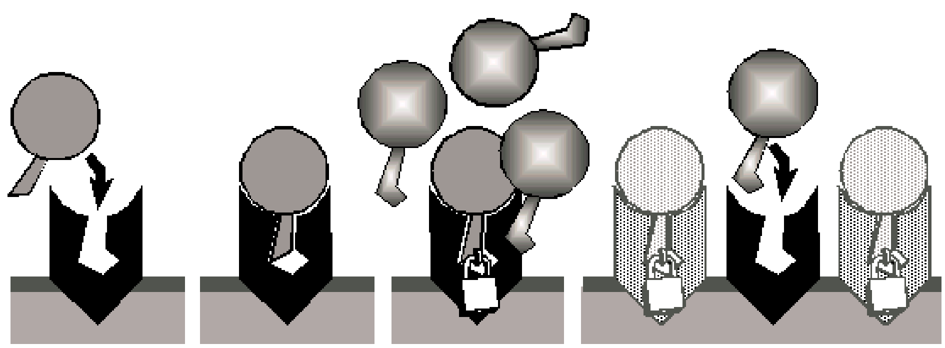

Figure 5: 1-MCP mechanism, (Adapted from: (Blankenship 2001) diagram by Jenny Bower, Dept. of Pomology, UC Davis.

2.3.2. Effect of 1-MCP

The 1-MCP compound is proven to slow down ethylene production as it blocks the ethylene receptors and inhibits ethylene action (Sisler and Serek 1997; Watkins 2002). 1-MCP exposure prevents quality loss of fresh produce and has been demonstrated to have a positive effect on physiology of fruits and vegetables. Reduction in color changes has been reported in broccoli (Ku and Wills 1999; Gong and Mattheis 2003), pear (Trinchero, Sozzi et al. 2004), peach (Kluge and Jacomino 2002), plum (Manganaris, Crisosto et al. 2008) and orange (Porat R, Weiss B et al. 1999). Reduced loss in firmness has also been reported in jujube (Xie, Gu et al. 2009), peach(Kluge and Jacomino 2002), plums (Menniti, Gregori et al. 2004; Manganaris, Crisosto et al. 2008), persimmon (Luo 2007), strawberry (Jiang, Joyce et al. 2001) and banana (Jiang and Joyce 2003; Baez-Sañudo, Siller-Cepeda et al. 2009).

During the peak of ripening in climacteric fruits, the level of ethylene and CO2 is also at its highest, at which point the production has been temporarily suppressed by 1-MCP (Golding, Shearer et al. 1999; Jiang, Zhang et al. 2004; Lippert and Blanke 2004). 1-MCP has also decreased ethylene production and respiration rate in apple (Fan and Mattheis 1999), plum (Abdi, McGlasson et al. 1998; Dong, Lurie et al. 2002; Manganaris, Crisosto et al. 2008), pear (de Wild, Woltering et al. 1999; Trinchero, Sozzi et al. 2004), and avocado (Jeong, Huber et al. 2003).

2.3.3. Current 1-MCP treatment for tomatoes

1-MCP has positive effects on fruit quality of fresh produce such as delay ripening and senescence, reduce decay and weight loss, and lastly extend the shelf life of a wide range of climacteric fruits including tomato (Blankenship and Dole 2003).

Based on literature review, Figure 6 below shows the flow chart of the current practice of 1-MCP treatment for tomatoes. Generally there are two ways to treat tomatoes with 1-MCP:

1) Tomatoes exposed with 1-MCP gas in a closed room for several hours and then stored until sold.
2) Tomatoes immersed in 1-MCP aqueous solution for 1 to 12 minutes and then quickly dried and stored until sold.

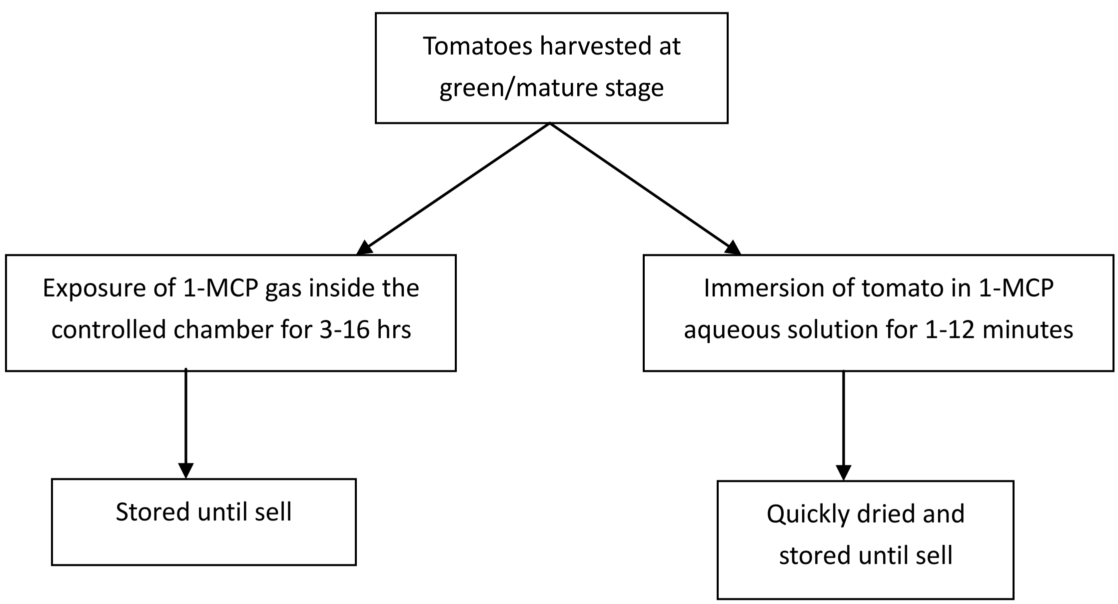

Figure 6: Two types of current 1-MCP treatment practice for tomato

Although the existing 1-MCP treatment is effective to inhibit ethylene action and delay tomato ripening, it does have certain drawbacks as listed below:

1) Tomatoes are typically harvested at green immature stage to increase their shelf life and reduce damage during transportation. However due to this act, tomatoes lose their ability to respire by itself and results in uneven ripening. Therefore to initiate ripening and ripening related changes tomatoes need exogenous ethylene treatment before or after 1-MCP application. But since both these treatments require a controlled atmosphere room it adds additional cost and time.
2) 1-MCP is a volatile gas and commercially available in encapsulated cyclodextrin powder form and it requires an additional step to release the 1-MCP gas from the cyclodextrin into the chamber prior to its action on fresh produce.
3) The formation of receptor sites on plant cell is a continuous process. So after the treatment when 1–MCP is no longer available to bind with receptor sites, ethylene regains sensitivity to trigger ripening thus re-enabling faster ripening.

Therefore there is a need for a system which provides continuous replenishment of 1-MCP to bind with constantly forming receptor sites and also avoids the additional gas preparation step as well as saving costs in creating controlled chambers. This need can be fulfilled using the CRP technology that is explained in the next section.

2.4. Concept of controlled release packaging

Traditionally, active compounds such as antimicrobials and antioxidants are incorporated into food formulations; however, once these active compounds get consumed in reaction, the quality of food degrades rapidly. To overcome this problem, an innovative concept of controlled release packaging (Yam and Schaich 2005) was developed to achieve slow release of active compounds from packaging material into the food in a controlled manner to enhance food safety and quality.

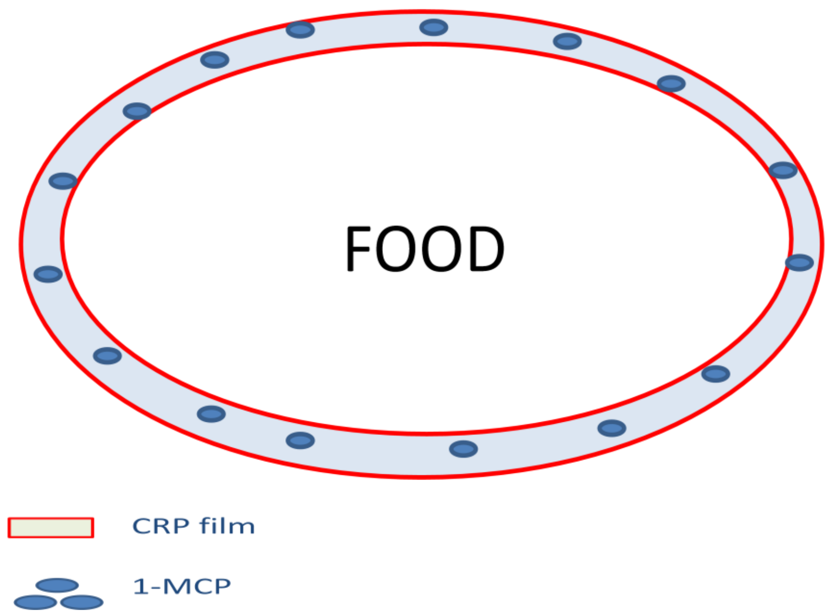

Figure 7: Illustration of Controlled Release Packaging

The controlled release packaging technology is effective to release antioxidant and antimicrobial active compound but no evidence is found which proves its effectiveness in releasing 1-MCP gaseous compounds. This research focuses on evaluating the effect of gaseous active compound 1-MCP over fresh produce by imitating CRP technology.

Through the CRP technology 1-MCP molecules would be released from the package at a controlled rate over longer period of time thus ensuring the presence of 1-MCP to bind with continuously formed ethylene receptor cites and preventing its reaction with ethylene.

There are many ways by which CRP technology can be designed. Some of the methods include:

i. Incorporation of 1-MCP CD directly into active layer of polymer matrix.

ii. Addition of sachet containing 1-MCP CD into package.

iii. Coating of 1-MCP CD onto polymer surface.

As we know that fresh produce generates moisture by itself, this allows us to make an assumption that the moisture of the fresh produce would help in releasing 1-MCP from the cyclodextrin. When the 1-MCP releases in the above manner, the compound would diffuse through the polymer into the package and inhibit ethylene action.

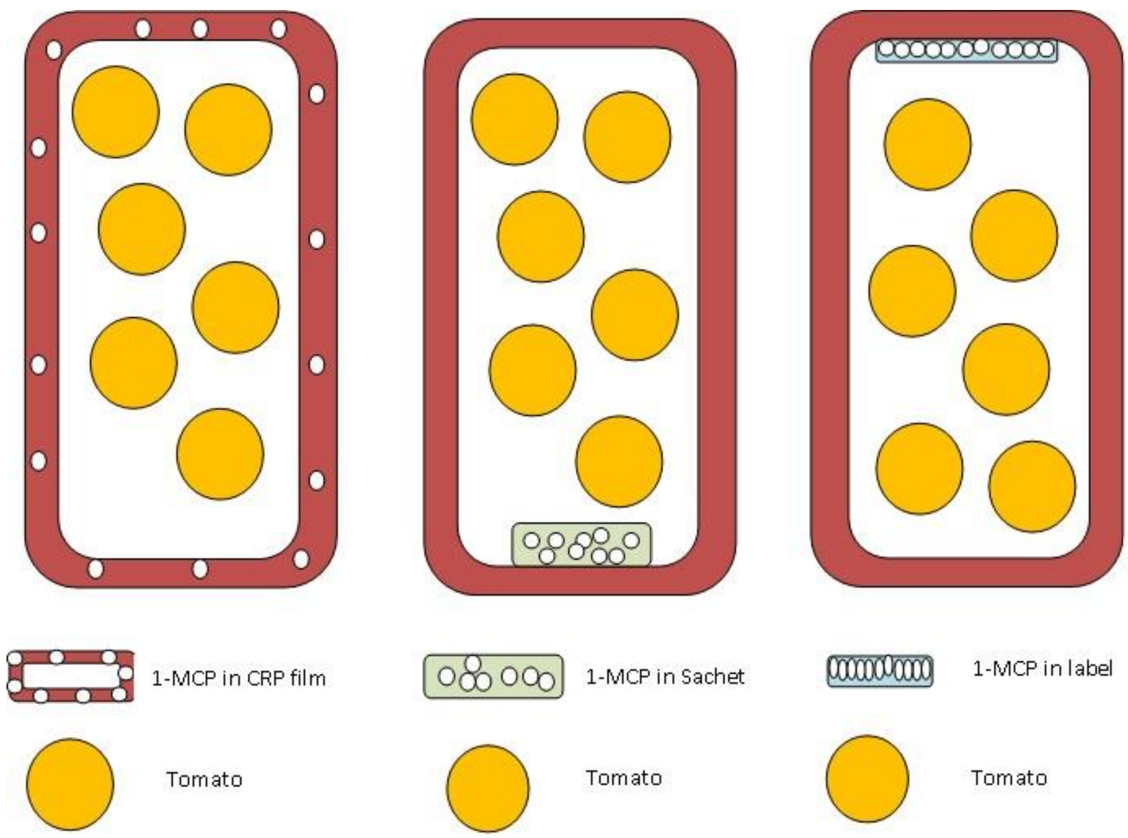

Figure 8: Different ways to incorporate 1-MCP to make CRP film

1-MCP incorporate in a) polymer matrix, b) polymer sachet and c) into labels

2.5. Single and multiple 1-MCP treatments

Single 1-MCP treatment is defined here as a treatment of tomatoes by1-MCP inside a package in which a syringe is used to inject a known amount of 1-MCP into the package one time only, with no additional injections.

Multiple 1-MCP treatment is defined here as a treatment of tomatoes by 1-MCP inside a package in which a syringe is used to inject a known amount of 1-MCP into a package multiple times. Multiple 1-MCP treatment provides prolonged replenishment of 1-MCP by multiple injections into a package which mimics the CRP application as shown in Figure 9 below.

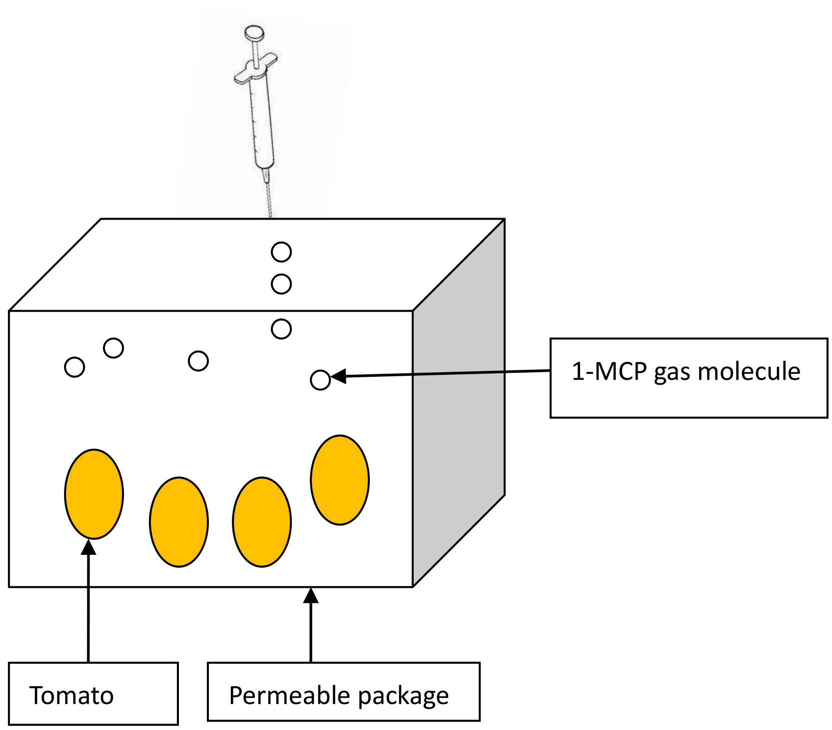

Figure 9: Mimic the controlled release packaging

Single 1-MCP treatment and the current 1-MCP treatment (shown in Figure 6) provide shorter 1-MCP exposure time compared to that of multiple 1-MCP treatment.

3. OBJECTIVES

3.1. Scope of the research

Although the current 1-MCP treatment is effective in delaying the respiration rate and ripening process to increase shelf life of fresh produce, this treatment does have some drawbacks and is only effective for initial period of time which creates a need to develop an alternative method to deliver 1-MCP to the fresh produce. Delivery of antioxidant and antimicrobial from the package has been studied to develop Controlled Release Packaging (CRP), but the delivery of 1-MCP using CRP has not been studied. It has been reported (Lee, Beaudry et al. 2006; Hotchkiss, Watkins et al. 2007) that 1-MCP is released from sachets and different heat pressed polymer films but there is no data found to evaluate the effect on fresh produce except in another research (Mir, Canoles et al. 2004) in which continuous replenishment of 1-MCP inhibited color development of tomato at breaker stage.

In this study we hypothesized that there is a noticeable difference between single and multiple 1-MCP treatments delay the ripening process and maintain quality attributes of fresh produce for a longer period of time.

If the hypothesis is supported by the data, two possible conclusions can be drawn:

I. If single 1-MCP treatment is better than multiple 1-MCP treatment, then CRP may not be a good alternative for the current 1-MCP treatment.

II. If multiple 1-MCP treatment is better than single 1-MCP treatment, then CRP can be a good alternative to provide better shelf life extension.

If the hypothesis is not supported by the data, this means that there is no noticeable difference between the two treatments, but CRP may still be used as an alternative in situations where the current 1-MCP treatment is not available.

3.1.1. Objective

Our long term goal is to design controlled release packaging system which provides prolonged replenishment of 1-MCP to inhibit ethylene action on fresh produce. This research is focused on the initial step to achieve this long term goal. Before designing a package there is need to study the effect of multiple 1-MCP treatment on respiration rate and quality attributes of tomato.

To test the hypothesis the main objectives of this research are as follows:

1. Evaluate the effect of multiple 1-MCP treatments (prolonged replenishment) versus single 1-MCP treatment on the
    - ✓ Respiration rate of tomato
    - ✓ Quality attributes of tomatoes
2. Evaluate the release profiles of 1-MCP from selected polymers.

4. MATERIALS AND METHODS

4.1. Single and multiple 1-MCP treatment on respiration rate and quality attributes of tomatoes

Ethylene production and respiration are an interrelated process. When ethylene production is highest, fresh produce respiration reaches its peak. The goal of this experiment is evaluating the effect of single 1-MCP versus multiple 1-MCP treatments on respiration and quality attributes of tomato.

4.1.1.1. 1-MCP gas preparation

1-MCP is an unstable gas and it is commercially available encapsulated in cyclodextrine powder form. For this study 1-MCP powder containing 0.14% active ingredient by weight was obtained from AgroFresh Inc. (Rohm and Hass Co., Philadelphia, PA, U.S.A). To obtain desired 1-MCP concentration, EthylBloc (1-MCP) powder was dissolved in 1 ml of de-ionized water at 25° C in 25 ml vial.

The concentration of 1-MCP was analyzed by using Gas chromatography (GC). Gas chromatography (HP series 5890A, Hewlwtt Packard) fitted with 80/100 mesh poropak N column with flame ionization detector was used. The temperatures were set at 120° C, 150° C and 150° C for column, injector and detector respectively. Helium gas was used as a carrier gas and flow rate was set at 40 ml/min.

4.1.1.2. Respiration rate

To evaluate the effect of single 1-MCP and multiple 1-MCP treatment on respiration rate of tomatoes experiment was performed in a closed glass jar. The tomatoes were bought from supermarket at turning stage (10% to 30% tomato surface shows red or pink color) according to the USDA chart.

Tomatoes having the same approximate weight were chosen for the experiment. They were individually placed in a 475ml tightly closed glass jar fitted with rubber septum as shown in Figure 10 below and stored at room temperature. The experiment was performed in triplicates. Tomatoes were divided into three treatment groups as below:

1) Control (no 1-MCP treatment),

2) Single 1-MCP treatment (1200nl 1-MCP gas) and

3) Multiple 1-MCP treatments (100nl 1-MCP every day).

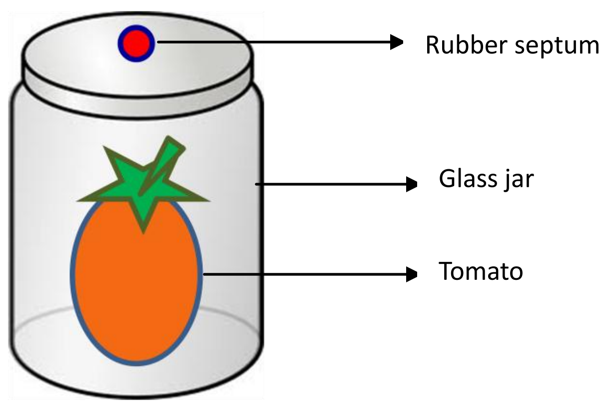

Figure 10: Closed jar system

All jars were closed tightly in the morning and 1-MCP gas was injected through the syringe from the stock gas solution into the jar. After 1 hour, 10ul headspace air (for CO_2 measurement) of the jar was injected into GC. This injection procedure was repeated four times and a graph was plotted to calculate the respiration rate of tomatoes. Carbon dioxide concentration was measured using gas chromatogrphy (HP Series 5890A, Hewlett Packard) fitted with thermal conductivity detector and CTR I column. The oven was set at 50°C and temperatures for injection and detector were set to 90°C and 110°C respectively. The carrier gas was hydrogen and the flow rate was set as 60ml/min. All the jars were opened after 7 hours to maintain normal atmosphere inside the jar.

4.1.2. Quality attributes

Fresh produce is a complex material. The effect of 1-MCP on tomatoes is linked to its respiration rate and quality attributes. Consumers judge the fruit quality depending on certain parameters such as appearance, color, uniformity, shape, size, texture, flavor, aroma, taste, nutritive value and defective mark on the skin (Thompson 1996). The quality of fresh produce can be measured by two ways – subjective and objective. Subjective method involves sensory evaluation whereas objective method uses visual inspection and other instruments. A number of such instruments are extensively used to measure and analyze quality parameters such as weight loss, firmness, color and chemical composition.

To measure quality attributes plastic vented hinged clamshell trays were used as shown in Figure 11 below. The dimension of the tray was 4 15/16 x 7 7/16 x 3 1/2 in^3 and was covered with Hefty storage bags (1 gallon) of dimension 11.5 x 12.18 in^2. The bags were then sealed with the help of sealer to make a permeable package.

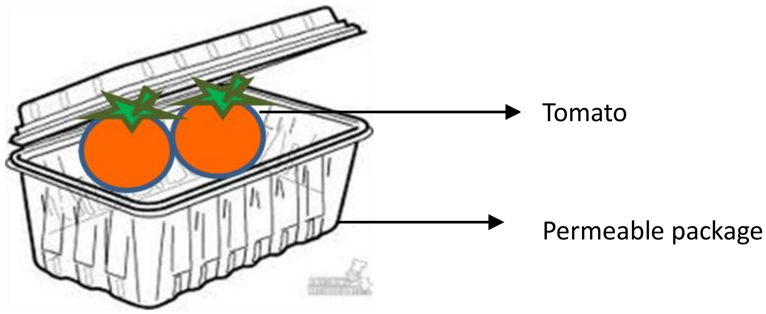

Figure 11: Permeable package

The above treatment was divided into three groups as below:

1) Control (no 1-MCP)

2) Single 1-MCP treatment (1200nl/L) and

3) Multiple 1-MCP treatments (100nl/L everyday for 12 days).

The experiment was performed in triplicates to measure quality such as weight loss, color change, texture, pH and total soluble solid.

4.1.2.1. Weight loss

Weight loss occurs in the fresh produce due to water loss by transpiration which leads to the wilted or wrinkled appearance and makes fruits unacceptable for consumer. The easy way to measure weight loss is by observation and weighing the fruit.

Figure 12: Weight scale

Weight loss in this experiment was determined by weighing fruit at the beginning and again at the end of the experiment. The formula used is:

Weight loss= initial weight – final weight

4.1.2.2. Fruit color

Color represents the quality and freshness of fresh produce. Consumer's judgment depends on overall appearance including color to purchase fresh produce. Color can be measured by using color chart where the fruit color development is visually checked. Color can also be measured using instruments which require calibration to allow the changes with each measurement. For this experiment Konica Minolta Colorimeter was used to measure L, a, b values of tomato.

- *L* (lightness) axis – 0 is black, 100 is white
- *"a"* (red-green) axis – positive values are red; negative values are green and 0 is neutral
- *"b"* (yellow-blue) axis – positive values are yellow; negative values are blue and 0 is neutral

Figure 13: Konica Minolta Colorimeter

In this experiment we are concerned about the “a” value which represents the degree of red and green color.

4.1.2.3. Texture

Firmness is an important attribute of fresh produce to measure ripeness and quality (Kagan-Zar, Tieman et al. 1995). It can be measured by various methods. One way is by inserting a hand held metal probe into fruit and measuring the resistance during insertion. Another method is by using a mechanical instrument fitted with probe and applying load to insert the probe into fruit to measure its resistance. Non-destructive instruments are also available that can be used to determine firmness of fresh produce.

Figure 14: Texture analyzer

For this experiment TA-XT2 Texture Analyzer (stable micro system, UK) was used to measure firmness of the tomato. A 5mm stainless steel probe at 1mm/s test speed with 10.01N trigger force was inserted into a 2cm thick slice of tomato at 10mm distance. The maximum force generated during probe travel was measured in grams.

4.1.2.4. TSS and pH

The sweetness of fresh produce is an important attribute to determine ripeness. Sugar content is higher in soluble solid part of the fruit and is measured with a Brix hydrometer or refractometer.

Tomatoes are homogenized at 20000 1/min speed for 20 mins. The resulting supernatant was filtered by using cheese cloth for later analysis.

Figure 15: Homogenizer

Figure 16: pH meter

Figure 17: Refractometer

Fisher scientific pH meter standardized with pH 4.0 and 7.0 buffers was used to measure the pH of supernatant. To measure total soluble solid one to two drops of the supernatant was placed on the prism of a digital ABBE refractometer (model 10480, Mark Abbe II).

4.2. Release profiles of 1-MCP from selected polymers

As we have seen in our first set of experiments above that multiple 1-MCP treatment is effective in increasing shelf life of fresh produce, hence there is further scope in designing a Controlled Release Packaging (CRP) system for fresh produce.

Controlled release packaging application is a form of delivery method to release active compounds for enhancing food safety and quality. The CRP application can be in the form of sachet, CRP film or label. The purpose for setting up this experiment is to determine which polymer is effective in releasing 1-MCP and also evaluate the release profiles of 1-MCP.

4.2.1. Experimental design

A known amount of EthylBloc (1-MCP) containing 0.14% active ingredient was incorporated into three sachets each made of polymer film LDPE, Tyvek and PLA respectively. The sides of the sachet were sealed using a sealer. All the three sachets were suspended inside 90% RH 475ml glass jar as shown in Figure 18 below. For control sample the same amount of EthylBloc was placed in an aluminum cup with no barrier layer and the cup was kept inside the 90% RH glass jar to quantify the release of 1-MCP gas. The experiment was performed in triplicates.

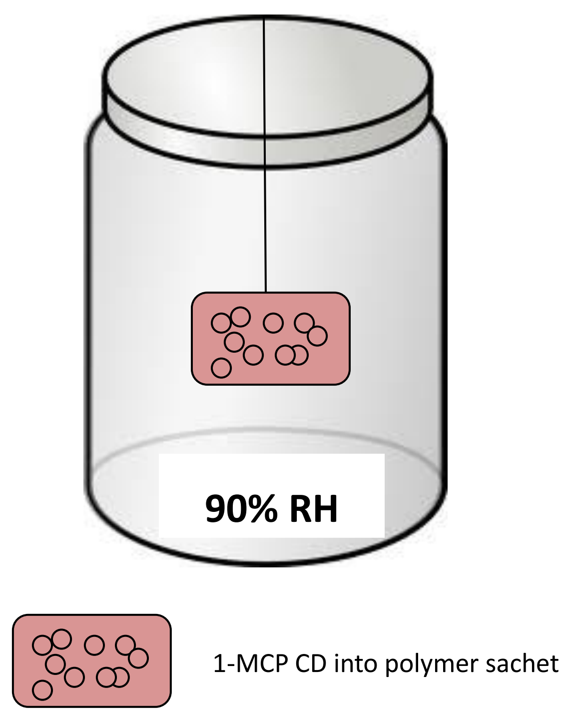

Figure18: Release study of 1-MCP

The release mechanism of 1-MCP gas into the glass jar occurs in three steps as below:

i. In the first step the moisture content from the glass jar penetrates into the polymer sachet.

ii. The penetrated moisture in the sachet helps to release 1-MCP from cyclodextrine into the headspace of the sachet.

iii. Finally the 1-MCP gas diffuses through the polymer matrix into the glass jar.

The 1-MCP gas molecules in the jar as were quantified at regular interval by drawing 1ml headspace air from the jar and injecting it into gas chromatography (HP Series 5890A, Hewlett Packard) fitted with flame ionization detector having 80/100 mesh Porapak N column. The GC oven was set at 210°C and the temperature for injection and detector was set to 210°C and 150°C respectively. The carrier gas helium was set at 40 ml/min flow rate.

5. RESULTS AND DISCUSSION

5.1.1. Respiration rate

The respiration rate of tomatoes varies based on the treatment type during the storage period. Overall the control sample tomatoes produced more CO_2 compared to the single 1-MCP and multiple 1-MCP treated samples.

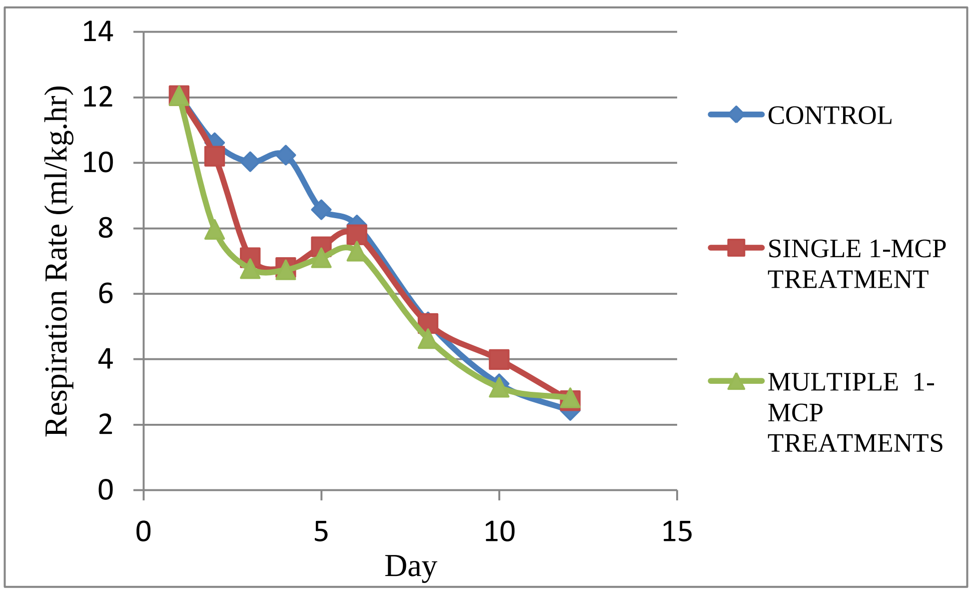

Figure 18: Respiration rate of plum type tomato at turning stage with 1-MCP in closed jar at room temperature.

Tomatoes were exposed to 1200nl (single 1-MCP treatment) for 6 hours, 100nl/day for 6 hours (multiple 1-MCP treatment), and no exposure (control sample). As seen in Figure 18 above, the respiration rate of turning stage plum tomatoes was 12.5 ml/KG.hr for all the samples on the first day of the experiment. On the second day single 1-MCP treated sample and control sample had almost the same respiration rate of 10 ml/Kg.hr whereas the multiple 1-MCP treated sample had 8ml/kg.hr respiration rate. This indicates that 1-MCP multiple treatments have an instant effect to slow down the respiration rate compared to single 1-MCP treatment and control sample. The respiration peak for control sample was reached on the 4th day of the experiment where the tomato produced 10.2 ml/kghr CO_2. On the same day single and multiple 1-MCP treated samples produced 5ml/kghr CO_2 which was one half of the respiration rate of control sample. From day 3 to 5, the single and multiple 1-MCP treatment maintained the same respiration rate. The respiration peak of the single and multiple 1-MCP treated samples was observed on the 7th day and produced 7ml/kghr CO_2. After crossing the respiration peak, CO_2 production decreased steadily from the day 6 to 12 to 3.4 ml/kg.hr for multiple 1-MCP treatment whereas the respiration rate was almost 4 ml/kg.hr for the single 1-MCP treated samples.

In conclusion it was observed that single and multiple 1-MCP treatment does have an effect to delay the respiration peak and rate. However the difference is too small to conclude which one is more effective. Further analysis is required to study the effect of multiple 1-MCP treatments over single 1-MCP treatment on quality attributes of tomatoes.

5.1.2. Weight loss

Weight loss was measured using weight scale. On the first day of the experiment, initial weight of the tomatoes were taken and then packed into permeable package. Weight loss was measured every other day and graph plotted.

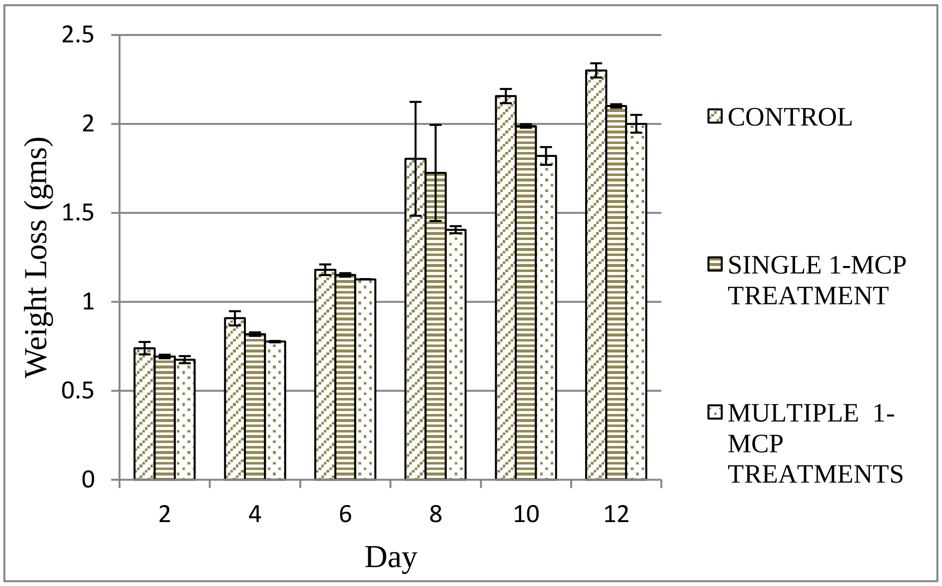

Figure 19: Effect of 1-MCP on weight loss of tomatoes in permeable package.

Based on Figure 19, it can be inferred that during the entire experiment weight loss in control sample was higher compared to single and multiple 1-MCP treated samples. Until the 6th day, the difference in the weight loss between the three samples was negligible. On the 8th day of the experiment, the control sample had the highest weight loss followed by single 1-MCP and multiple 1-MCP treated sample. This trend in weight loss continued from the 8th day until 12th day. It was also observed that on the 10th day the weight loss for single 1-MCP treated sample was 2 gms whereas it took 12 days for multiple 1-MCP treated sample to obtain the same amount (2 gms) of weight loss. It can be further concluded from the figure above, that multiple injections of 1-MCP over time is more effective in reducing the weight loss of tomatoes compared to single 1-MCP treatment as well as to control sample where no 1-MCP treatment is given.

5.1.3. Color measurement

Color skin evaluation of tomatoes was performed using Konika Minolta Colorimeter which measures the “L”, “a” and “b” values. In this research study we are only concerned with the “a” value which represents green to red axis. The value approaching 100 represents red color and the value towards zero or negative represents green color.

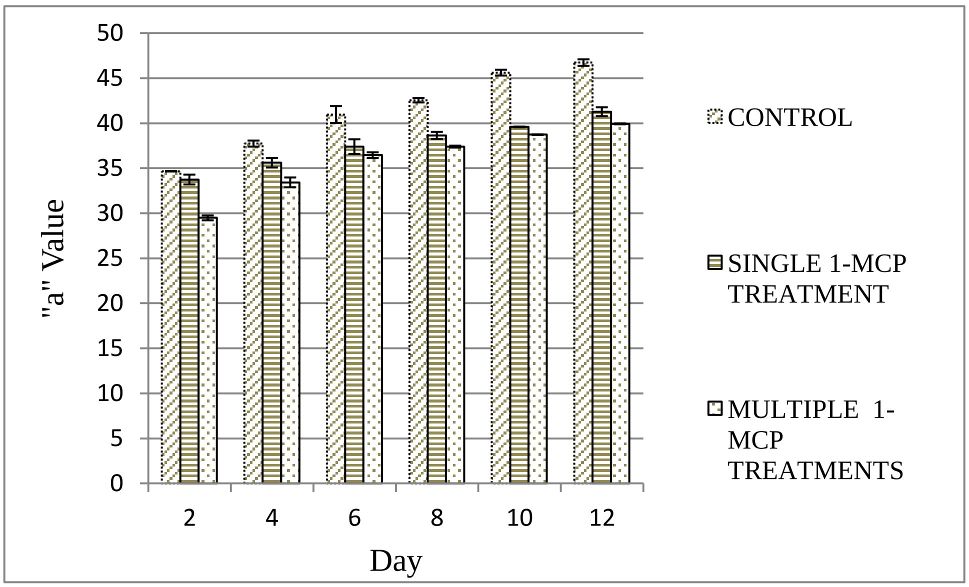

Figure 20: Effect of 1-MCP on “a” value of tomatoes in permeable package.

During the course of the entire experiment, the “a” value remained higher for control sample compared to single and multiple 1-MCP treated samples which indicates that control sample tomatoes are more red in color compared to the other two sample types. On the 2^{nd} day of the experiment there was no significant difference in color observed between control and single 1-MCP treated samples. From days 4 to 12 of the experiment, the “a” value was higher for single 1-MCP treated sample compared to multiple 1-MCP treated samples. However the difference of “a” value was relatively less between the two treated samples, and therefore it was hard to conclude that multiple 1-MCP treatment had a better effect over single 1-MCP treatment.

5.1.4. Texture

Texture loss was measured using texture analyzer which measures the force required to penetrate probe into the sample.

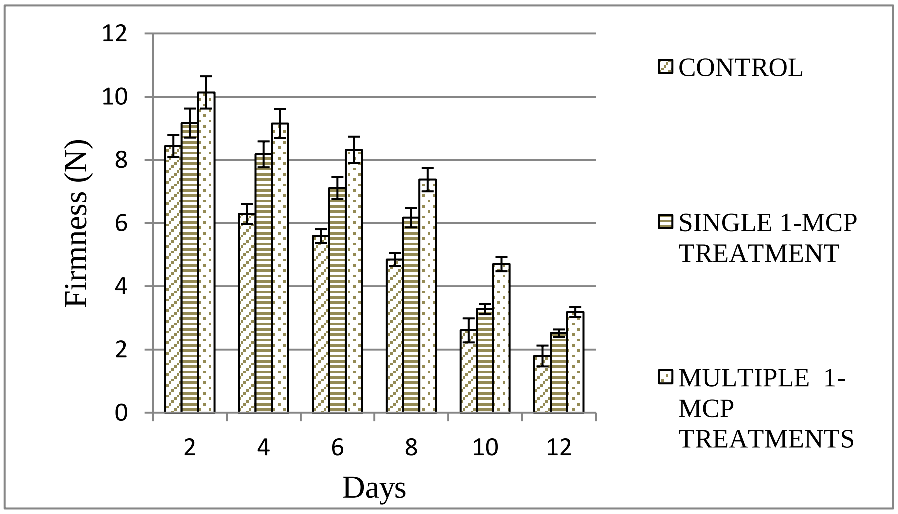

Figure 21: Effect of 1-MCP on firmness of tomatoes in permeable package.

A tomato normally softens during ripening. On the 1st day of the experiment the average firmness of 9 tomatoes at turning stage was measured to be 13.4 N. On the 2nd day the firmness for control sample was observed to be approximately 8 N which was also the same firmness level that was measured for single 1-MCP treated sample on the 4th day and multiple 1-MCP treated sample on the 6th day. This trend continued throughout the entire experiment duration up until the 12th day. From Figure 21, it was observed that on the 12th day of the experiment the firmness for control sample was close to 2 N whereas firmness of 2.5 N and 3 N were noted for single 1-MCP and multiple 1-MCP treated sample, respectively. Multiple 1-MCP treated sample shows promising result to maintain firmness better than control and single 1-MCP treated samples. Based on the experiment results, it can be further concluded that the multiple 1-MCP provided in small amount everyday shows effectiveness to maintain firmness of the tomatoes compared to high amount 1-MCP provided in single 1-MCP treatment and control sample where no 1-MCP treatment is given.

5.1.5. pH

To measure pH of the tomato juice pH meter was first calibrated. The pH measurement was taken and graph plotted.

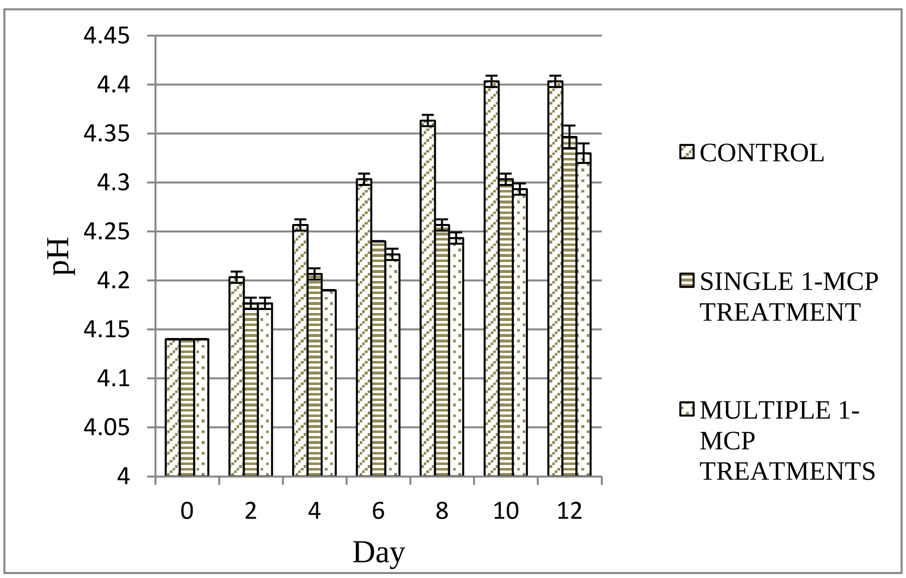

Figure 22: Effect of 1-MCP on pH of tomato in permeable package.

As seen in Figure 22, the control sample pH was higher than the other two sample types during the entire experiment. The difference in the pH level was not much between the single and multiple 1-MCP treated samples. It was difficult to conclude that multiple times 1-MCP treated tomatoes fared better in maintaining the pH level compared to one time 1-MCP treated tomatoes.

5.1.6. TSS

The sugar content of tomato juice was measured using refrectometer.

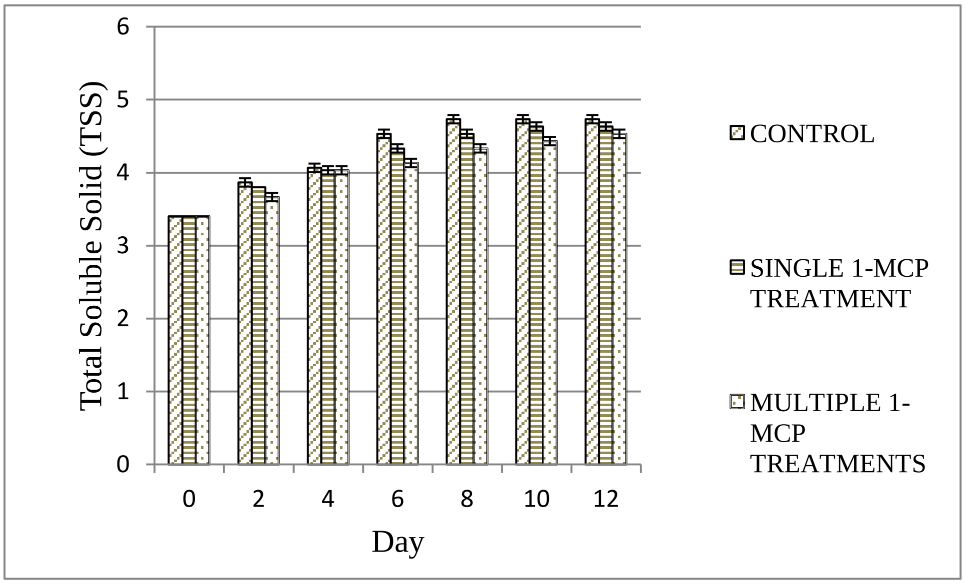

Figure 23: Effect of 1-MCP on TSS of tomato in permeable package.

The sugar content of the control sample was observed to be higher throughout the experiment duration compared to the single and multiple 1-MCP treated sample as shown above in Figure 23. More over there was no significant difference in the sugar content between single and multiple 1-MCP treated samples and thus it could not be concluded that multiple 1-MCP treated tomatoes had a positive effect on maintaining the sugar content for longer period of time.

Based on the above set of experiments it can be concluded that single 1-MCP and multiple 1-MCP treatment has a positive effect in delaying the respiration rate and peak of the tomatoes compared to the control sample. It is also important to note that there exists a minor difference in the respiration rate and peak delay time between the two treated samples. Based on the above set of experiments it can be concluded that multiple 1-MCP treatment has a better effect to maintain weight, color and texture of tomatoes for a longer period compared to single 1-MCP treated and control samples. However, not all quality parameters were affected in the same magnitudes by 1-MCP. The strongest effect of multiple 1-MCP treatment was found on firmness of the tomato. There was no difference observed between the three sample types for the TSS and pH of tomato juice, hence it leads to an assumption that the tomato eating quality remains the same as preferred by the consumer. Further study is needed to evaluate the effect of various concentration levels of 1-MCP that is used in the multiple 1-MCP treatments on tomato.

5.1.7. Release profile of 1-MCP from different polymers

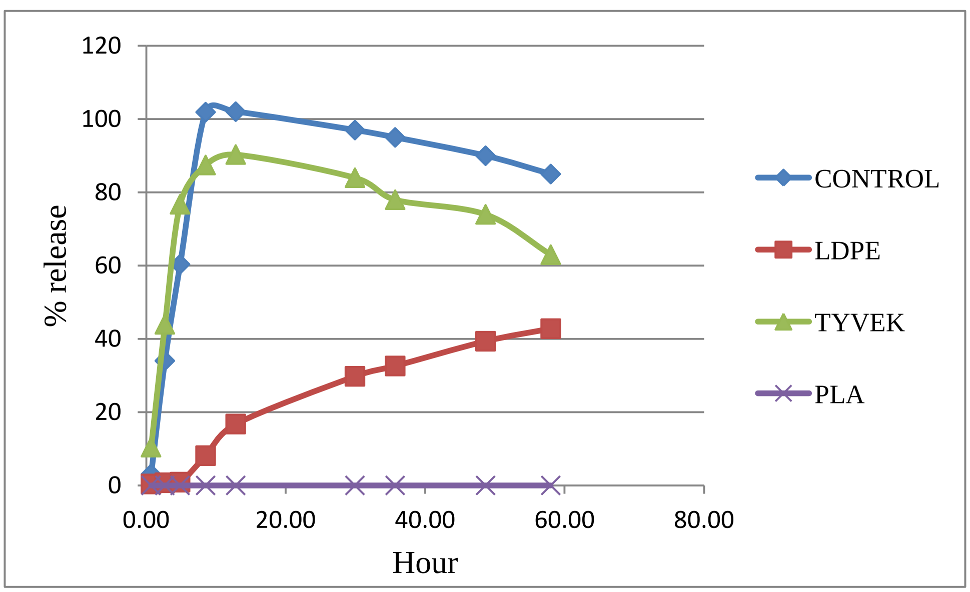

Figure 24: Release profile of 1-MCP from control (no barrier layer), Tyvek, LDPE, PLA

As seen in Figure 24, the control sample had the fastest release of 1-MCP into the glass jar headspace, almost 100% release in the first 10 hours. Tyvek polymer sachet clocked a 90% 1-MCP release in 10-12 hours, LDPE polymer had a slower release rate of around 40% 1-MCP in 60 hours and PLA polymer showed no release at all.

Designing a Controlled Release Packaging system that supports the mechanism of prolonged exposure of 1-MCP requires evaluating the release study from various polymers. From the above it is clear that Tyvek sachet provides the fastest release which was expected due to the porous structure of Tyvek polymer. The release mechanism of 1-MCP from Tyvek also characterizes the single 1-MCP treatment, thus Tyvek can be a favorable polymer to design packages for fruits which have positive consequences of single 1-MCP treatment. As LDPE polymer (water vapor transmission rate 16-23 g/m^2/24hrs and oxygen transmission rate 2000 g/cm^2.hrs) releases 1-MCP at a slower rate and provides a continuous replenishment of 1-MCP into the package, LDPE can therefore work as an active layer to design CRP application for fresh produce. It was also observed that the sachet made of PLA polymer (water vapor transmission rate 18-22 g/cm^2.hrs and oxygen transmission rate 38-42 g/cm^2.hrs) does not show any release of 1-MCP, hence it can act as a barrier layer in CRP application and prevent 1-MCP from escaping from the package. The PLA polymer will also maintain 1-MCP environment within the package for a longer period of time. Further study is needed to define a suitable polymer for designing CRP application.

6. CONCLUSION

Although the current 1-MCP treatment is effective in delaying the respiration rate and increasing shelf life of green stage tomatoes, the treatment does have some drawback which creates a need to develop an alternative method. Under the condition of this experiment, we have shown that compared to single1-MCP treatment, multiple 1-MCP treatment is more effective in maintaining firmness and weight loss and has the same effects on color, pH, and TSS. The biggest difference between both treatments was found between 8^{th} and 12^{th} day. From days 8 to 12 it has been clearly shown that multiple times 1-MCP treatment helps to reduce dehydration of tomato and maintain firmness. Based on these facts, CRP technology can be used as an alternative to eliminate the step which required a closed room to treat tomatoes with 1-MCP.

Based on the experiments performed on sachets, it can be concluded that sachet system could be a helpful technology to design packages which releases 1-MCP into the package to inhibit ethylene action and provide exposure of 1-MCP for a prolonged period. The results from the experiment also shows that Tyvek sachet produces fast release of 1-MCP, almost 90% in 10-12 hours compared to LDPE sachet which had a slower 1-MCP release around 10% in 10-12 hours. Therefore both Tyvek and LDPE polymers can work as an active layer to design CRP application for fresh produce. It was also observed that PLA sachet does not release 1-MCP which makes it a good barrier layer element of the CRP system thus maintaining the 1-MCP environment for extended period of time.

7. FUTURE WORK

Multiple times 1-MCP treatment seems to have commercial potential for growers and traders to delay the ripening and for retailers and consumers to delay the senescence of tomato. It also contributes in increasing postharvest life of tomato.

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.

Findings of this research proves that Controlled Release Packaging (CRP) can be a good delivery method or a better alternative to treat fresh produce with 1-MCP which has positive effect to increase shelf life and maintain quality attributes of tomatoes.

Designing a Controlled Release Packaging system also requires certain additional analysis such as defining suitable polymers based on their physicochemical properties (WVTR, Gas permeability), quantifying 1-MCP release from the suitable polymers and testing the system on various fruits.

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