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DEVELOPMENT OF A 1-METHYLCYCLOPROPENE
PACKAGE DELIVERY SYSTEM
TO CONTROL TOMATO RIPENING

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DEVELOPMENT OF A 1-METHYLCYCLOPROPENE PACKAGE DELIVERY SYSTEM TO CONTROL TOMATO RIPENING

By

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ABSTRACT

DEVELOPMENT OF A 1-METHYLCYCLOPROPENE PACKAGE DELIVERY SYSTEM TO CONTROL TOMATO RIPENING

By

Youn Suk Lee

1-methylcyclodextrin (1-MCP) was used to prolong the freshness of tomatoes during postharvest storage. The effect of storage temperature and 1-MCP treatment conditions on the ripening process of tomatoes was evaluated. Change in two major tomato pigment (chlorophyll and lycopene) contents in tomatoes was also investigated. Sorption behavior for the 1-MCP/adsorbing agents was studied using inverse gas chromatography. The specific retention volume and various thermodynamic parameters relating to adsorption of 1-MCP on the adsorbing agents were calculated. Adsorption and 1-MCP release studies from the adsorbing agents in the sachet system were conducted to evaluate delivery of the 1-MCP gas to the tomatoes under the environmental conditions within the package.

Skin color, firmness, fruit weight, and ethylene production were used as quality indicators for the stored tomatoes. Total chlorophyll and lycopene contents in tomatoes were quantified using a specific extinction coefficient method. Sorption isotherms of 1-methylcyclopropene (1-MCP) on silica gel, Tenax-TA, and activated clay were determined at low sorbate concentration. Sachets made from Tyvek®, paper, LDPE, and PVA materials were fabricated to contain silica gel and activated carbon. The 1-MCP release study was performed using a closed system under two different environmental

release study was performed using a closed system under two different environmental conditions, dry air (0%RH) and 90%RH. The partitioning of 1-MCP between the gas/polymer matrix was determined for several adsorbing agents, and in sachet materials to estimate the adsorb ability of 1-MCP in dry air at 23°C. The water and 1-MCP permeability of the sachet pouch film were measured.

Once-a-day 1-MCP treatment at 10°C was very effective in retarding changes in the skin color of the tomatoes. Exposure of tomatoes to 1-MCP gas at 10°C, using a once-a-day method was the most effective in delaying chlorophyll degradation and lycopene synthesis. The sorption isotherms followed Henry's law, and behaved according to the binding site theory. Silica gel had a much higher number of binding sites for 1-MCP, compared to Tenax-TA and activated clay agents. PVA sachets containing silica gel indicated slow release of 1-MCP. The amount of 1-MCP released from the PVA sachet containing silica gel at 90%RH was larger than the amount of 1-MCP released at dry air condition.

The results showed that combination of 1-MCP treatment with low storage temperature was very effective in delaying color change in tomatoes. PVA sachets containing silica gel have potential use for slow release of 1-MCP from the experimental results in a closed system. Delivering the 1-MCP gas to the tomatoes, from the sachet containing an adsorbing agent may help maintain the freshness of tomatoes during postharvest storage.

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SECTION I: INTRODUCTION

Ethylene is a major factor regulating plant development and can cause a significant increase in the respiration rate. Ethylene is a ripening hormone for climacteric fruits because the gaseous compound stimulates other ripening processes in the postharvest environment. Ethylene responses include desirable reactions such as softening, and color change in ripening. On the other hand, ethylene promotes undesirable reactions inducing abscission and physiological damage, and tends to shorten storage life (Lelievre, 1997a).

Tomatoes are representative of climatic fruits and can be affected by environmental conditions such as temperature, light, relative humidity, ethylene, and CO_2 concentration (Ryall, 1979). Tomatoes have a moisture content over 90% and are a nutritionally valuable source of pro-vitamin A (β-carotene) and vitamin C (ascorbic acid). The color of the tomato is used to predict the degree of its maturity in terms of fruit quality. Change in color is caused mostly by the loss of chlorophyll and synthesis of lycopene throughout ripening (Watada, 1976a). Most tomato fruit is picked either at the green mature stage for long-distance transport or the ripened stage for the fresh market. For long-term storage or transport of the tomato fruit, a temperature of 13°C at 80%RH is recommended to delay the ripening process (Haard, 1975).

In the fresh produce industry, postharvest treatments are the most important processes used to extend freshness during storage, transport, and handling. Biochemical and physiological changes in fresh produce depend mostly on environmental conditions. Postharvest environmental treatments include low temperature storage under air and

controlled atmosphere (CA) and/or by treating them with chemicals which affect plant hormone action (Floros, 1993).

Many fruits are subjected to a wide range of temperatures during storage and transportation. Optimal storage temperatures of fruits differ because their responses to lower temperatures vary widely dependent upon fruit variety and the required length of storage. Lower storage temperature reduces respiration and fungal growth in fruits. However, very low temperature treatment causes chilling injury (Efiuvwevwere, 1988), or can stimulate autocatalytic ethylene synthesis (Lelievre, 1997b).

CA storage refers to storage under modified from the O_2 and/or CO_2 concentrations in the atmosphere surrounding the fresh produce. CA storage is used commonly to delay the ripening process of fruits, and can prevent certain diseases and some disorder symptoms. Tomato ripening can be prevented by extremely low O_2 or high CO_2 concentrations in storage (Ratanachinakorn, 1997). Temperature control is also important because low O_2 or high CO_2 levels do not control all diseases or disorders effecting fruits. Thus, CA storage needs to be used with selected temperatures for certain fruits in order to achieve ideal storage conditions.

The chemical treatments silver thiosulphate (STS), diazocyclopentadiene (DACP), and 2,5 norbornadine (NBD), inhibit ethylene action in fresh produce. These chemicals reduce the effects of ethylene, by blocking the receptor site that signals ethylene action. STS, DACP, and NBD compete with ethylene for the binding receptor and are very effective inhibitors of ethylene response. However, these inhibitors are limited in their commercial application in the horticultural industry because of their unstable and explosive potential (DACP), toxicity and odor (NBD), and environmental

contamination (STS) (Serek, 1994). Thus, there has been a need to find alternatives to these compounds.

One such alternative is 1- methylcyclopropene (1-MCP). Recently, 1-MCP has begun to be commercially used as an agent to extend storage life of fruits and vegetables, and flowers. This compound is a nontoxic gas, and been shown to prevent some aspects of ripening in climacteric fruits (Fan, 1999). 1-MCP acts by inhibiting binding of the ethylene to its receptor sites and is effective at low concentrations (parts per billion levels) (Serek, 1994). Several studies have been conducted on the efficacy of 1-MCP as an inhibitor of ethylene action in ethylene-sensitive cut flowers, vegetables, and fruits (Serek, 1995a and 1995b; Wills, 2002).

Pure 1-MCP has the problems of its stability, and the potential explosive hazard associated with a compressed gas (Daly, 2000). A convenient and safe means of delivering 1-MCP to fresh produce is to trap it using alpha-cyclodextrin as the molecular encapsulation agent. This has been developed as a commercial product "Smart Fresh". This product can also be delivered by dissolving the complex in a suitable solvent in order to release the 1-MCP.

Another technique which can be used to deliver 1-MCP gas is the addition of an inert carrier system such as silica, talc, and dust. This produces a product that has greater stability (Daly, 2000). In addition, use of a packaging system with appropriate permeation properties can provide for slow release of 1-MCP into the headspace of the fresh produce to obtain the desired shelf life.

In packaging applications with an inert carrier system, a sachet may be used to deliver the1-MCP gas to the fresh produce. The sachet technique has been developed to

extend shelf-life of food (Smith, 1995). This system includes an ethanol vapor generator as an antimicrobial agent, and an oxygen absorbing agent to prevent oxidation and mold growth.

The sachet technique can be used with postharvest produce containing high moisture contents under the environmental conditions within the package. Figure 1 shows the proposed 1-MCP package delivery system. The proposed delivering mechanism utilizes a three-step process: (1) release of active molecules from the absorbing surface layer within an inert material; (2) permeation of active molecules through the sachet material into the free volume of the pouch; and (3) sorption of active molecules onto the surface of the packaged produce. In this proposed mechanism, an adsorbing agent, can absorb and/or desorb active molecules. Investigation into the sorption behavior, and thermodynamic parameters of this interaction can provide valuable information. Inverse gas chromatography is a very powerful technique which can be used to characterize interactions between two-phase materials (Lloyd, 1989). Material porosity in conjunction with material barrier properties can control the release of active molecules from the sachet pouch matrix to the produce.

The hypotheses of the current research are the following: (1) The shelf life of a packaged fresh tomato was extended by maintaining an established level of active compounds such as 1-methylcyclopropene. (2) The potential proposed delivery system allowed release of 1-MCP gas at an acceptable rate into the headspace of packages containing fresh tomatoes.

The present study focuses on the efficient treatment of the 1-MCP gas for ripening on tomatoes, and the development of a 1-MCP delivery system for packaged

tomatoes. This information may be helpful in maintaining the quality of packaged tomatoes.

Research Objectives

Objective 1: Application of 1-methylcyclopropene (1-MCP), with temperature control, to the tomato fruit during the ripening process to extend storage life.

The effects of 1-MCP treatment and temperature on the ripening process of the tomato fruit during postharvest storage were investigated. In this study, 1-MCP treated fruits using single and once-a-day applications compared to control fruits without 1-MCP treatment. The tomato fruit storage study was performed at three maturity stages, mature green, pink, and red, and at three storage temperatures, 10, 15, and 20°C.

Objective 2: To observe change in pigment concentration in the tomato fruit treated with 1-MCP and held at various temperatures.

Changes in chlorophyll and lycopene content in tomatoes treated with 1-MCP gas during the ripening process were investigated. Total chlorophyll and lycopene contents were quantified using a specific extinction coefficient absorbance technique. Three parts of the tomato fruit were used to study pigment changes, the pericarp, the locular gel, and the placenta.

Objective 3: To observe interactions between 1-MCP and selected adsorbing agents using inverse gas chromatography.

Sorption behavior and thermodynamic parameters related to 1-MCP interaction with selected adsorbing agents were studied using inverse gas chromatography. Determination of thermodynamic parameters provided for a better interpretation of the 1-MCP/adsorbing agent interactions. Calculation information of the specific retention volume (V^o_g), free energy (ΔG_s), isosteric enthalpy (ΔH_s), and entropy (ΔS_s) of sorption were used to characterize 1-MCP/adsorbing agent interactions.

Objective 4: To observe 1-MCP release from the adsorbing agents and through the packaging system.

This objective focused on the release of 1-MCP from the adsorbing agents in the sachet pouch system. The release study was performed under two conditions, dry (0%RH) and high relative humidity (90%). The water and 1-MCP permeability values of the sachet pouch films were measured. Partition coefficients of 1-MCP ($K_{s/g}$) with the adsorbing agent, or the film phase in air were obtained as the ratio of the equilibrium levels of 1-MCP in these different media.

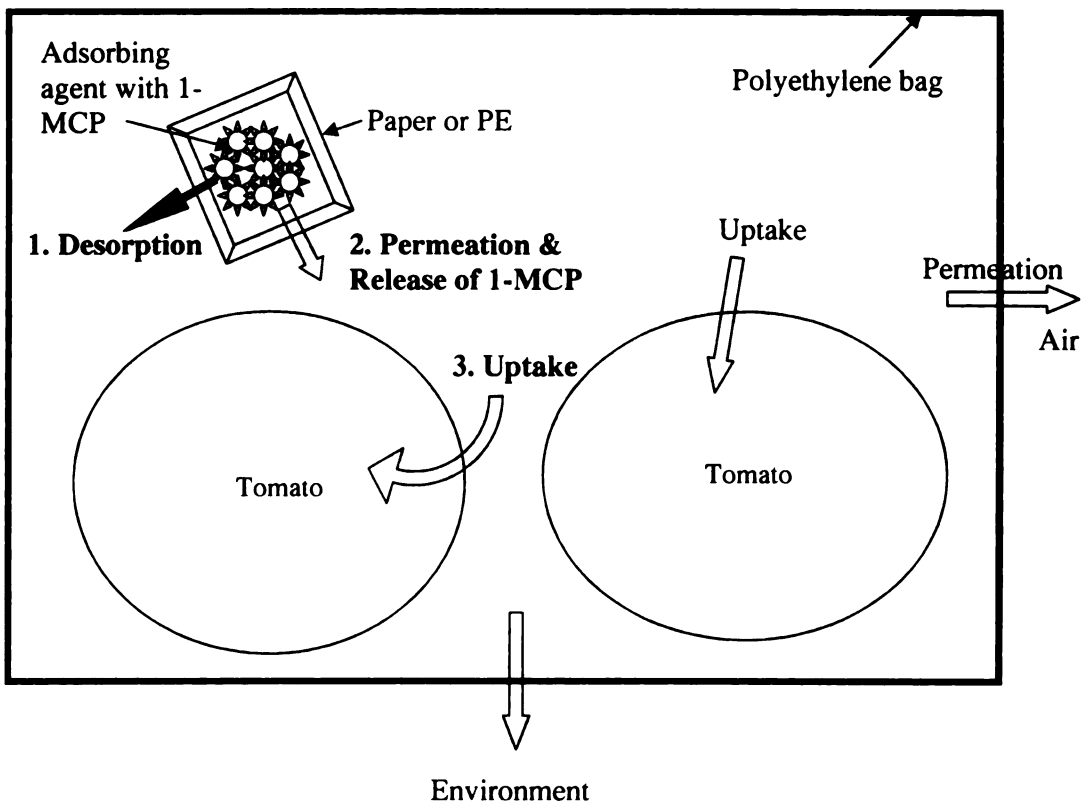


Figure 1. The proposed 1-MCP delivery mechanism in the packaging system for fresh tomatoes.

SECTION II: REVIEW OF LITERATURE

1. Tomato fruit

Tomato *(Lycopersicon esculentum)* is an annual plant cultivated in temperate regions or short-lived perennials in the tropics. Most tomato species are native to the coastal areas of Ecuador, Peru, and Northern Chile (Male, 1999). The tomato fruit is a fleshy berry having two or more cavities that contain seeds imbedded in a gelatinous tissue that becomes a smooth and soft surface when it is fully developed to mature conditions. The shape of the tomato fruit is dependent on cultivar; there is cherry-, pear-, and egg-shaped fruit, but most cultivars have the globose shape. The tomato fruit consists of pericarp, placenta tissue, and seeds (Figure 2).

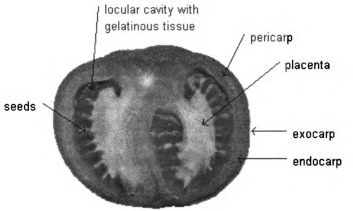


Figure 2. Longitudinal structure of a tomato fruit

The skin of the pericarp consists of an epidermal layer which contains all three cell layers of collenchymous tissue, with a relatively thick cuticle. The wall of the pericarp is separated into the exocarp and endocarp. The gel is a gelatinous tissue containing the seeds. It is a light cream to brown color and fills the locules. The placenta is composed of parenchyma cells that surround the ovules with the seeds embedded in the gel.

Tomato fruits are climacteric, a class that also includes apples and bananas. Climacteric products undergo a significant increase in ethylene production at the onset of ripening. Exogenous ethylene causes the rate of ethylene production to increase and induces a respiratory rise. The ethylene stimulates other ripening processes such as softening, color changes, compositional changes, and aroma production. Non-climacteric crops include fruits like citrus and grape which have relatively low respiration rates and levels of ethylene production. During ripening, non-climacteric fruits are considered to have an ethylene-independent metabolism in which ethylene does not promote fruit ripening.

2. Fruit ripening

Ripening represents physiological changes in a number of biochemical pathways including an increase in respiration, ethylene output, carotenoid synthesis, chlorophyll degradation, production of cell wall hydrolases, and texture softening (Hobson, 1972). The result of each physiological behavior in ripening occurs independently and is normally an irreversible process. Figure 3 shows the general pattern of physiological changes that occur during ripening in tomatoes. Among several physiological changes,

color is the most important visual standard of quality in tomatoes. The maturation process in tomatoes develops from the mature green stage to the breaker stage, then the full red stage with good texture, and then arriving at the final stage, in which most tomatoes have a dark red color and the tissue is softened. The main factor in the color change of fruits occurs in the transition of chloroplasts into chromoplasts which are rich in yellow or red carotenoid pigments, and an increase in the water-soluble pigments such as anthocyanins. Maturity of tomatoes is classified into six levels using a subjective assessment of the external fruit color (Ryall, 1979; Edwards, 1967). The maturity stages are: Stage 1-'Green': totally green color in internal and external tissues, showing no evidence of red or yellow coloration; Stage 2-'Breaker', most green color, not more than 10% of the surface is yellow; Stage 3-'Turning', over 10 to 30% of the surface area is yellow or pink color, but showing some green areas; Stage 4-'Pink', between 30 and 60% of the surface is pink, showing no traces of green coloration; Stage 5-'Light red', more than 60% of the surface is pinkish and red color; Stage 6-'Red', more than 90% of the surface is red color.

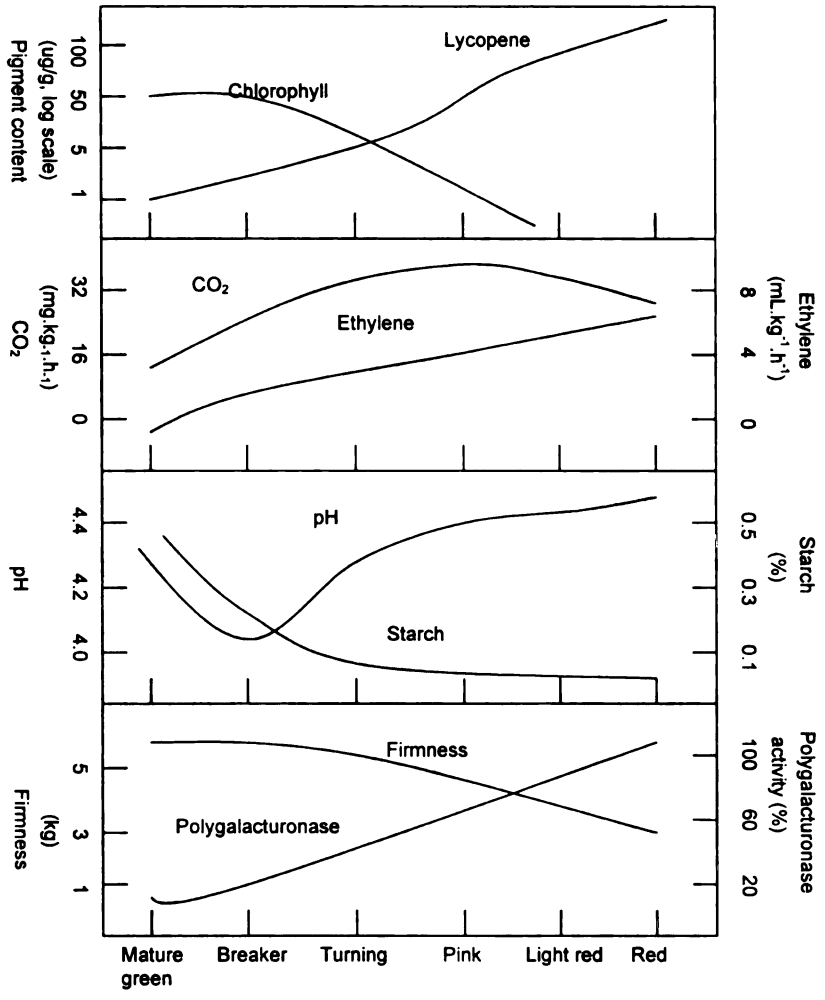


Figure 3. Patterns of some physiological changes during ripening of tomatoes. (Wills, 1989)

Softening of fruit texture can be caused by the partial degradation of cells by polygalacturonase (PG) which is present in the cell walls, and through hydrolytic changes

in the cellular constituents. The ripening process increases the activity of polygalacturonase (PG), causing pectin degradation in the cell walls. It also causes a large increase in ethylene production in tomato fruits at the onset of ripening (pink color stage). After the ripening process is under way, ethylene production reaches a peak, and continues at a relatively high level throughout the ripening period. Nakatsuka (1997) and Della Penna (1986) characterized the genes involved in ethylene synthesis as 1-aminocyclopropane-1-carboxylate (ACC) synthase, and ACC oxidase. Control of fruit ripening is important for the successful transport and marketing of fresh produce. Fruit that ripens too early is easily damaged during transport, and produces ethylene, which can adversely affect other commodities. Various techniques have been used to delay or manage ripening of fresh produce, including cold storage, controlled atmosphere storage, ethylene addition and removal, and chemical inhibitors of ethylene action. The optimum storage temperature for ripening of the mature green tomato is approximately 15°C to 20°C. Throne (1982) reported that the firmness and surface color of stored tomatoes are adequate quality indicators for temperatures above 12°C and below 27°C. Fruit firmness of various tomato cultivars was significantly different at time of harvest and after storage for 1 and 2 weeks at 19-23°C (Gormley, 1978). Temperatures less than 10°C will cause chilling damage, and the fruit softens, color development slows, and flavor is reduced. Mature-green tomato fruit stored for 9 days at 7°C, subsequently ripened to an acceptable color (Efiuvwevwere, 1988). At 12 and 19°C, color development was more even than at lower temperatures.

3. Pigments for color development in tomato fruits

Chlorophylls

Chlorophylls are the green colored pigments in photosynthetic tissues that convert light energy into chemical energy. Chlorophylls produce carbohydrates and other organic compounds from carbon dioxide and water in the presence of light, and release molecular oxygen. All green plants contain the blue-green pigment chlorophyll *a*, and yellow-green chlorophyll *b*. They occur concurrently within the same plant. The ratio of chlorophylls varies with growth conditions and environmental factors. Chlorophyll *a* differs from chlorophyll *b* due to in the presence of a single methyl group instead of an aldehyde group (Figure 4). Chlorophylls are porphyrins which contain four pyrrole rings surrounding a central Mg^{2+} ion. Chlorophyll *a* and *b* contain a phytol chain with 20 carbons ($C_{20}H_{39}OH$) attached to the propionyl group of a pyrrole ring.

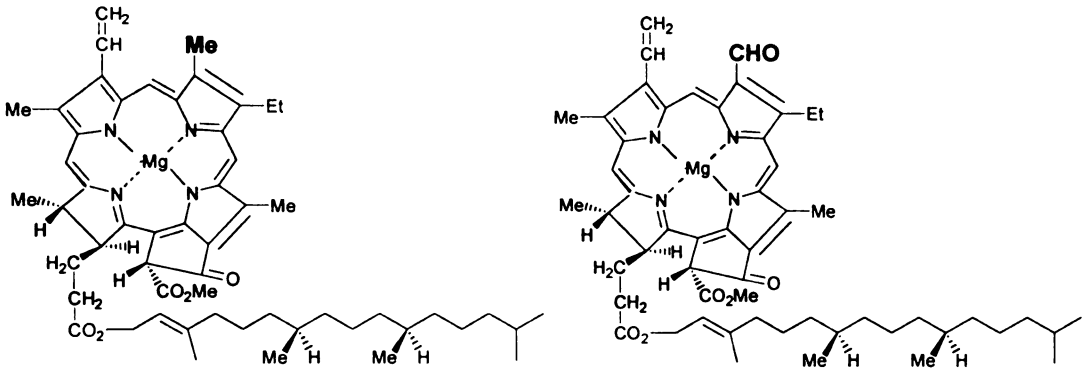


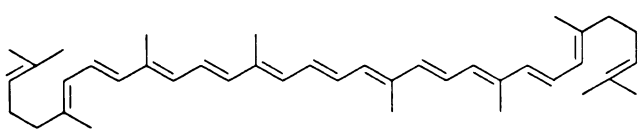
Figure 4. The chemical structures of chlorophyll *a* and chlorophyll *b*

The degradation of chlorophyll can occur through mechanisms such as loss of magnesium, and loss of the phytol group, through the action of the enzyme chlorophyllase. Loss of the phytol group results in the formation of chlorophyllide, abstraction of both magnesium and phytol produces pheophorbide, and finally cleavage of the porphyrin ring system by a dioxygenase results in the formation of pyropheophorbide (Van Boekel, 2000). In many fruit tissues the loss of chlorophyll leads to the transformation of chromoplasts from chloroplast, which has capacity to form carotenoids. The end products of chlorophyll degradation are colorless, low-molecular-weight compounds such as CO_2, NH_3, and H_2O. Watada (1976a) estimated the total chlorophyll content of green 'Walter' tomatoes by light absorbance. Chlorophyll content changed from 13.4mg/kg in immature-green fruit to 0.3mg/kg in partially ripe fruit. Chlorophyll is sensitive to light, heat, humidity and enzymes which can cause

degradation. Cold storage of cabbage resulted in a color change from green to white due to chlorophyll degradation (Heaton, 1996a and b). Ethylene also increases chlorophyll degradation and transforms the green chloroplasts to colored chromoplasts. Liu (1989) found that 2,5-norbornadiene inhibited the loss of chlorophyll in the pericarp tissue of 'Dahong' tomato fruit. Controlled-atmosphere storage of tomatoes with low O_2 and high CO_2 slows down the degradation of chlorophyll (Sozzi, 1999).

Lycopene

Carotenoids are important natural pigments synthesized by higher plants and microorganisms. Lycopene is the major carotene (red color) in tomatoes and may comprise as much as 90 percent of the total carotenoids. Lycopene is an acyclic carotenoid with 11 linear conjugated carbon-carbon double bonds, making it soluble in lipids. Figure 5 illustrates the structural form of lycopene typically found in tomatoes.



All-*trans* Lycopene

Figure 5. The chemical structure of lycopene

Lycopene is susceptible to oxidation by physical and chemical factors such as elevated temperature, exposure to light, and oxygen (Nguyen, 1999). In common tomato varieties lycopene exists predominantly in the all-trans configuration which is the most thermally stable form. Its concentration varies significantly with ripening and in the different varieties of tomatoes (Edwards, 1967). Matinez-Valverde (2002) reported that lycopene content ranged from 18.60 to 64.98mg/kg in 'Liso' and 'Durina' tomato varieties, respectively. Lycopene content of ripe tomato fruit varied between 12.1mg/kg in 'Urbana' and 198.6mg/kg in 'San Marzano' tomatoes (Edward, 1967). Lycopene is used to predict the degree of maturity since it influences the color of tomatoes, and is found in greater amounts in the chromoplast of plant tissues. Lycopene biosynthesis increases dramatically during ripening at which time the color of the tomato turns from green to white to yellow to pink to red. As chloroplasts are transformed into chromoplasts, the chlorophylls are degraded. Carotenoids are not uniformly distributed within fruits. The chromoplast in the outer pericarp contains predominantly lycopene, while the gelatinous part of the pericarp has a substantial amount of the major β-carotene (Nguyen, 1999). The majority of carotenoids, including lycopene are very efficient singlet oxygen quenchers (Di Mascio, 1989). Viljanen (2002) demonstrated that lycopene, β-carotene, and lutein acted as antioxidants in photooxidation by quenching singlet oxygen which prevented the formation of methyl linoleate hydroperoxides. Lycopene showed the greatest quenching ability at a concentration higher than 40ppm and was more stable than the other carotenoids tested. Lycopene is an efficient scavenger of oxygen radicals and acts as an antioxidant against oxidative damage associated with carcinogenesis (Khachik, 1995). Dietary lycopene acts to prevent cancers of the prostate and gastrointestinal tract

(Clinton, 1998).

4. Ethylene

Ethylene is a plant hormone that plays an important role in many aspects of plant growth, development, and senescence. It also stimulates ripening processes resulting in changes in color and softening, and effects respiration of fruits. Ethylene can be used to change the uniform color in the ripening of tomatoes, apples, and bananas. On the other hand, this gas may shorten the storage life of fruits by accelerating the ripening process. Ethylene is formed according to the following biosynthetic pathway in plant tissue: L-methionine → S-adenosyl-L-methionine (AdoMet) → 1-aminocyclopropane-l-carboxylic acid (ACC) → ethylene (Adams, 1979). In this pathway, two enzymes react to regulate ethylene production. First step is the conversion of AdoMet to ACC which is catalyzed by ACC synthase. Second step is the oxidation of ACC to ethylene which is catalyzed by ACC oxidase (EFE). These biosynthetic processes are designed to promote the rate of ethylene production even when the amount of the amino acid methionine is small. Besides converting ACC to ethylene, ACC synthase produces 5'-methylthioadenosine (MTA). This can then be used for the synthesis of new methionine via the Yang cycle (Figure 6). ACC synthase plays a key role in regulating ethylene production and can respond to many inducers such as ethylene, wounding of the fruit, temperature and presence of metal ions such as Cd^{2+} and Li^{+}. ACC oxidase activity also increases in some plant tissues in response to internal or external factors that induce ethylene production, thus its activity may be limiting. ACC can also be metabolized to l-

(malonylamino)cyclopropane-1-carboxylic acid (MACC), a biologically inactive end-product catalyzed by ACC malonyltransferase. The rate of formation of MACC affects the endogenous levels of ACC which have low ACC synthase activity.

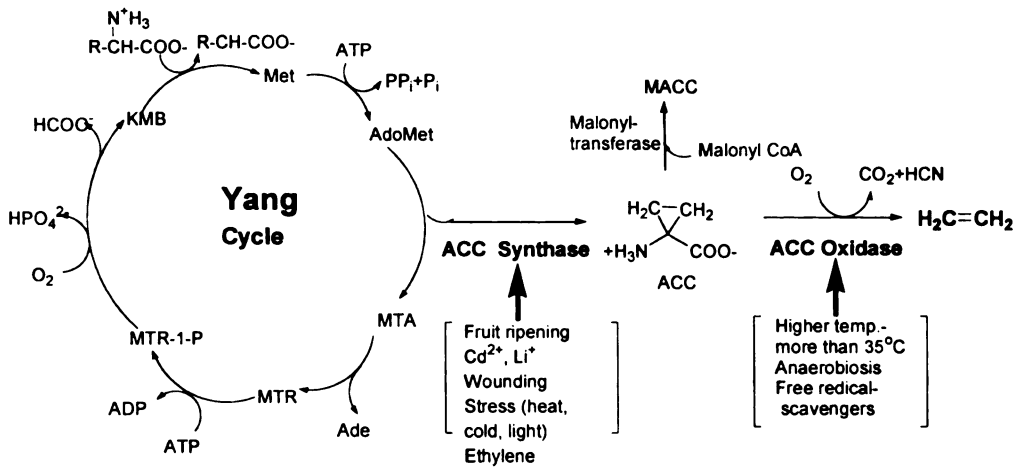


Figure 6. Ethylene biosynthesis pathway (Yang, 1987)

s-adenosyl-L-methionine (AdoMet), 5'-methylthioadenosine (MTA), 5'-methylthioribose (MTR), MTR-1-phosphate (MTR-1-P), 2-keto-4-methylthiobutyrate (KMB), methionine (Met), 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC), 1-aminocyclopropene-1-carboxylic acid (ACC)

It is well-known that ethylene biosynthesis is subject to positive and negative feedback regulation in plants (Kende, 1993). Positive feedback regulation of ethylene biosynthesis is a characteristic feature of ripening fruits. A large increase in ethylene production is triggered by exposure to exogenous ethylene with activation of ACC synthase and ACC oxidase. Negative feedback has been recognized in immature climacteric fruit. Exogenous ethylene significantly inhibits endogenous ethylene

production induced by ripening (Nakatsuka, 1997). The Yang cycle is responsible for ethylene synthesis during ripening. Ethylene is the key regulatory molecule effecting fruit ripening.

5. 1-methylcyclopropene (1-MCP)

1-MCP (Figure 7) was discovered by Drs. Sylvia Blankenship and Ed Sisler of North Carolina State University (Blankenship, 1996). It has been found to be an effective blocking agent, and thus, inhibiting the ethylene response (Sisler, 1996). However, 1-MCP gas is quite unstable and will react with oxygen and other organic compounds. It is also explosive at high concentrations. Recently, 1-MCP, encapsulated with alpha-cyclodextrin, has been marketed under the trade names EthylBloc® and Smartfresh™ as a commercial product. It acts by occupying the ethylene-binding site which prevents ethylene from binding to the active site. 1-MCP is an irreversible, nontoxic, and odorless inhibitor of the ethylene binding and action. Treatment with 1-MCP at concentrations of less than 1 ppm eliminates the effect of ethylene on most ethylene-sensitive plants.

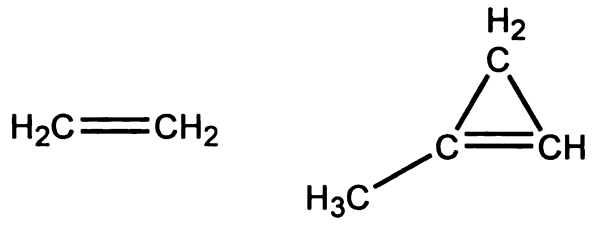


Figure 7. Chemical structures of ethylene and 1-methylcyclopropene

Ethylene antagonists, such as 1-MCP can be a useful in investigating the role of ethylene in the ripening of climacteric fruit. Although many studies have evaluated the influence of 1-MCP and its effect on ripening, its mode of inhibition relative to ethylene is still not verified. Sisler (1997) proposed a model to describe the effect of 1-MCP on the ethylene receptor. The biologically active form of the ethylene receptor led to the theory that binding takes place at an active site containing a metal, and that 1-MCP binds to a metal ion in the ethylene receptor. It was proposed that the competitive interaction of ethylene with its inhibitor prevented the ethylene from binding to the site. Ethylene binds a metal in the ethylene receptor, and electrons are withdrawn. A ligand substitution process with ethylene then induces an active response, and the ethylene is expelled from a metal on the receptor. An active receptor complex is then formed. 1-MCP withdraws electrons in a way similar to ethylene, but since the binding action of 1-MCP is stronger than that of ethylene, it remains bound to a metal on the ethylene receptor surrounded by ligands of unknown structure. 1-MCP may effectively block the active receptor, thus preventing the formation of an active receptor complex.

1-MCP has been found to reduce ethylene-related response in many fruits such as apples, bananas, plums, kiwifruits, and strawberries. Golding (1998) reported that treating mature green bananas with 1-MCP significantly delayed the onset of ethylene production, respiration, and aroma volatile production. Watkins (2000) showed that the efficacy of 1-MCP in apples was affected by storage conditions, and concentration level. Kim (2001) reported that kiwifruit responded to 1-MCP levels of 1, 10, and 100 ppm (v/v) by reducing ethylene production, and fruit softening at 20°C. Higher concentrations of 1-MCP were needed to affect an ethylene inhibitor response. Mir (2001) showed that 1-MCP effectively reduced the rate of apple fruit softening with a treatment of 0.7 ppm 1-MCP compared with the control at 0, 5, 10, 15, and 20°C storage temperature. Skog (2001) demonstrated that 1-MCP treatment decreased the firmness of plums significantly compared to untreated fruit during postharvest storage and ripening. Porat (1999) evaluated the effect of ethylene and 1-MCP on oranges, a non climacteric fruit, during postharvest storage. 1-MCP concentrations of 50 and 100 ppb (v/v) were very effective and markedly inhibited the de-greening process, but were ineffective in preventing chilling injury and decay development. Jiang (1999) investigated the effect of 1-MCP on bananas packed in polyethylene bags. 1-MCP with modified atmosphere (MA) packaging markedly delayed peel color change and fruit softening during ripening.

6. Other ethylene inhibitors

Aminoethoxyvinylglycine (AVG) is an inhibitor of ethylene biosynthesis in plant tissues that prevents the conversion of S-adenosylmethionine to 1-amino cyclopropane-1-carboxylic acid (Yang, 1984). Fan (1998) reported that AVG inhibited production of some volatile esters in climacteric apple fruit.

Diazocyclopentadiene (DACP), a light-activated compound, has been shown to irreversibly inhibit ethylene action. DACP can inhibit softening, starch degradation, ethylene production, and color change during fruit ripening. A single exposure of DACP to mature green tomatoes prevented the ripening process for about 10 days at 25°C and for 20 days at 14.5°C (Sisler, 1993). Exposure at five-day intervals resulted in longer ethylene inhibition. DACP works at very low concentrations, and since a single exposure protects the fruit for several days, it is easier to study the effects of blocking the receptor with a minimum of side effects. However, DACP has not been selected for commercial application because of its explosive potential (Serek, 1994).

2,5-norbornadiene (NBD) is a cyclic olefin compound, which blocks ethylene action. It has been used to study ethylene action in stimulation of respiration, fruit softening, and flower senescence. Stimulation of endogenous ethylene by NBD also tends to interfere with ethylene by competing for the binding site. However, the effect of its inhibition is not permanent because the binding is reversible. This compound also has bad odor and toxicity concerns. Sisler (1984) demonstrated that NBD is a competitive inhibitor of ethylene by inhibiting ethylene's binding to its receptor. NBD blocks the receptor, but requires continuous exposure at higher concentrations. Liu (1989) reported

that NBD reduced loss of chlorophyll, lycopene synthesis, and increased PG activity in tomato fruits. NBD also inhibited ethylene production, which increased the ACC content, and decreased the MACC content in ripening stages from green to pink colored fruits.

Silver thiosulphate (STS) is one of the best-known ethylene inhibiting compounds. Silver ions prevent exogenous ethylene from competing with ethylene for the binding receptor. STS very effectively inhibits the ethylene response, and its application prevents the deteriorative effects of ethylene on flower plants. Newman (1998) showed that STS was effective in extending the life of 'gypsophila' flowers. While STS is highly effective, it has been prohibited for commercial use in many countries, because the silver component may cause environmental contamination.

7. Active packaging applications for fresh produce

Fresh produce consists of living tissue that respires after harvest for climacteric tissues. High concentrations of CO_2 with relatively low O_2 concentrations can be beneficial at extending freshness during storage, transportation, and distribution (Sozzi, 1999). Controlled atmosphere (CA) storage creates an environment in which the desired atmosphere is maintained throughout the storage period. Modified atmosphere packaging (MAP) is packaging which can result in a desirable headspace mixture of gases (Zagory, 1988). Maximum storage quality can be maintained by creating the optimum concentration of gas or gas mixtures in the headspace of the food product or produce. Active package systems include oxygen, carbon dioxide, and ethylene

absorbers, and materials which release antimicrobial and antioxidant compounds. Active packaging applications are summarized in Table 1.

Table 1. Some published active packaging applications

Type	Function	Form	Active components	Reference
Ethanol release	Prevent microbial growth	Microencapsul-ation, Sachets	Silicon dioxide powder	Ooraikul, (1990)
Ethylene absorbers	Control horticultural produce ripening	Sachets and Films	Potassium permanganate and Clay powder	Jayaraman, (1992); Shorter, (1992); Hobson, (1981); Liu, (1970)
Oxygen absorbers	Prevent lipid oxidation and mold growth	Sachets, Films, and Bottle closures	Ascorbic acid, $Ca(OH)_2$, and Iron oxide	Day, (2002); Brody, (2002, 2001); Rooney, (1981)
Antioxidant release	Prevent lipid oxidation or off flavor	Films	BHT, BHA, and Tocopherol	Laermer, (1994); Ho, (1994)
CO_2 and water vapor absorbers	Control horticultural produce freshness	Sachets	$Ca(OH)_2$ and $CaCl_2$	Jenkins, (1991); Vogt, (1985)

Applications of modified atmosphere packaging to fresh produce have been studied by many researchers. Examples include the gas exchange system (Zagory, 1988), the use of $CaCO_3$ and SiO_2 filled microporous films (Mizutani, 1993), and application of ceramic filled polymer films (Lee, 1992). Generally, most applications use absorbers in sachets or films to remove or replace the gas in the atmosphere surrounding the fresh produce in the package (Jaeger, 1999). A gas releasing system, which uses an ethylene inhibitor in conjunction with modified atmosphere packaging, has not been reported. A releasing system which uses an ethylene inhibitor such as 1-MCP gas can be used as an alternative to ethylene absorbers to delay the fruit ripening process. Absorbers can also be incorporated directly into plastic structures, such as bottle closure

liners and as films to form pouçhes (Teumac, 1995). Multiple layers can help absorb or release gas compounds on the inside of the package (Zeoli, 1983). Permeable systems which include either sachets or a multi-layer film allows for controlled release of small concentrations of active compounds into the target zone.

8. Inverse gas chromatography (IGC)

To develop active packaging, a permeable sachet and appropriate absorbing agent are required to deliver an active gas compound to the surface of the postharvest fruit. These interactions include the sorption/desoprtion of 1-MCP gas on the adsorbing agents. The physiochemical interactions of 1-MCP and the adsorbing agent can be described using a powerful tool, inverse gas chromatography.

Inverse gas chromatography (IGC) can be used to study the characteristics of the stationary phase of specific solid materials and their interaction with sorbate molecules. This is in contrast to the analytical gas chromatography system, where the stationary phase is only used to separate and quantify the injected compound. A typical IGC system is similar to a conventional gas chromatography because it separates the injected compound. However, in IGC a specific material is mixed in the column or coated on the inner wall with an inert supporting material. The sorbate is then injected at the inlet of either a packed column or a capillary column. The carrier gas carries the injected compound through the column, and its motion is delayed by interaction with the sorbant. The peak profile of the interaction between the two phases is recorded in a chromatogram. The information can then be used to deduce the physicochemical and

surface properties of a specified solid material by plotting sorption isotherms and calculating thermodynamic parameters. IGC is a rapid and accurate way to measure sorption properties and dynamic interaction parameters of the vapor/solid system. Many researchers have used this technique to measure polymer surface adsorption (Kontominas, 1994), interaction of polymer with its monomer (Demertzis, 1987; Kontominas, 1993), transition temperature and crystallinity in a polymer structure (Guillet, 1989), interaction of volatile flavor compounds in the food matrix (Delarue, 2000), and water sorption in plastic packaging materials or food products (Kalaouzis, 1993; Demertzis, 1989). IGC has been used to evaluate solid materials for measurement at infinite dilution (zero surface coverage), where the adsorption isotherm is essentially linear, and at finite concentration, where the shape of the adsorption isotherm reflects the build-up of multi-layers on the surface (Vukov, 1989). In addition to the sorbate-stationary phase interactions, the amount of an injected sorbate can become a significant factor affecting the shape of the chromatogram. At high concentrations, most isotherms become nonlinear. The elution profiles observed under high concentrations of sorbate are often skewed and have a sharp front and diffuse tail or vice versa. At low sorbate concentrations, these isotherms are linear and thus the elution profiles are symmetric and easily interpreted.

IGC analysis techniques

The elution technique is the most common method and is referred to as the pulse technique, the other is a frontal analysis method referred to as the step technique. These

IGC techniques are described briefly as follows:

Elution analysis involves the injection of a known amount of the sorbate molecules into a column packed with the adsorbent materials. The injected sorbate moves in the direction of the carrier gas with an initial velocity. The molecules of the injected sorbate collide with the surface of the adsorbent. If there is no interaction, the velocity remains the same throughout the column. If interaction takes place, the velocity will be retarded and the area response and shape of the pulse will be affected depending on the strength of the interaction. The IGC elution technique was used to determine the sorption isotherms for a series of n-alkanes with polystyrene at 30°C (Kontominas, 1994). Demertzis (1987) investigated the interaction of vinylidene chloride (VdC) with vinylidene chloride (VdC-VC and VdC-AcN) copolymers using the elution technique.

Frontal analysis uses a continuous flow of carrier gas and sorbate vapor at a constant concentration through a column packed with an adsorbing agent. After the sorbate breaks through the stationary phase a frontal chromatogram is developed. When the adsorbing agent in the column is saturated, the detector response reaches a maximum step-height establishing a plateau. This frontal analysis doesn't depend on the shape of the response, but on the area bounded by the chromatogram not affected by the kinetic band broadening process. The isotherm profile can be derived from frontal analysis using the mixture stream as a function of time. Apostolopoulos (1988b) used frontal IGC to determine the water sorption isotherms of commercial freeze-dried coffee at given temperatures. A modified frontal IGC method was used to obtain water sorption isotherms of sucrose and starch at 25°C (Paik, 1986). The results showed that water sorption isotherms (for sucrose and starch) obtained by modified frontal IGC were similar

with those of the static equilibrium relative humidity method using saturated salt solutions.

Sorption isotherms (elution chromatography technique)

The procedure is carried out by injecting a quantity of sorbate at the inlet of a column containing the stationary phase. By analyzing the corresponding elution peak (Kiselev, 1969), sorption isotherms can be calculated as follows.

$$a = \frac{1}{W}\int_0^c V_c dc \qquad (1)$$

where a is the amount of the adsorbed sorbate per unit mass of adsorbing agent, W is the amount of adsorbing agent in the column, and V_c is the retained volume (V, m^3) of sorbate at the gas phase concentration (c)

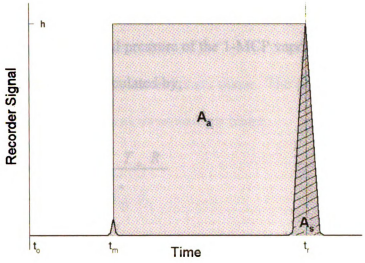


Figure 8. Typical gas elution chromatogram showing the areas used in calculating sorption isotherm data. A_a is the area limited by the non-interacting time (t_m), height (h), time axis, and the diffuse side of the sorbate peak. A_s is the peak area of sorbate. t_0 is the time of sorbate injection. t_r is the retention time of a sorbate peak.

Figure 8 illustrates the typical plot of a gas elution chromatogram from which a data point in the isotherm (concentration of 1-MCP in the column and partial pressure) can be calculated from symmetrical peaks. The concentration of 1-MCP adsorbed in the column, M_a, is given (Kiselev, 1969) by

$$M_a = \frac{m\ A_a}{w\ A_s} \qquad (2)$$

where M_a is expressed in moles of sorbate gas per weight unit of adsorbing agent (mole/g), m is the injected amount of 1-MCP in moles, A_a and A_s are the areas indicated in Figure 8, and w is the weight in grams of adsorbing agent in the column.

The partial pressure of the 1-MCP vapor entering the gas chromatographic detector, p, is calculated by,

$$p = \frac{m \quad q \quad h \quad T_c \quad R}{v \quad A_s} \qquad (3)$$

where p is expressed in Pascal (Pa), q is the integrator chart speed (cm/min), h is the recorder peak height as in Figure 8 in cm, T_c is the column temperature (K), v is the corrected carrier gas flow rate (mL/min), and R is the universal gas constant.

Specific retention volume (V^o_g) values and thermodynamic parameters

A typical chromatogram for a single sorbate (A_s) contains a retention volume (V_R) at the sorbate retention time (t_r) and an additional small peak (t_m) early in the chromatogram. The retention volume is shown in Figure 8 as the distance from the point of injection to the peak maximum. At constant flow rate (F_c), V_R is given by,

$$V_R = t_r \times F_c \qquad (4)$$

Equation 5 (McNair, 1998), relates the retention volume to the theoretical distribution constant as follows.

V^*_R is the adjusted retention volume excluding the void volume corresponding to the adjusted retention time (t'_r). t'_r is the portion time (t_r-t_m) resulting from interactions of the sorbate with the stationary phase. The small early peak (V_M) represents inert air that does not sorb in the stationary phase.

$$V_R - V_M = V_R^* = K_d \cdot V_s \qquad (5)$$

where the volume of the air peak (V_M) is called the retention volume of an unretained compound. V_s is the volume of the stationary phase. K_d is the distribution constant.

The net retention volume (V_N) represents the value obtained by subtracting the non-retained air peak (V^o_M) from the portion of the sorbate (V^o_R) defined in equation 6 (Lundqvist, 1997).

$$V_N = J \cdot (V_R - V_M) = J \cdot F_c \cdot (t_r - t_m) = J \cdot V_R^* = V_R^o - V_M^o \qquad (6)$$

where t_r is the retention time of sorbate, t_m is the retention time of an unsorbed species (air), and J is the James and Martin compressibility factor.

The corrected retention volumes (V^o_R and V^o_M) are defined as the retention volume of sorbate and the air peak correcting for the compressibility of the carrier gas, based on the correct flow rate.

$$V_R^o = J \cdot t_r \cdot F_c \quad (7)$$

$$V_M^o = J \cdot t_m \cdot F_c \quad (8)$$

$$J = \frac{3}{2}\left[\frac{(p_i / p_o)^2 - 1}{(p_i / p_o)^3 - 1}\right] \quad (9)$$

where p_i is the column inlet pressure, p_o is the column outlet pressure, and F_c is the correct gas flow rate (ml/sec) according to the following,

$$F_c = F_r \cdot (\frac{T_c}{T_r}) \cdot \left(\frac{p_o - p_w}{p_o}\right) \quad (10)$$

where T_c is the column temperature (K), T_r is the room temperature (296 K), and F_r is the flow rate (ml/sec) measured using a soap-bubble flow meter and p_W is the partial pressure of water at room temperature present in the soap-bubble flow meter.

Specific retention volume (V^o_g)

Specific retention volume (V^o_g) is the amount of carrier gas required to elute a given amount of sorbate from a column containing a given weight of interacting stationary phase at 273 K per gram of adsorbing agent (Delarue, 2000).

$$V_g^o = \frac{V^o{}_R - V^o{}_M}{W} \times \frac{273}{T_c} = \frac{J \cdot F_c \cdot (t_r - t_m)}{W} \times \frac{273}{T_c} \quad (11)$$

$$V_g^o = \frac{V_N}{W} \times \frac{273}{T_c} \quad (12)$$

where V_N is the net retention volume, W is the weight of adsorbing agent in the column, and T_c is the column temperature.

Thermodynamic parameters

Determination of the thermodynamic parameters provides a better insight into the sorption mechanism. Thermodynamic parameters were calculated by defining specific retention volume (V^o_g) at an infinite dilution.

Free energy of sorption (ΔG_s)

The free energy indicates the adsorbing agent's affinity for 1-MCP gas in the sorption process. It provides a means to tell whether the 1-MCP sorption is stronger (negative ΔG_s value), or weaker (positive ΔG_s value) (Apostolopoulos, 1983). The more negative the values of ΔG_s, the stronger the sorption is, ΔG_s can be calculated as,

$$\Delta G_s = -R \cdot T_c \cdot \ln K_d \quad (13)$$

Enthalpy of sorption (ΔH_s)

Enthalpy of sorption, called isosteric heat, represents the quantity of heat released during sorption (Riganakos, 1989). Negative values of ΔH_s are associated with strong binding forces in the sorption mechanism. Positive values are an indication of stronger self attraction than adsorption.

$$-\frac{\Delta H_s}{R} = \left(\frac{\partial \ln V_g^o}{\partial \frac{1}{T_c}} \right) \tag{14}$$

Entropy of sorption (ΔS_s)

Entropy is related to the number of different equilibrium energy states occurring at the 1-MCP and adsorbing agent interface at a defined temperature and pressure. The entropy (ΔS_s) characterizes the structural properties that exist when 1-MCP is absorbed. Entropy values are useful in the interpretation of processes such as dissolution, crystallization, and swelling which occur during 1-MCP sorption (Apostolopoulos, 1988a). Ordered systems have negative ΔS_s values, whereas more random systems have positive values, ΔS_s is calculated as,

$$\Delta S_s = \left(\frac{\Delta H_s - \Delta G_s}{T_c} \right) \qquad (15)$$

where ΔG_s is the free energy of sorption (kcal/mol), ΔH_s is the enthalpy changes (kcal/mole), and T_c is the column temperature (K).

9. Adsorbing agents

The sorption of a gas on a solid surface may take place between the sorbate and adsorbing agent. The sorption occurs in the gas/solid system provided that the temperature and pressure are suitable, and the gas is capable of forming a chemical bond with the solid (Engewald, 1991). Commercial adsorbing agents are listed in Table 2. Silica gel is a uniformly porous adsorbent, it is an amorphous condensation of polysilicic acid with a high specific surface area. The surface of silica gel is covered by hydroxyl groups to various degrees (Kiselev, 1969). Silica gel is a common adsorbent and is chemically inert, non-corrosive, and non-toxic. Activated carbon is a good adsorbent that has a large surface area. The most widely used activated carbon has a surface area of 800-1500m^2/g. This surface area is predominantly within the micropores (Smisek, 1970). Tenax-TA is a porous polymer that is based on 2,6-diphenyl-p-phenylene oxide. Tenax-TA has a low affinity for polar compounds such as water and high affinity for

non-polar compounds. The average pore size is 200nm. The density of Tenax-TA is 0.25g/cc. The specific surface area of Tenax-TA is $35m^2/g$ (Loffe, 1984). Activated clay has been used as an adsorbent for oil pigments (Boki, 1992; Chapman, 1992). Pinnavaia (1983) and Lan (1994) showed the structure, properties, and applications of various clay minerals.

Table 2. Commercial applications for adsorbing agents

Adsorbing agent	Main function	Application	Reference
Silica gel	Desiccant	Food and packaged goods	Rix, (1994); Satomi, (1988)
Activated carbon	Filtration of odor, contaminated air, water	Food and consumer product	Leggott, (2001); Pendyal, (1999); and Roy, (1995)
Activated clay	Desiccant, filtration, pollutant adsorbent, additives in polymer	Healthcare, food, and packaging	Boki, (1992); Habile, (1992)
Tenax-TA	Trapping of volatiles and semi-volatiles	Water, air, and consumer product	Piringer, (1993)

11. Partitioning in air and adsorbing agent matrix

The change in sorbate concentration in a food packaging system can be described by partition coefficients between a food, its packaging and the environment (Giacin, 1995). The interaction phenomena are particularly relevant for product development where sorbate compounds are important. Headspace sampling methods can be used to measure the partition coefficients between air and the matrix for aroma components in the gas phase. Headspace sampling is generally classified into two types (Chaintreau,

1995): (1) static headspace method where the vapor phase is directly injected into a gas chromatograph and (2) dynamic headspace method, which requires trapping of the volatiles onto an adsorbent prior to GC injection. For headspace sampling, the experiments should satisfy the following rules: sampling of the vapor phase at equilibrium; no pressure change during sampling; and no dilution while sampling (Chaintreau, 1995). Partition coefficients studied between a two-phase of food packaging system are as follows: various volatile organic compounds between air and water (Kolb, 1992; Amoore, 1978; Buttery, 1965); gas/solution (Nahon, 2000); between food (solid) and air or film and air producing off-flavor from plastic packaging film (Halek, 1988a and b; Biran, 1979a and b); water and polystylene in a polymer packaging system (Gavara, 1996). Liang (1996) examined the characteristics of gas/particle (G/P) partitioning of organic compounds in environmental tobacco smoke. Partition coefficients were used to estimate diffusion. Lee (1996) investigated the sorption of cadminum on 15 types of soil using a partition coefficient model which related the adsorption of the Cd(II) to the soil components: iron oxides, manganese oxides, aluminum oxides, and organic matter.

12. Gas permeation

Permeability is the rate at which a quantity of gas or vapor passes through a surface of a film structure. The amount of permeant transported via the permeation process, is expressed by the transmission rate or flux (F).

$$F = \frac{Q}{A \cdot t} \tag{16}$$

where Q is the amount permeated through area (A) during time (t).

The permeability model can be expressed by the three processes involved in permeation such as absorption, diffusion, and desorption according to the movement of permeating molecules through a film structure (Rogers, 1985). Permeation processes are affected by several factors, including the nature of the polymer, including its chemical composition and molecular structure, the nature of the permeants such as size and polarity, and the environmental conditions, such as temperature and relative humidity. Other factors affecting permeation include permeant concentration, polymer fillers, and plasticizers.

The linear relationship between the concentration of a permeant in the polymer and the concentration or partial vapor pressure of permeant in the gas phase is expressed by Henry's law.

$$C = S \cdot p \tag{17}$$

where C is the concentration of gas in the polymer, p is the partial pressure of permeant, and S is Henry's law constant, which is the solubility coefficient.

The diffusion process of a permeant is described by Fick's first and second laws of diffusion (Crank, 1975). Fick's first law describes the process in which a gas or vapor diffuses through a polymer structure under steady state conditions.

$$F = -D(\frac{\partial c}{\partial x}) \tag{18}$$

where F is the flux or the rate of transfer of permeant per unit area, c is the permeant concentration in the polymer, x is the direction of the diffusion process, t is time, and D is the molecular diffusion coefficient.

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial^2 x} \tag{19}$$

Fick's second law shows the non-steady diffusion condition, in which the concentration of the diffusing permeant across the polymer structure is changed over time.

The permeation of a gas through a polymer structure is generally described by the permeability coefficient (P). The permeability coefficient is a measurement of the permeation rate of a gas or vapor through a polymer structure at steady state which is related to the diffusion coefficient (D) and solubility coefficient (S).

$$P = D \times S \tag{20}$$

where D is the diffusion coefficient that describes how fast the permeant molecules move in a polymer structure in terms of a kinetic parameter. S is the solubility coefficient that measures the concentration of permeant molecules sorbed in the polymer matrix as a thermodynamic parameter.

By integrating equation 18 where D is independent by concentration of the permeant,

$$F = D \cdot \frac{(C_1 - C_2)}{\ell} \tag{21}$$

where C_1 and C_2 are the steady state concentrations of the permeant at the two surfaces of the polymer film with thickness (l).

Eqn. 21 can be rewritten to Eqn. 22 by substituting Eqn. 21 for Eqn. 16.

$$Q = D \cdot \frac{(C_1 - C_2) \cdot A \cdot t}{\ell} \tag{22}$$

By combining Eqn. 22 and Eqn. 17, it gives,

$$Q = D \cdot \frac{S \cdot (p_1 - p_2) \cdot A \cdot t}{\ell} \tag{23}$$

Eqn. 20 and Eqn. 23 can be rewritten as Eqn. 24 to determine the permeability coefficient.

$$P = \frac{Q \cdot \ell}{A \cdot t \cdot \Delta p} \tag{24}$$

$\Delta p = p_2 - p_1$, pressure difference across the film

13. Permeability measurement methods

Permeability, diffusion and solubility coefficient values can be determined using the following procedures.

Gravimetric method

In the gravimetric procedure, the permeation value of a permeant through a packaging material is calculated as the weight change of a total test cell. ASTM E-96 is described as an example of a gravimetric procedure to determine the water vapor transmission rate. An aluminum dish containing dried desiccant is covered with a packaging material, weighed and placed in a constant temperature and humidity chamber. The samples are weighed periodically, and the water vapor transmission rate is determined by the weight gain over time. The gravimetric method in general (ASTM E-96 procedure) has low sensitivity, especially for high barrier polymeric materials and is applicable over a limited vapor pressure range. Among the gravimetric methods, an electrobalance system can continually monitor over time the weight change of a test specimen at equilibrium vapor pressure (Barr, 2000; Hernandez, 1994). An electrobalance system among the gravimetric method has been used to measure the sorption and diffusion of organic vapors by polymer films (Nielsen, 1994; Hernandez-Munoz, 1998). The electrobalance method has excellent sensitivity and application over a wide range of temperature and sorbate vapor pressures.

Isostatic method

In the isostatic method, a permeation cell is used, which consists of two chambers, divided by a test film. A permeant flows continuously through the high concentration chamber, and an inert carrier gas flows through the low concentration chamber. The partial pressure gradient of the permeant provides the driving force causing penetration from the high concentration chamber to the low concentration chamber. The transmission rate of the permeant through the test film is monitored until steady state is reached under constant conditions of temperature and permeant vapor pressure (Hernandez, 1986). The permeability coefficient (P) is determined from the transmission rate at steady state by the expression:

$$P = \frac{C_{ss} \cdot f \cdot \ell}{A \cdot \Delta p} \tag{25}$$

where C_{ss} is steady state concentration of permeant, in mass per unit volume; f is the rate of carrier gas flow in the low concentration cell chamber, in volume per unit of time; *l* is the film thickness; and A is the surface area.

The diffusion coefficient D is calculated using the following expression (Ziegel, 1969):

$$D = \frac{\ell^2}{7.199 \cdot t_{0.5}} \tag{26}$$

where $t_{0.5}$ is the time required to reach a transmission rate value that is equal to half of

that at steady state.

Quasi-isostatic method

In this procedure, the permeability coefficient is obtained by quantifying the amount of permeant accumulated in the low concentration chamber after passing through the film (Liu, 1991; Sajiki, 1993). This method is also called the accumulation method. The gas or vapor being tested is allowed to flow from the high concentration to the low concentration chamber. The partial pressure gradient provides the driving force for the permeant to pass into the low concentration chamber. The permeant in the low concentration chamber is quantified periodically using a gas chromatograph. As the quantity of permeant accumulates in the low concentration chamber, the rate of transfer of permeant becomes a constant.

The permeability coefficient can be determined using the flux value at steady state and is given by,

$$P = \frac{Q \cdot \ell}{t \cdot A \cdot \Delta p} \qquad (27)$$

where Q is the total quantity of permeant that has penetrated during a unit time (t). A is the film area exposed to the permeant, l is the film thickness and Δp is the driving force (concentration or partial pressure gradient).

The quasi-isostatic method can be used to measure the permeation rate of organic vapors at any desired low concentration level.

Diffusion coefficient (D) can also be determined from the lag time. It is obtained at the intersection between the steady state projection of the transmission curve and the time axis (Hernandez, 1986), and expressed as:

$$D = \frac{\ell^2}{6\theta} \tag{28}$$

where θ is the lag time and l is the film thickness.

14. Active compound release systems

The active compound delivery system either physically traps or chemically binds a suitable material to release a constant quantity of the active compound. Applications of a controlled release system are found in the drug release mechanism of many of pharmaceutical products (Shao, 2001), herbicide release of agricultural materials (Zhu, 2001), flavor/fragrance release of consumer products (Greenblatt, 1993), and the food ingredient release of food products (Meyers, 1998). The delivery technologies of controlled release system include (Zeoli, 1983): encapsulation, hollow fiber, monolithic system, and laminated structure. Encapsulation is a process that coats a polymeric material with the active agent (liquids, solids, or gases), thus providing a barrier to undesirable environmental or chemical interactions until the release action is desirable (Greenblatt, 1993; Risch, 1995). The hollow fiber active agent system holds the active agent in tiny open tubes where the agent releases by diffusion through the air layer above

the liquid in the solid interface (Sirkar, 1999; Zeoli, 1983). In the monolithic system, the active agent is physically blended with polymeric or elastomeric materials and remains dispersed within the polymer matrix after it is molded or extruded. The active agent diffuses from the inner region to the surface of the monolithic materials as the active agent evaporates (Opperman, 1999). In a laminated structure, the active agent is impregnated in a layer between two outer plastic layers. Controlled amounts of the active agents move from the inner layer to the surface layer and subsequently diffuse through the plastic layer (Zeoli, 1983).

In conventional control release systems, the active agent is absorbed on materials such as silica gel, activated carbon, or activated clay. The active agent in such systems is released by desorption, so it cannot control the release rates of the compounds (Patwardhan, 1983). Use of polymer barriers combined with these adsorbing materials gives an alternative controlled release application for the active agent (Yoshida, 1979). The release mechanism for the active agent includes two processes: a desorption process from an adsorbing material and diffusion through a controlling polymer structure.

SECTION III: EFFECT OF SINGLE AND REPEATED 1-METHYLCYCLOPROPENE (1-MCP) EXPOSURES ON THE RIPENING OF TOMATOES (*LYCOPERSICON ESCULENTUM* MILL.) DURING STORAGE AT 10, 15, AND 20°C

ABSTRACT

The effects of storage temperature and 1-MCP treatment on the ripening process of tomatoes were evaluated using the following parameters: skin color, firmness, total weight, and ethylene production. Skin color and firmness changes were measured manually using a chromameter and a durometer, respectively. The total weight of a tomato was measured using a digital balance. The tomato ethylene production was measured on a single fruit using a static gas collection system. Tomatoes stored at 10°C had longer ripening periods than tomatoes stored at 15°C and 20°C. 1-MCP treatment was very effective in retarding changes in the skin color of tomatoes at the respective temperatures. In addition, once-a-day 1-MCP treatment was more effective than an initial single 1-MCP treatment. The weight loss rate of fruit showed no significant difference between 1-MCP treatment and the untreated control. The total weight loss rate for whole fruit at 20°C was greater than that at 10°C and 15°C. After 24 days of treatment, the external ethylene production of fully ripened tomatoes was higher at 20°C than at 10°C and 15°C.

INTRODUCTION

An important effect of ethylene on climacteric fruits is to induce the ripening process by coordinating the expression of genes, responsible for increasing ethylene production, respiration, color change, conversion of starch to sugars, tissue softening, and other related metabolisms (Lelievre, 1997a). Tomato (*Lycopersicon esculentum* Mill.) is a climacteric fruit that has a significant response to ethylene. The rapid ripening of fruit after harvest limits storability and is a concern during transportation and marketing. To counter balance this process several procedures have been used to extend postharvest life of fruits: low temperature storage (Hall, 1961); controlled atmosphere (Sozzi, 1999); and treatment with chemical agents such as diazocyclopentadiene (Sisler, 1994), 2, 5-norbornadiene (Liu, 1989), and silver thiosulfate (Newman, 1998). However, in fruits, such as pears, very low temperature storage can still induce ripening and produces chilling injury. The fruit loses firmness and develops off colors (Efiuvwevwere, 1988), or stimulates autocatalytic ethylene synthesis (Lelievre, 1997b). On the other hand, chemical agents like 2, 5-norbornadiene and silver thiosulfate are unsafe and can cause environmental contamination (Serek, 1993).

Recently, 1- methylcyclopropene (1-MCP) was evaluated as an agent to extend the freshness of horticultural produce by inhibiting ethylene production (Serek, 1994). 1-MCP is a non toxic gaseous compound that, at a very low concentration, retards the ripening of fresh produce. Studies on 1-MCP have been carried out with strawberries (Ku, 1999), apples (Watkins, 2000), bananas (Jiang, 1999), kiwifruits (Kim, 2001), and plums (Skog, 2001).

The objective of this study was to determine the effect of 1-MCP on the ripening of tomatoes during postharvest storage. The tomato fruits were treated with 1 ppm(v/v) 1-MCP for once-a-day, and applied a single time at the same 1-MCP concentration during storage. 1-MCP treated fruits using single and once-a-day applications compared to control fruits without 1-MCP treatment. Quality changes were evaluated as a function of storage time. The following quality factors were considered: fruit weight, skin color, firmness, and ethylene production.

MATERIALS AND METHODS

Materials

'*Plum dandy*' tomatoes grown during the summer of 2000 at the MSU Horticulture Research Center in Holt, MI were picked by hand to observe quality changes of the fruits in storage. The tomato fruits were classified into three development stages of maturity by skin color based on standard maturity grading (Ryall, 1979): mature green stage (green color tomatoes); pink stage (early ripened tomatoes); and red stage (rapidly ripened tomatoes).

1-MCP was obtained from Floralife Inc., (Walterboro, SC) as EthylBloc® powder with 0.14% active ingredient. 1-Butene (99.9% pure), was purchased from AGA Specialty Gas Inc., (Maumee, OH) to use in quantitative analysis of 1-MCP. A Carle gas chromatograph Series 100 AGC (Loveland, CO) equipped with a flame ionization detector and a 30cm × 2.2mm i.d. Porapak-N stainless steel packed column (Aldeich Co., Milwaukee, WI) was used for the analysis. Latex-free plastic syringes, 1mL, (B-D. Co.,

Franklin Lakes, NJ) were used to inject samples into GC, and a 20 mL polypropylene syringe (B-D. Co., Franklin Lakes, NJ) was used for exposing tomatoes to 1-MCP.

Arrangement of tomato fruit for storage

Defect-free fruits with uniform appearance in color, shape, and size were sorted, rinsed with distilled water to remove any dirt, and dried in air. Between eight to ten tomatoes were separated by color and placed in a 10 L glass desiccator (Figure 10). A rubber port placed on the lid connected the inlet and outlet ventilating lines with constant air flow of 12 mL/min which was bubbled through water as indicated in Figure 9. The glass desiccators with tomato samples were then stored in a temperature-controlled chamber in order to perform the storage study. The temperatures in the three chambers were set at 10, 15, and 20±1°C respectively.

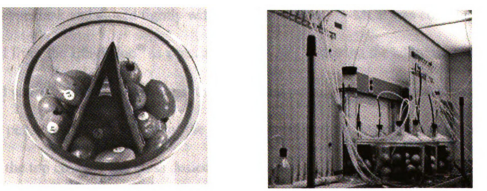


Figure 9. The storage arrangement of tomato fruits

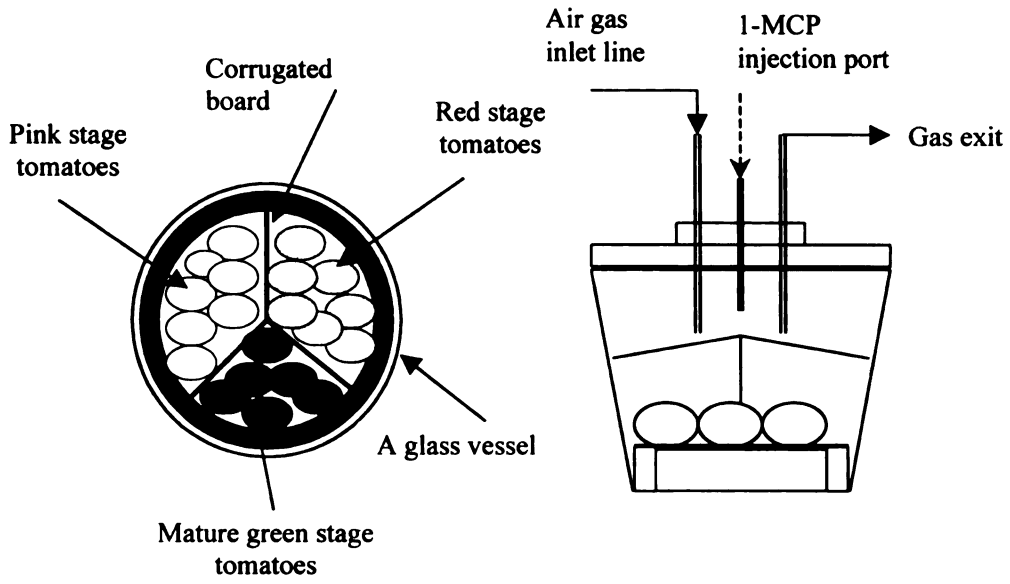


Figure 10. A schematic diagram of the apparatus for arrangement of tomato fruits in the glass vessel

Preparation of 1-MCP gas

Aliquots of 1-MCP as EthylBloc® powder were weighed and placed in a 500 mL Erlenmeyer flask and closed tightly with a rubber stopper. 1-MCP was completely released from the EthylBloc® as a gas after two hours following the addition of 40 mL of distilled water. At these conditions, the headspace volume had a concentration of 480μL 1-MCP per L. A 10 mL aliquot of the headspace volume was injected through a rubber port on the top cover in the glass desiccator to expose the tomatoes to 1 μL 1-MCP per L of air. The concentration of 1-MCP in the sealed desiccator was measured by GC and quantified using a calibration curve constructed with certified pure 1-butene gas. For all measurements the gas chromatographic oven was set at 85°C.

1-MCP treatments of tomato fruit

Tomatoes were treated with 1-MCP gas using either a single or once-a-day application. 1-MCP treatments were prepared as follows. (1) For the single application, 10 mL of headspace gas stock, containing 480 μL of 1-MCP per L, was injected manually into the glass desiccator containing the tomatoes to provide a concentration of about 1-MCP 1 $\mu L \cdot L^{-1}$. After exposing the tomatoes to 1-MCP for 10 hours, humidified air was supplied through the desiccator to allow the tomatoes to respire. (2) For the once-a-day application, 10 mL of the same gas stock was manually injected daily with humidified air flowing through the desiccators. The experiment was carried out, for up to nine weeks depending on the temperature. Control fruit, not exposed to 1-MCP, was stored under a continuous stream of humidified air.

Skin color, firmness, total weight loss, and ethylene production of tomato fruit

Changes in the skin color, firmness, and total weight loss of tomatoes were monitored periodically. Measurements were performed at marked locations on the fruit's surface. Skin color was measured at the one-third position from top stem on a defect-free surface area and recorded with a chromameter Model CR-300 (Minolta, Japan). Hue angle was used to describe the skin color change. A decrease in the value of the hue angle represented a change in skin color from green to pink and to red. Firmness was measured manually using a Shore® hand-held durometer (Shore Co., NY) at the marked positions. A plunger-tip probe fitted in the durometer was pressed against the surface tissue of the fruit and the value after 3 second was measured. The durometer

values were recorded between 0 and 100, a decrease in value represented a decline in firmness, converted to Newton·mm^{-1} unit. All experiments were performed on three tomatoes, respectively. The total weight of a tomato was measured using a digital balance with 10 mg sensitivity. The tomato ethylene production was measured on a single fruit using a static gas collection system at room temperature. A tomato was placed in a 475 mL wide mouth glass jar, purged using ethylene-free air, and sealed with a closure containing a rubber septum at the top. After one hour, 1 mL of gas from the headspace was extracted from the sealed glass jar using a 5 mL plastic syringe. The gas sample was injected into the gas chromatograph for quantification. The concentration of ethylene production was determined in triplicate for each time interval. Skin color, firmness, and ethylene production of tomatoes were measured as shown in Figure 11.

(a) Skin color test

(b) Firmness test

(c) Ethylene production test

Figure 11. Measurements of the skin color, firmness, and ethylene production for stored tomato fruits

The level of the external ethylene in a tomato was calculated by substitution into the following equation:

$$\text{Concentration of ethylene } (\mu L/kg.hr^{-1}) = \frac{C_i \times V_t}{W_t \times T} \quad (29)$$

where, T is the collecting time (hrs) of external ethylene produced by the tomato; V_t is the total headspace volume (475mL); W_t is the tomato weight (g); C_i is the amount of injected ethylene (ppm, v/v), corresponding to the known level of standard ethylene using GC

Statistical analysis

Statistical analyses were performed using SAS for Windows, version 6.08 (SAS Institute, Cary, NC). Appropriate comparisons of 1-MCP treatments and storage time on the quality of tomatoes were made using a Tukey test for multiple comparisons by a one-way analysis of variance ($p<0.05$).

RESULTS AND DISCUSSION

1-MCP has recently been shown to be an effective ethylene inhibitor, binding at the ethylene receptor in climacteric fruits. In this experiment, treatment with 1-MCP greatly delayed the ripening process during storage from 10°C to 20°C. In addition, low storage temperature at 10°C also greatly delays the biochemical ripening of tomato fruit with 1-MCP application. Color development of tomato fruit at 12°C and 19°C was much more regular than at lower temperature where chilly conditions induced poor development (Efiuvwevwere, 1988). Atta-Aly (1992) found that pink tomatoes showed maximum ethylene production at 20°C.

The combination of 1-MCP treatment and optimum temperature storage may result in consistent control of color development and firmness of postharvest tomato fruits. 1 ppm(v/v) of 1-MCP could be sufficient to inhibit ethylene action based on the work on tomato (Nakatsuka, 1997), strawberry (Ku, 1999), apple (Mir, 2001), and banana (Jiang, 1999).

Skin color

Mature green tomatoes at the harvest stage showed the greatest difference in color development with 1-MCP treatment compared to control fruits (Figures 12, 13, and 14). Thus, 1-MCP treatment of green tomatoes at harvest was more effective in delaying skin color development than at the pink and red stages.

The hue angle values of the control mature green tomatoes began to decrease rapidly at day 10, 7, and 5 for 10°C, 15°C, and 20°C respectively. For mature green

tomatoes, the highest rate of color change occurred at day 18 at 10°C, day 15 at 15°C, and day 10 at 20°C as shown in Table 3.

The results of skin color change indicate that the single 1-MCP treatment greatly retarded the ripening process. For example, at 10°C a single treatment delayed the onset of color development for mature green stage fruit from 7 to 28 days. The rate of color change was slower in mature green stage tomatoes treated with 1 μL per L 1-MCP at one day intervals for the once-a-day 1-MCP treatment than in single 1-MCP treated fruits. The once-a-day 1-MCP treatment was even more effective in delaying skin color change up to 45 days at 10°C. 1-MCP action was limited by fungal growth and mottled appearance on the skin of the tomatoes after 50 days.

Green tomato fruits treated with 1-MCP at 10°C were most effective in delaying color development. A single treatment with 1-MCP on fruits at 20°C delayed the color change for 15 days (when a hue angle value began to decrease to 110), compared to the controls at 7 days.

Skin color change involves loss of chlorophyll, and synthesis of other pigments, such as carotenoids and lycopene, during the ripening period. Thus, color change is often used as an index of the degree of ripeness, and provides primary information about the physiological condition of the fruit. Thorne (1982) used equations to predict the rate of fruit surface color change at any temperature. Color and firmness changes were adequate quality indicators and could be used to predict the storage life of 'Nemato' tomato fruits at temperature conditions between 12 and 27°C. A very small amount of ethylene induced color development. The rate of color development varied depending on the particular cultivar of the same fruit or of different species. Porat (1999) reported

that treatment with 1-MCP effectively inhibited the effect of ethylene on orange fruit degreening. Jiang (1999) showed that treatment with 1-MCP delayed the skin color change of bananas when exposed to 0.01-1 ppm (v/v) for 1 day. Increasing concentrations of 1-MCP were generally more effective for longer periods of time.

Table 3. Days of storage until hue angle began to decrease (the breaker stage) for single and once-a-day treated mature green fruit. For comparison, values of the control tomatoes are included.

Temperature °C	Control No 1-MCP Treatment	Single 1-MCP Treatment	Once-a-day 1-MCP Treatment
	Time (in days) at which color started developing (hue angle=110)		
10	11	25	53
15	8	17	38
20	7	15	25

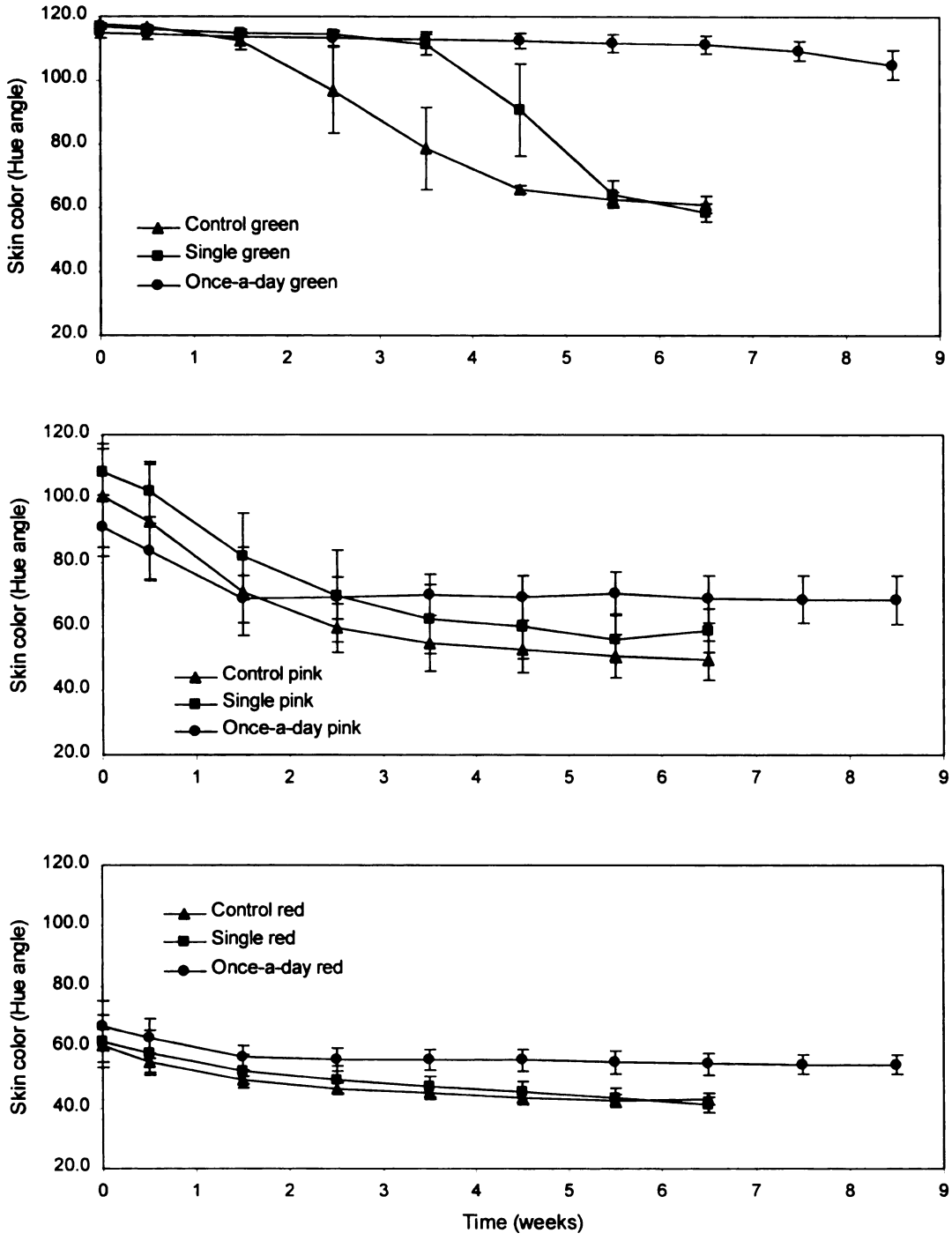


Figure 12. Change in skin color of tomatoes at 10°C. The error bars represent the standard errors of the means (n=3).

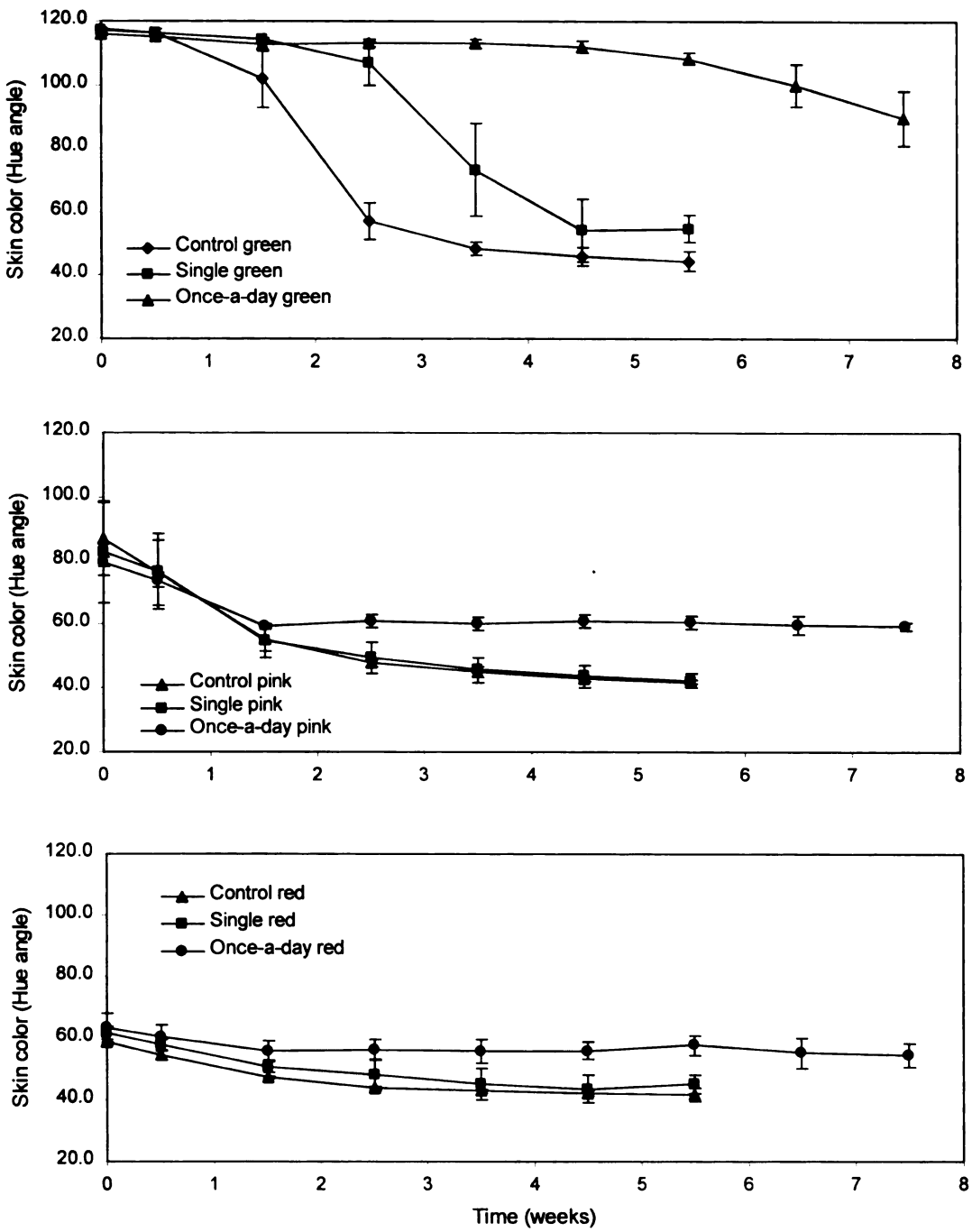


Figure 13. Change in skin color of tomatoes at 15°C. The error bars represent the standard errors of the means (n=3).

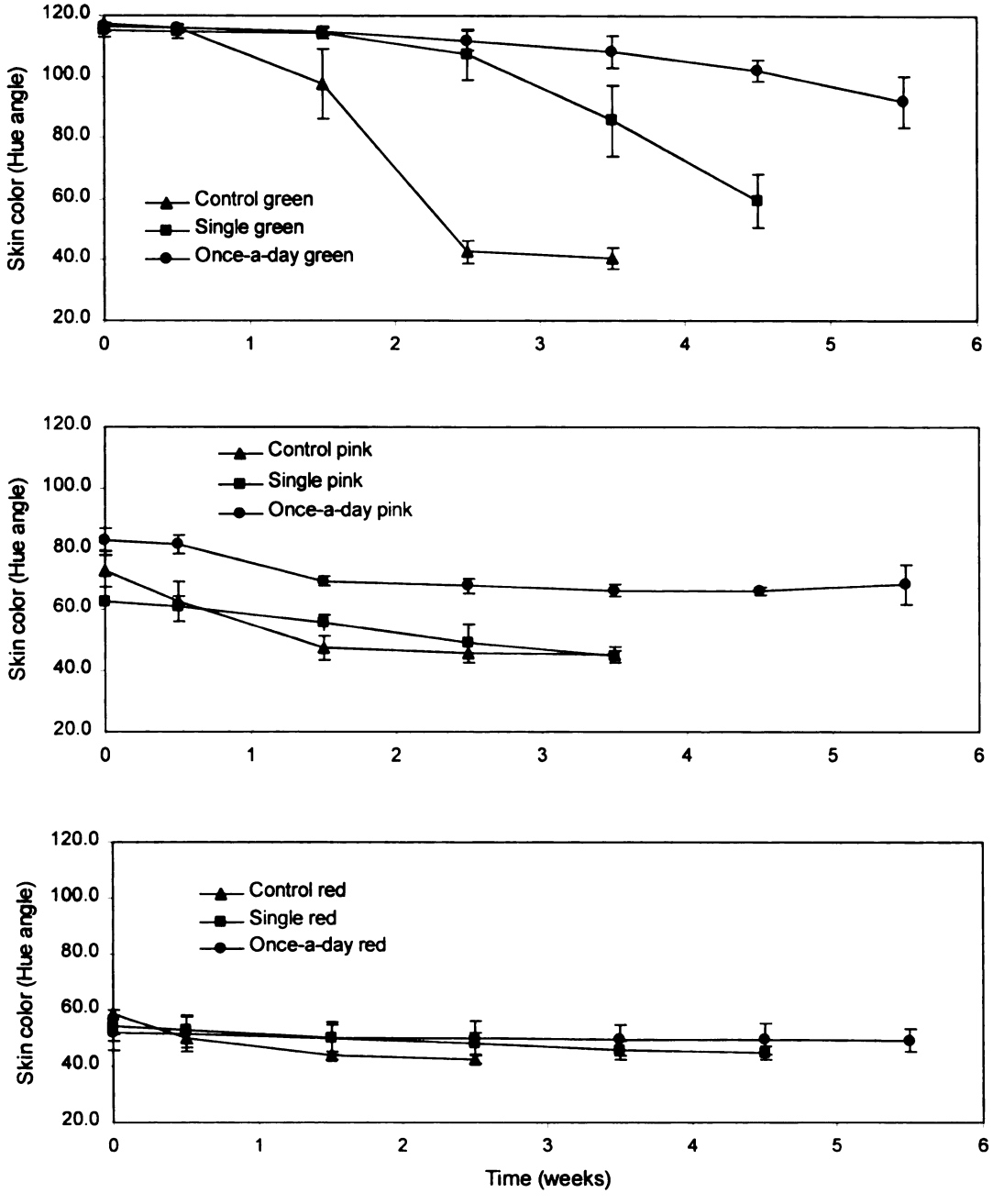


Figure 14. Change in skin color of tomatoes at 20°C. The error bars represent the standard errors of the means (n=3).

Firmness

Firmness in most fruits varies according to the type of fruit, and its maturity, and represents an irreversible process once initiated. The texture of fruits is affected by the composition of their cell walls, cellular constituents, and degree of hydration. Firmness is affected by storage temperature and the time associated with ripening. As shown in Figures 15, 16, and 17, both the 1-MCP treated, and the control fruits showed a constantly decreasing firmness at all temperatures.

On the initial day, firmness values were approximately 7.3 $N \cdot mm^{-1}$ in the mature green stage tomatoes, 4.2 $N \cdot mm^{-1}$ in the pink stage fruits, and 3.2 $N \cdot mm^{-1}$ in the red stage fruits, all with an error of $\pm$ 0.5. At 20°C the firmness of the control green stage fruit rapidly decreased to 4.1 $N \cdot mm^{-1}$ on day 10, as compared to 5.7$N \cdot mm^{-1}$ in the single 1-MCP treated, and 6.9 $N \cdot mm^{-1}$ in the once-a-day 1-MCP treated fruit. At 6.5 weeks (10°C) once-a-day 1-MCP treated stage pink fruit had a value of 3.06 $N \cdot mm^{-1}$, while the control fruit was softer at 1.87 $N \cdot mm^{-1}$. Once-a-day 1-MCP treated red stage fruit at 15 and 20°C was more firm than the controls, but had similar results to the controls at 10°C.

Both the single and the once-a-day 1-MCP treatments significantly delayed the loss of firmness in green skin tomatoes as compared to the control fruit at 20°C. Moreover, the once-a-day 1-MCP treatment was more effective in maintaining firmness than the single treatment. Green fruits stored at 10°C were also significantly firmer than those at 20°C for the storage period. This can be attributed to the lower storage temperature and treatment with 1-MCP.

At 20°C, the control pink fruit firmness began to decrease rapidly and had a value of 1.59 $N \cdot mm^{-1}$ on day 10. The firmness of the controls, at relatively low storage temperatures of 10°C and 15°C, began to slowly decrease over the storage period compared to storage at 20°C. Loss of firmness in the pink tomatoes was greatest at 20°C and least at 10°C for 10 days of storage.

1-MCP treatments significantly extended the storage time. Once-a-day 1-MCP treatment was more effective than the single treatment. In addition, green fruit stored at 10°C were significantly firmer over a longer period.

During ripening of most fruits, enzymatic degradation of the cell walls results in a significant decrease in firmness. A significant decrease in firmness is mostly caused by loss of tissue integrity in the cell wall polysaccharides pectin and hemicellulose due to the softening related tomato β-galactosidase (Smith, 1998).

Mir (2001) reported that 1-MCP reduced the softening rate of apple fruit. The frequency of 1-MCP application affected the rate of fruit softening, with more frequent applications resulting in a lower rate of firmness loss. Kim (2001) also reported that 1-MCP affected the firmness of kiwifruit at 20°C. Similarly 1-MCP treated tomato fruit for both single and once-a-day 1-MCP treatments significantly delayed firmness loss of the fruit skin compared with the controls at 10, 15, and 20°C.

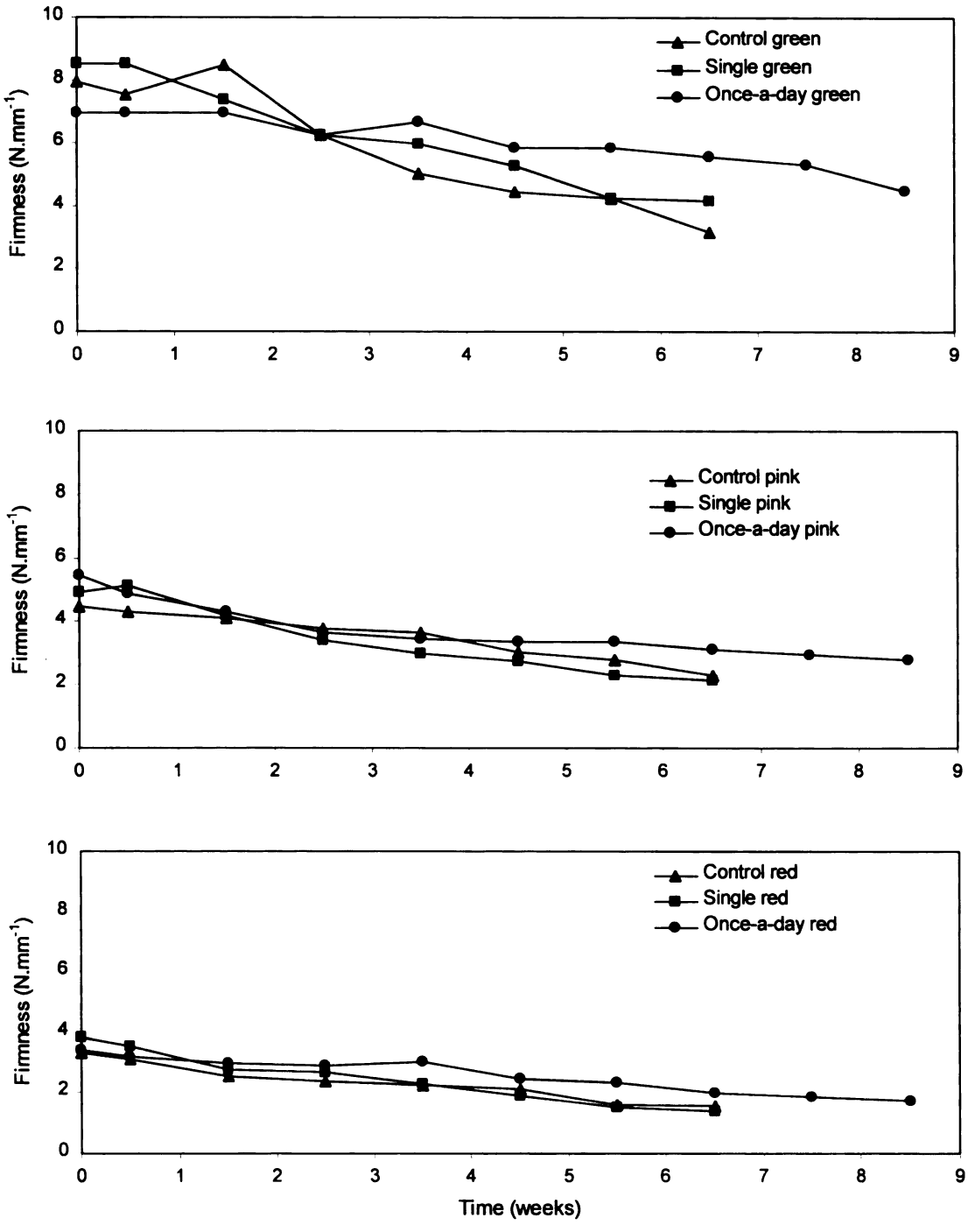


Figure 15. Change in tomato firmness at 10°C. The error bars represent the standard errors of the means (n=3).

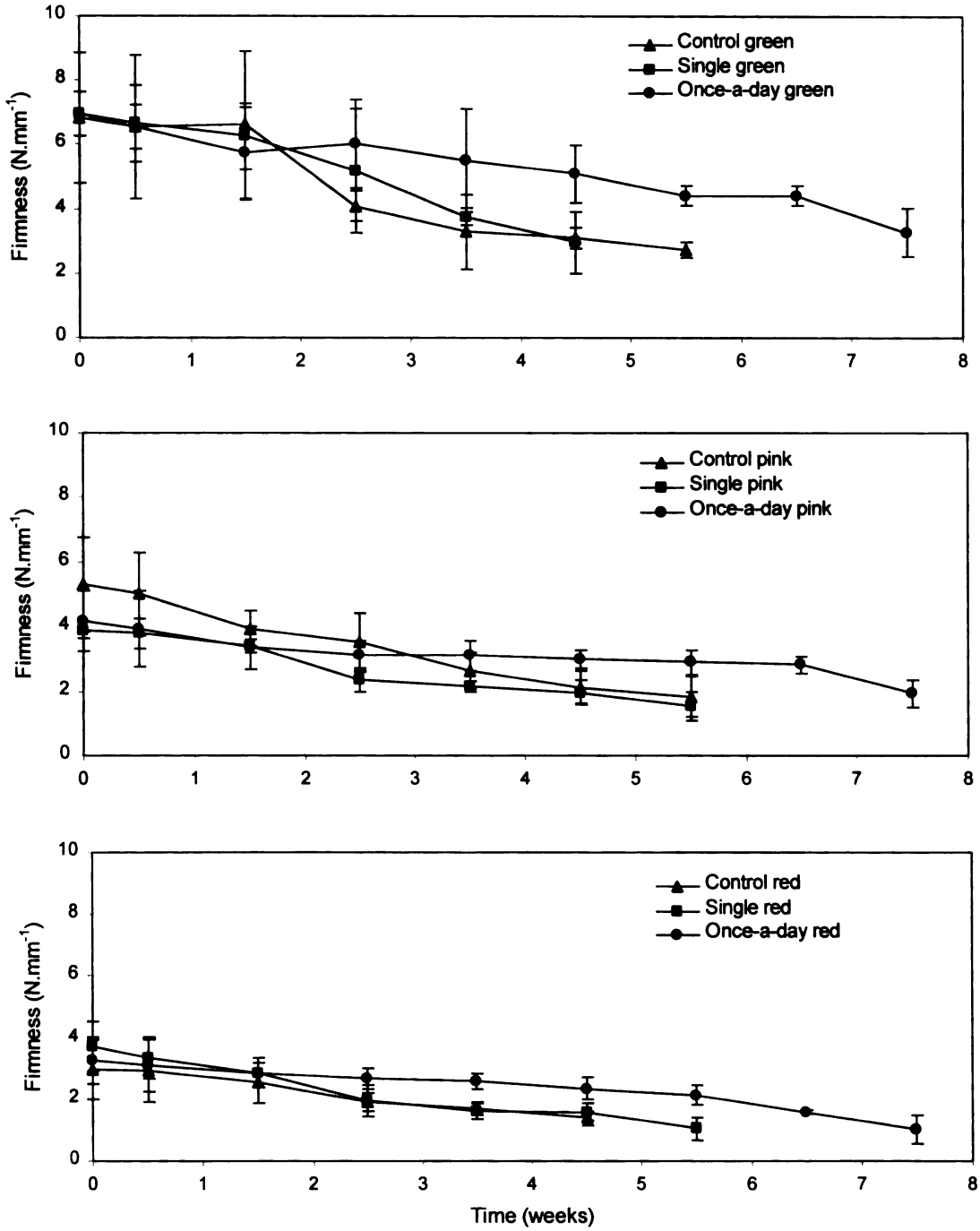


Figure 16. Change in tomato firmness at 15°C. The error bars represent the standard errors of the means (n=3).

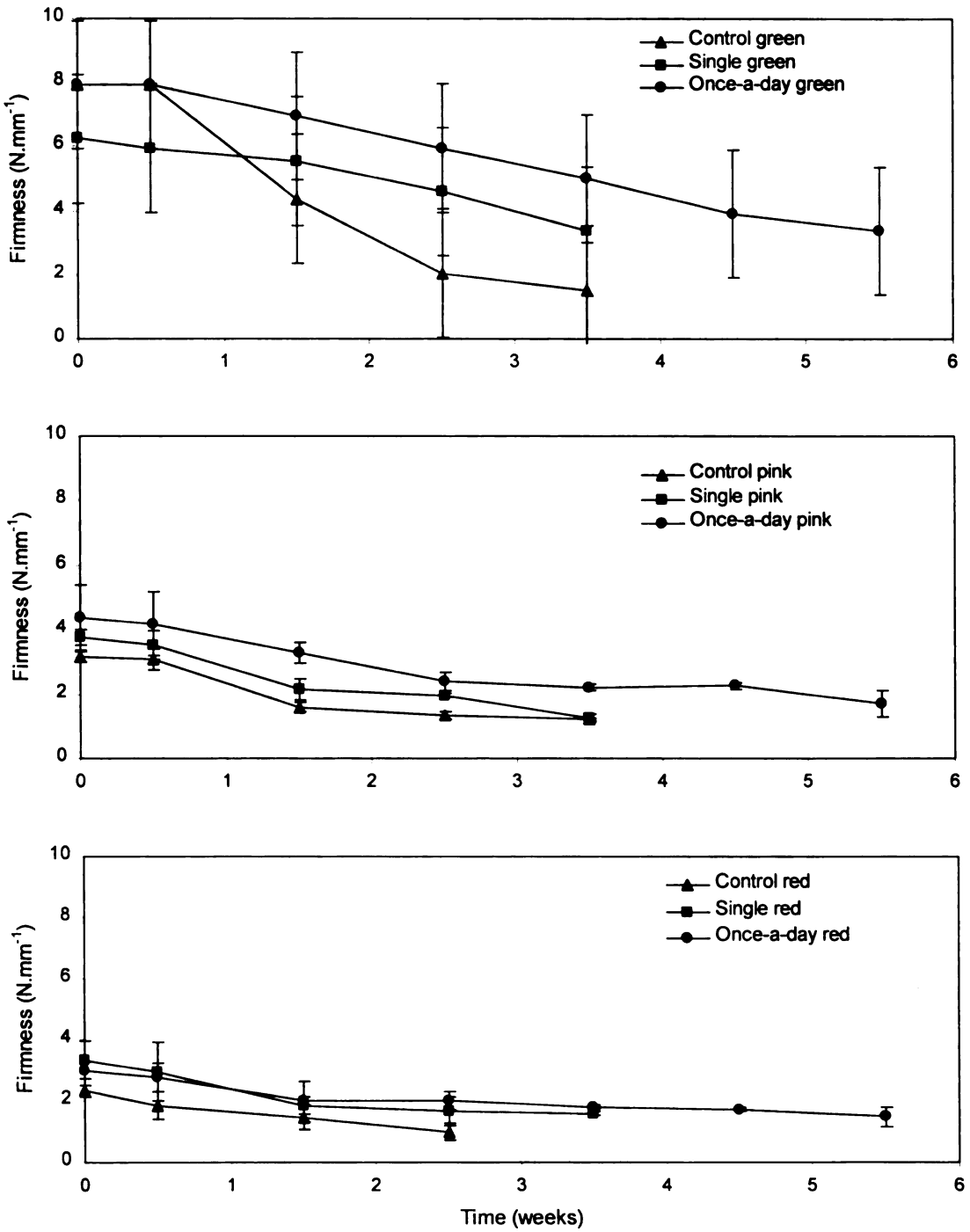


Figure 17. Change in tomato firmness at 20°C. The error bars represent the standard errors of the means (n=3).

Total weight loss

Weight loss by dehydration is an important quality factor. Moisture loss in postharvest produce causes changes in the structure of the cell tissue (Woods, 1990). The total weight of green tomatoes decreased slowly over the storage period at 10, 15, and 20°C as shown in Tables 4, 5, and 6. On day 10, the average weight loss of green fruits was about 0.64% at 10°C, 0.88% at 15°C, and 1.54% at 20°C. There were no clear differences between the once-a-day 1-MCP treated and the control fruit. Porat (1999) reported a difference in weight loss of the orange fruit with 1-MCP treatment, compared to the controls. At the end of 20°C storage for totally ripened fruit, total weight loss was about 2.71 percent. At 20°C the control red fruit had a short storage period because fungal growth and a mottled appearance on the skin caused leakage at surface cracks after 2.5 weeks.

Table 4. Weight loss of each maturity tomato at 10°C.

(grams)

Maturity	Storage time (weeks)	Control	Single 1-MCP treatment	Once-a-day 1-MCP treatment
Mature green stage	0	0.00±0.00	0.00±0.00	0.00±0.00
	0.5	0.37±0.15	0.27±0.12	0.23±0.06
	1.5	0.90±0.26	0.50±0.10	0.67±0.21
	2.5	1.17±0.29	0.77±0.15	0.93±0.31
	3.5	1.53±0.32	0.97±0.12	1.13±0.31
	4.5	1.73±0.32	1.27±0.15	1.60±0.56
	5.5	2.17±0.47	1.57±0.15	2.00±0.66
Pink stage	0	0.00±0.00	0.00±0.00	0.00±0.00
	0.5	0.40±0.10	0.23±0.06	0.27±0.12
	1.5	0.77±0.15	0.53±0.15	0.60±0.17
	2.5	1.10±0.20	0.77±0.21	0.87±0.12
	3.5	1.50±0.26	1.03±0.29	0.97±0.12
	4.5	1.73±0.31	1.47±0.51	1.27±0.06
	5.5	2.60±0.89	1.97±0.76	1.50±0.01
Red stage	0	0.00±0.00	0.00±0.00	0.00±0.00
	0.5	0.23±0.06	0.17±0.06	0.30±0.10
	1.5	0.53±0.06	0.40±0.10	0.60±0.10
	2.5	0.73±0.07	0.60±0.10	0.87±0.21
	3.5	0.97±0.05	0.83±0.06	1.13±0.23
	4.5	1.03±0.06	1.13±0.15	1.50±0.36
	5.5	1.53±0.23	1.50±0.01	2.10±0.44

Average ± standard deviation of each tomato (n=3) as a function of time.
All values in same row are not significantly different ($p>0.05$).

Table 5. Weight loss of each maturity tomato at 15°C.

(grams)

Maturity	Storage time (weeks)	Control	Single 1-MCP treatment	Once-a-day 1-MCP treatment
Mature green stage	0	0.00±0.00	0.00±0.00	0.00±0.00
	0.5	0.30±0.10	0.27±0.12	0.43±0.12
	1.5	0.77±0.23	0.77±0.15	1.07±0.40
	2.5	1.23±0.38	1.13±0.12	1.57±0.49
	3.5	1.47±0.40	1.40±0.10	1.80±0.62
	4.5	1.73±0.38	1.83±0.12	2.27±0.85
	5.5	2.17±0.49	N/A	2.53±1.00
Pink stage	0	0.00±0.00	0.00±0.00	0.00±0.00
	0.5	0.37±0.12	0.27±0.06	0.27±0.06
	1.5	0.80±0.20	0.77±0.23	0.73±0.12
	2.5	1.33±0.23	1.13±0.21	1.10±0.00
	3.5	1.70±0.35	1.40±0.26	1.30±0.10
	4.5	2.20±0.36	1.70±0.26	1.73±0.06
	5.5	2.73±0.55	2.33±0.25	2.10±0.10
Red stage	0	0.00±0.00	0.00±0.00	0.00±0.00
	0.5	0.33±0.15	0.27±0.12	0.27±0.06
	1.5	0.70±0.10	0.67±0.21	0.50±0.10
	2.5	0.97±0.15	0.90±0.26	0.77±0.15
	3.5	1.30±0.26	1.03±0.25	0.87±0.23
	4.5	1.80±0.30	1.27±0.31	1.17±0.32
	5.5	N/A	2.43±0.93	1.53±0.40

Average ± standard deviation of each tomato (n=3) as a function of time.
All values in same row are not significantly different ($p>0.05$).
N/A means it is not available due to a crack of tomato surface

Table 6. Weight loss of each maturity tomato at 20°C.

(grams)

Maturity	Storage time (weeks)	Control	Single 1-MCP treatment	Once-a-day 1-MCP treatment
Mature green stage	0	0.00±0.00	0.00±0.00	0.00±0.00
	0.5	0.43±0.12	0.43±0.12	0.63±0.06
	1.5	1.70±0.17	1.40±0.17	1.57±0.40
	2.5	2.17±0.15	1.70±0.26	1.87±0.40
	3.5	2.70±0.20	2.10±0.36	2.37±0.47
	4.5	N/A	2.37±0.38	2.63±0.42
Pink stage	0	0.00±0.00	0.00±0.00	0.00±0.00
	0.5	0.47±0.12	0.57±0.21	0.53±0.12
	1.5	1.40±0.20	1.43±0.23	1.43±0.32
	2.5	1.67±0.31	1.90±0.44	1.83±0.42
	3.5	2.13±0.49	2.53±0.51	2.23±0.51
	4.5	N/A	N/A	2.43±0.61
Red stage	0	0.00±0.00	0.00±0.00	0.00±0.00
	0.5	0.67±0.06	0.53±0.12	0.47±0.15
	1.5	1.33±0.15	1.17±0.29	1.07±0.23
	2.5	1.63±0.15	1.50±0.35	1.30±0.26
	3.5	N/A	1.93±0.42	1.57±0.32
	4.5	N/A	2.33±0.55	1.83±0.38

Average ± standard deviation of each tomato (n=3) as a function of time.
All values in same row are not significantly different ($p>0.05$).
N/A means it is not available due to a crack of tomato surface

Ethylene production

An increase of ethylene production in the control and 1-MCP treated mature green stage fruit was detected after 4, 12, and 27 days for 20, 15, and 10°C, respectively (Figures 18, 19, and 20).

Ethylene production of 1-MCP treated pink stage fruit was similar to the controls at 10°C. Ethylene production of the control and 1-MCP treated pink stage fruit began to increase rapidly after 2.5 weeks at 20°C. Once-a-day 1-MCP treated pink stage fruit also showed increasing ethylene production over the length of the storage period, but had a relatively lower production than the control and single 1-MCP treated fruit at 20°C. However, ethylene production in control pink stage fruit showed no significant difference in comparison with the 1-MCP treated fruit.

Ethylene production of the control red fruit began to slowly increase after 2.5 weeks at 10°C. Ethylene production of the control and 1-MCP treated red fruit increased rapidly over 2.5 weeks at 20°C and rose on week 4.5 at 15°C. However, ethylene production in red fruit treated with 1-MCP showed no significant difference in comparison with the control fruit. Thus, the ethylene production of the whole fruit was not significantly affected by 1-MCP treatment. The rate of ethylene production of pink tomato fruit increased at 20°C. This is in agreement with Atta-Aly (1992) who observed that the highest level of ethylene production was at the pink stage of ripening in the intact tomato fruit at 20°C. Mathooko (1995) reported that carbon dioxide and diazocyclopentadiene treated tomato fruit reduced ethylene production rates compared to the control fruit at 25°C. Maturity stage and storage temperature had only slight effect

on the ethylene production, compared to the controls throughout the experiment. Nakatsuka (1997) examined the regulation of gene expression of the ACC synthase and ACC oxidase in ripening tomato fruit. The activities of ACC synthase and ACC oxidase, ACC content, ethylene production, and red color development increased as maturity was reached in the fruit ripened from the turning stage. Nakatsuka (1997) showed that ethylene production from tomato fruit was greatly reduced by 1-MCP treatment at the turning and pink stages. Kim (1991) reported that internal ethylene concentration increased as tomato fruit ripened.

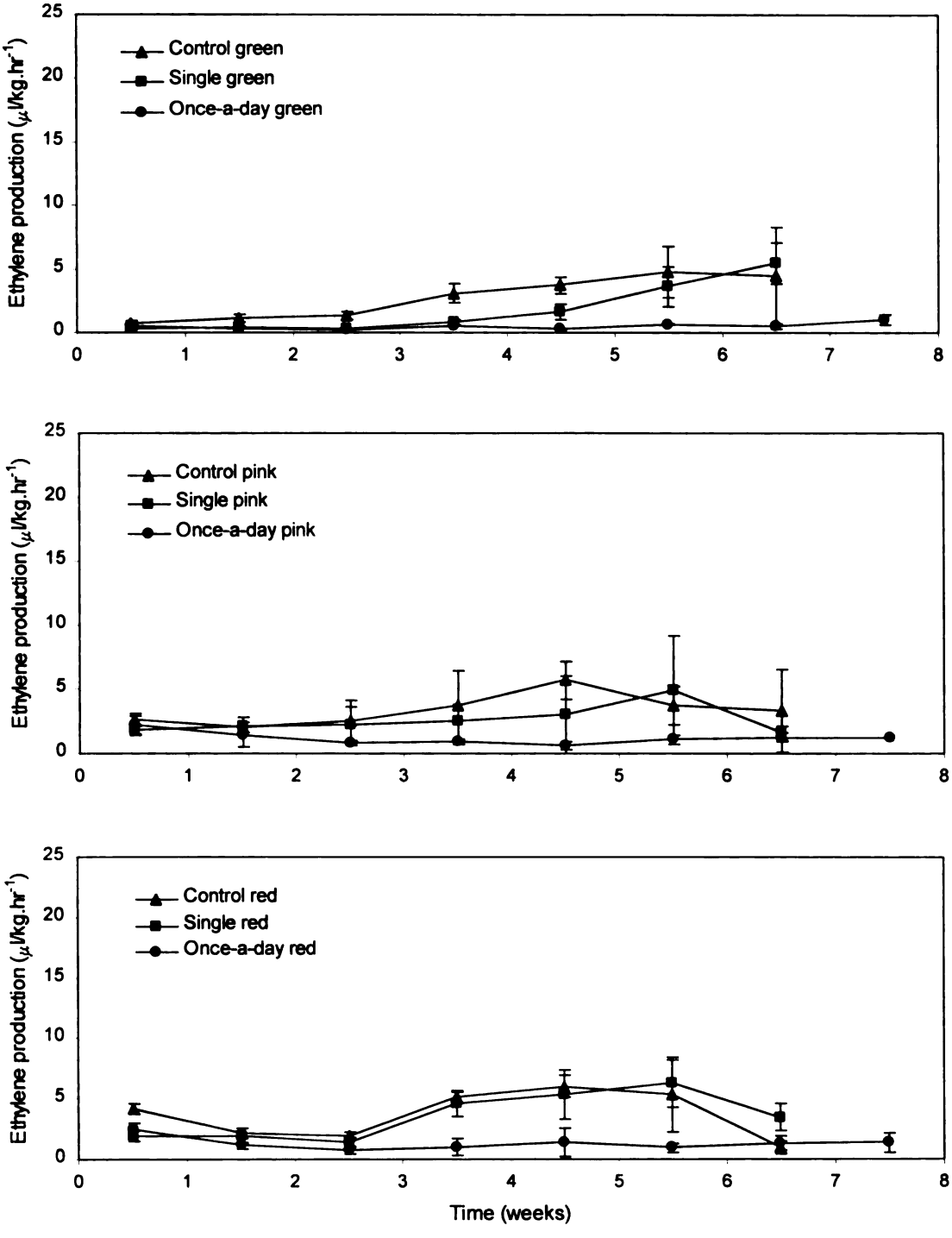


Figure 18. Ethylene production of tomatoes at 10°C. The error bars represent the standard errors of the means (n=3).

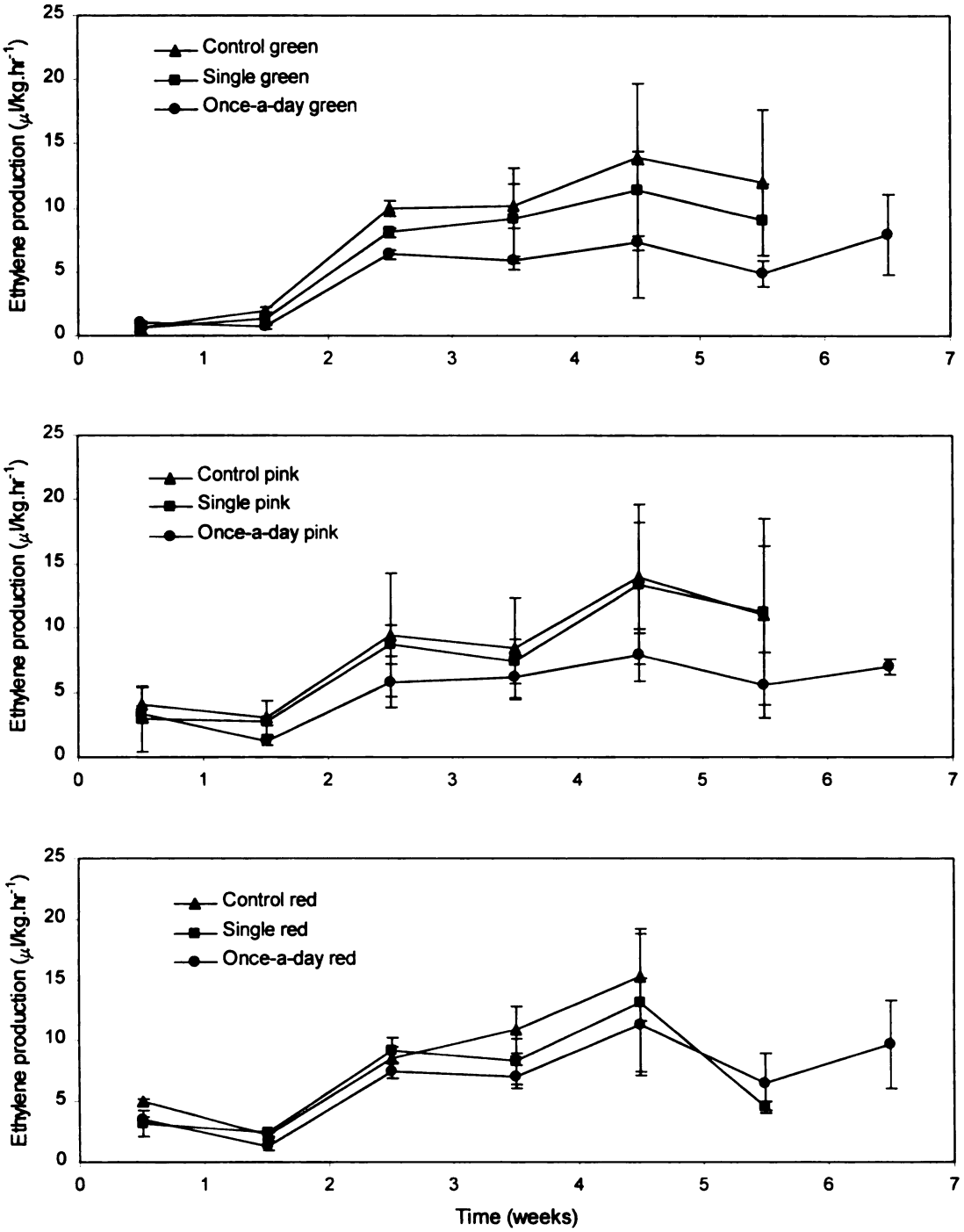


Figure 19. Ethylene production of tomatoes at 15°C. The error bars represent the standard errors of the means (n=3).

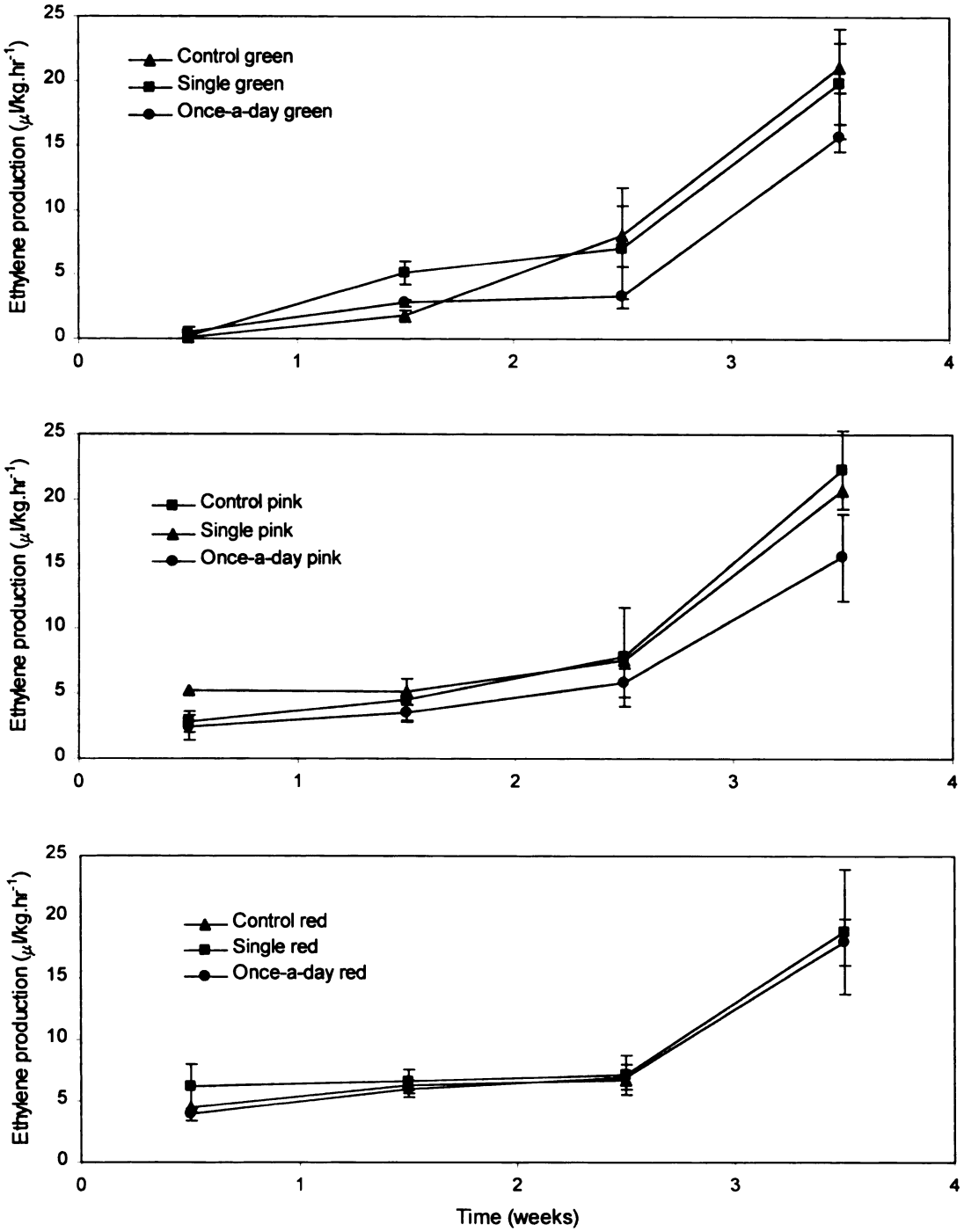


Figure 20. Ethylene production of tomatoes at 20°C. The error bars represent the standard errors of the means (n=3).

CONCLUSION

Treatment with 1-MCP, in addition to low storage temperature, showed good potential for extending the storage and distribution life of postharvest tomatoes. Both single and once-a day 1-MCP treatments proved to be very effective in delaying color change. However, the once-a-day 1-MCP treatment resulted in longer storage times than the single treatment. Long-term exposure provided benefits regarding to slowing color development.

This inhibitor role requires that a continuous 1-MCP treatment be applied over the storage period, which also means that the effects will stop when 1-MCP treatment is discontinued. The information obtained in this work maybe useful in developing a controlled-release packaging system for 1-MCP treatment of tomatoes.

APPEN

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APPENDIX A

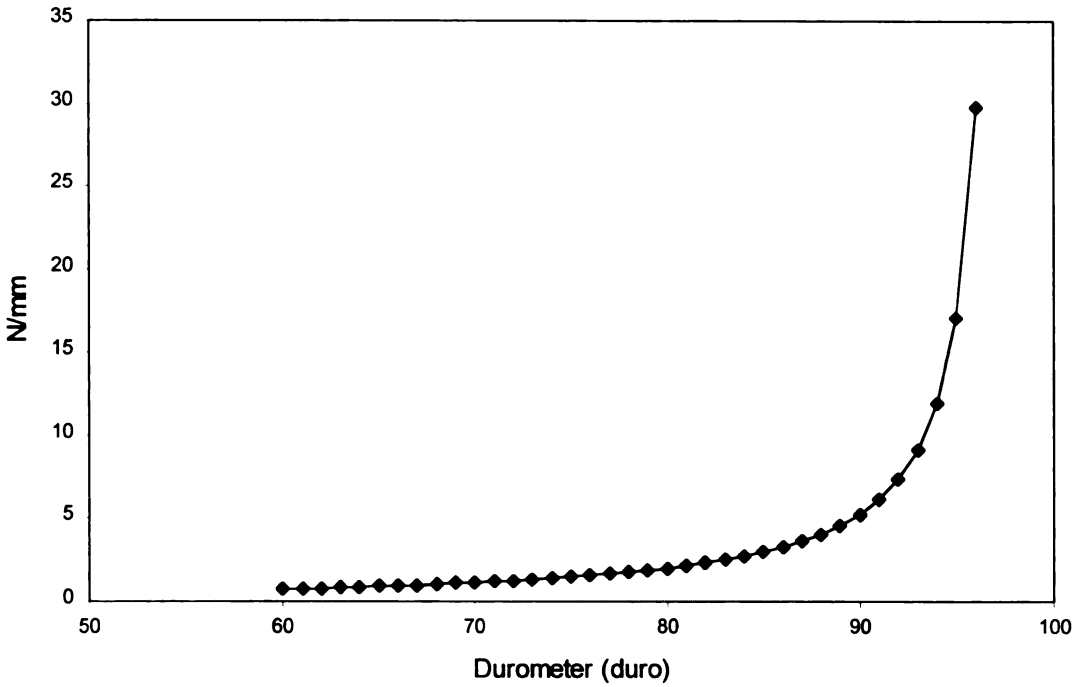

Figure 21. Conversion of the durometer duro unit to the force unit

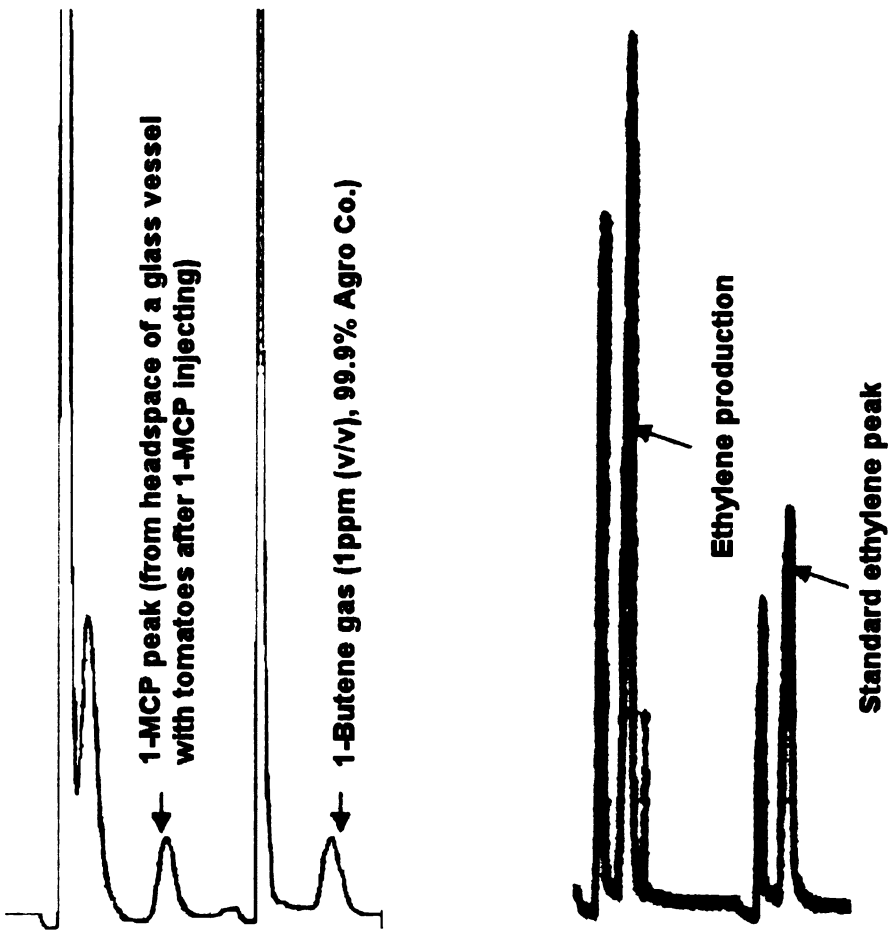

Figure 22. Chromatographic peak shapes of (a) 1-MCP and 1-butene standard, (b) ethylene produced from the tomato and ethylene standard using a Carle gas chromatograph series 100 AGC equipped with a 30 cm × 2.2 mm i.d. Porapak-N stainless steel packed column and a flame ionization detector

SECTION IV: EFFECT OF 1-METHYLCYCLOPROPENE (1-MCP) ON CHLOROPHYLL AND LYCOPENE CONTENTS IN STORED TOMATOES

ABSTRACT

To study the influence of storage temperature and 1-MCP treatment, changes in two major tomato pigments were observed in the pericarp, the locular gel, and the placenta tomato tissues during storage. Storage studies were performed at 10, 15, and 20°C. Harvested tomatoes were treated with 1-MCP once at the beginning of storage or daily during storage to observe inhibitory effects on color development. Total chlorophyll and lycopene contents were obtained by a solvent extraction method using the partial specific extinction coefficient. 1-MCP treated tomatoes had relatively low rates of chlorophyll degradation and lycopene synthesis during storage. Total chlorophyll and lycopene contents in the pericarp tissue of tomatoes were affected more by the 1-MCP treatment than those in the other tomato tissues. Exposure of tomatoes to 1-MCP gas at 10°C, using the once-a-day method was the most effective in delaying pigment changes.

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INTRODUCTION

Fruit color is one of the most important factors in determining market quality of tomato. Change in tomato color is used as an index to represent the ripening process during storage (Ryall, 1979). Chlorophyll degradation and carotenoid synthesis are affected by ethylene (Lelievre, 1997a).

The most significant change in tomato pigments can be represented by the chlorophylls and lycopene, which are the primary pigments responsible for the changes in visual color of the intact fruit (Edwards, 1967). Levels of chlorophylls for green colored tomatoes decreased while the ratio changes of each pigment's level of carotenoids such as lycopene, lutein, β-carotene, ζ-carotene, neurosporene, phytoene, and phytofluene increased during the ripening process. Total chlorophyll content during ripening is in the range of 13.4μg/g tissue in immature green to 0.3μg/g in ripened fruit (Watada, 1976a). Two main carotenoid pigments, β-carotene and lycopene, influence the color changes in tomatoes. Total lycopene content of tomatoes can vary significantly with variety and developmental stage. Edwards (1967) measured 198.6μg/g in *San Marzano* and 12.1μg/g in *Urbana* at the fully ripe stage.

Delay of color change during ripening has been achieved with short term controlled atmospheres with high CO_2 and low O_2 at 22°C (Ratanachinakorn, 1997), treatment with fluorescent-light exposure to diszocyclopentadiene (DACP) at 22°C (Sisler, 1994), treatment with temperatures above 30°C (Mitcham, 1992), and ethanol exposure of the whole tomato fruit at various maturity stages (Saltveit, 1992). Storing the mature green tomatoes at low oxygen atmosphere (3.05kPa, 3% O_2), also delayed

color development when compared to control fruit under atmosphere conditions at 20°C (Kim, 1999).

1-methylcyclopropene (1-MCP) is an inhibitor of the ethylene action and can delay color development in ripening fruits. Porat, (1999) found that "Shamouti" oranges treated with 100 ppb(v/v) of 1-MCP was very effective in inhibiting the ethylene-induced fruit degreening process. Jiang (1999) observed that treatment of bananas with 1-MCP at the levels of 0.01-1.0ppm (v/v) delayed ripening markedly in a polyethylene bags held at 20°C. Golding (1998) found that the application of 1-MCP to mature green bananas significantly delayed onset of peel degreening, which is associated with the ripening process at 20°C.

Therefore, the purpose of this study was to focus on changes in the major tomato pigments during storage after 1-MCP application.

MATERIALS AND METHODS

Materials

'*Plum dandy*' tomatoes grown during the summer of 2001 at the MSU Southwest Michigan Research and Extension Center in Benton Harbor, MI were picked by hand and sorted into two major maturity stages. The stages were defined based on surface color as follows: (1) the mature green stage (green color tomatoes), loss of chlorophyll measured; (2) the pink stage (early ripened tomatoes), formation of lycopene determined.

Total pigment content was based on the levels in the tomato pericarp, gel, and placenta (Figure 2). The pericarp tissue consists of an epidermal layer with a relatively thick cuticle. The gel is a gelatinous tissue containing the seeds. The placenta is composed of parenchyma cell that surround the ovules with the seeds embedded in the gel.

Storage of tomato fruit

Tomato fruits with defect-free appearance were sorted, rinsed with distilled water to remove any dirt, and dried in air. About eighteen tomatoes were separated by color and placed in a closed glass desiccator (10L).

Storage of tomato fruits was carried out using a ventilation system with a constant humidified air flow of 18 ml/min through the inlet and outlet ventilating lines connected a rubber port on the lid. Storage studies were conducted at 10, 15, and 20±1°C.

Preparation of 1-MCP and its treatment

Aliquots of 1-MCP as EthylBloc® powder were weighed and placed in a 500mL Erlenmeyer flask and closed tightly with a rubber stopper. 1-MCP was completely released from the EthylBloc® as a gas after two hours following the addition of 40mL of distilled water. At these conditions, the headspace volume had a concentration of 480μL 1-MCP per L. A 10mL aliquot of the headspace volume was injected through a rubber port on the top cover in the glass desiccator to expose the tomatoes to 1μL 1-MCP per L of air. The concentration of 1-MCP in the sealed desiccator was measured by Gas chromatography.

Tomatoes were treated with 1-MCP gas using either a single or once-a-day application. 1-MCP treatments were prepared as follows. (1) For the single application, 10mL of headspace gas stock, containing 480μL of 1-MCP per L, was injected manually into the glass desiccator containing the tomatoes to provide a concentration of about 1-MCP $1\mu L \cdot L^{-1}$. After exposing the tomatoes to 1-MCP for 10 hours, humidified air was supplied through the desiccator to allow the tomatoes to respire. (2) For the once-a-day application, 10mL of the same gas stock was manually injected daily with humidified air flowing through the desiccators. Control fruit, not exposed to 1-MCP, was stored under a continuous stream of humidified air.

Chlorophyll

(1) Extraction

A harvested green tomato (pre-ripening) was prepared by extracting the pericarp, placenta, and gel tissues. Each part was cut into small pieces. About 10 grams of the sample were transferred to a Coors Mortar (90mm diameter) and ground using a hand pestle for 10 minutes. After grinding, three-gram portions were transferred to a Kimax glass tube (25mm OD × 150mm L). N-N-dimethylformamide (DMF) (5 mL) was added to each sample. The mixture was stirred using a Vortex (Model K550G, Scientific Industries Inc. Springfield, MA) mixer and allowed to stand for approximately 10 minutes. The extraction mixture were filtered through filter paper (Whatman 1, 110 mm Diameter) using a Buchner Funnel. The pulp remaining in the filter paper was discarded after squeezing to remove the solution in the tissues. An extraction solution of 1 mL was placed into a glass cuvette for spectrophotometry analysis. The total chlorophyll content of the extracted sample was measured using an UV-Visible Spectrophotometer (Model U-300, Hitachi Ltd.). A flow diagram to determine the total chlorophyll in the tomato fruits is shown in Figure 23.

(2) Identification

The chlorophyll content in N-N-dimethylformamide (DMF) solvent was analyzed and calculated as described by Moran (1982). Its specific extinction coefficient (SEC) was based according to the following equation:

$$A_\lambda = \varepsilon_\lambda c l \qquad (30)$$

where, A_λ is the absorbance (OD units) at a given wavelength, ε_λ is the SEC of the solution at wavelength λ, c is the concentration ($g \cdot l^{-1}$) and l is the beam-path (1cm) in the measuring cuvette.

From the absorbance, the total chlorophyll content in DMF was calculated by substitution into the following equation:

$$\text{Chlorophyll total } (\mu g/ml) = 7.04A_{664} + 20.27A_{647} \qquad (31)$$

where total chlorophyll concentration is expressed in μg/mL.

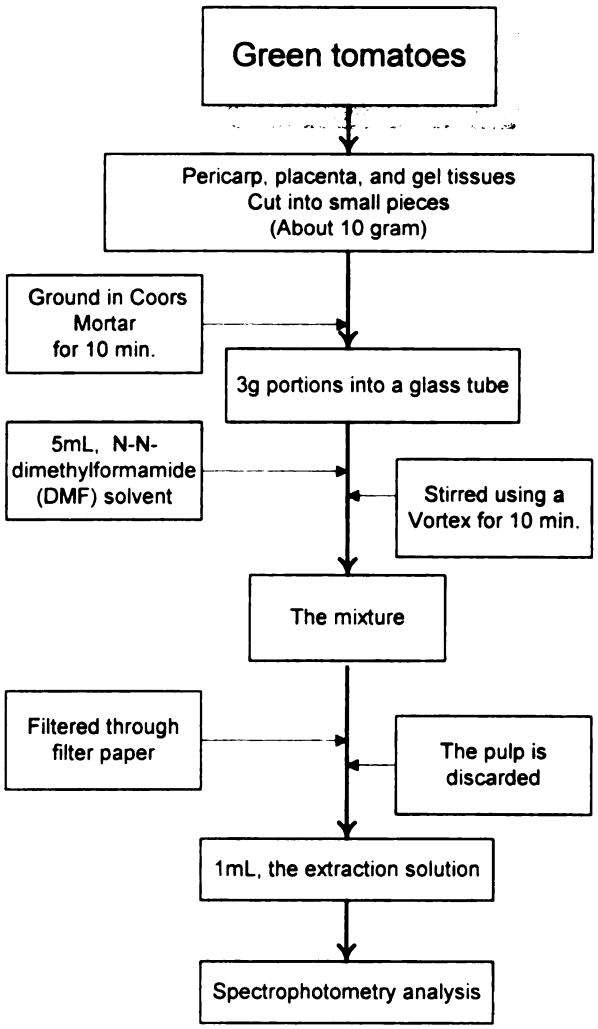


Figure 23. Flow diagram of total chlorophyll extraction in tomatoes

Lycopene

(1) Extraction

Tomatoes were separated into pericarp, and combined placenta and core tissues. Each tissue sample was cut into small pieces. About 10 grams of sample were transferred to a Coors Mortar (90mm diameter) and then ground using a hand Pestle for 10 minutes. After grinding, six gram portions were transferred to a Kimax glass tube (25mm OD × 150mm L). 10 mL of methyl alcohol was added to each sample. The mixture was stirred using a Vortex (Model K550G, Scientific Industries Inc. Springfield, Mass) mixer and allowed to stand for approximately 10 minutes. An acetone-hexane solution (1:1), 10 mL, was added to the mixture. The mixture was stirred again using the Vortex for 1 minute and then filtered through filter paper (Whatman 1, 110mm Diameter) using a Buchner Funnel into a 100 mL volumetric flask. The pulp remaining in the filter paper was washed with 5 mL of hexane. The washed pulp was discarded after squeezing. The extracts were combined and placed in a 125 mL separatory funnel. 100mL of distilled water was added and the mixture shaken gently for 1 minute to remove the acetone and the methanol-water soluble substances. The hexane extract (top phase in a separatory funnel) was collected in a glass tube (Figure 24).

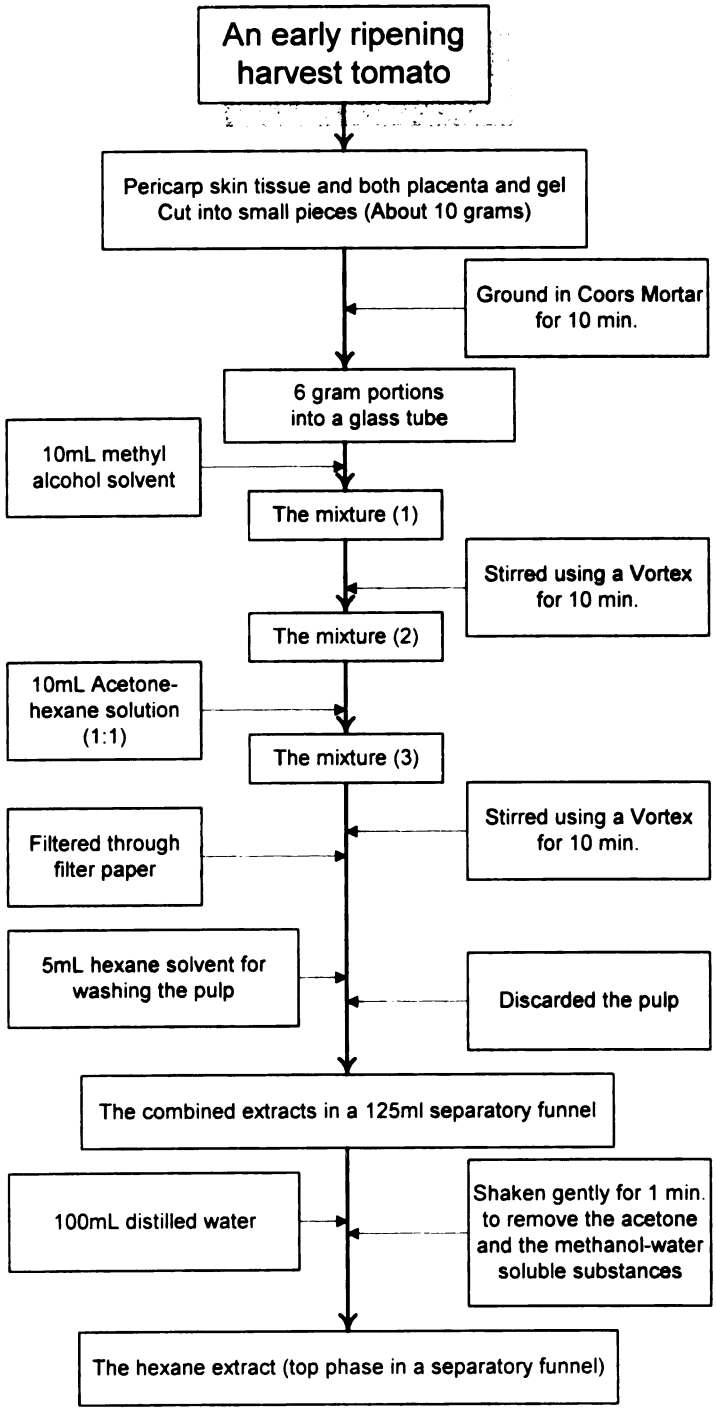


Figure 24. Flow diagram of lycopene extraction in tomatoes

(2) Saponification

The hexane extract was subjected to a saponification procedure to remove unwanted lipids and chlorophylls (Figure 25). The hexane extract was stirred using a Vortex mixer for 1 minute with one-eighth volume methyl alcohol saturated with potassium hydroxide. After 30 minutes, the hypophase was removed using a 5 mL glass pipette. 10 mL of methyl alcohol was added to wash the hexane epiphase. The extract was then washed with 50 mL of distilled water to remove the methyl alcohol using a separatory funnel. The hexane extract (top phase in a separatory funnel) was filtered through anhydrous sodium sulfate using filter paper and Buchner Funnels. The hexane extract was stored at -20°C, prior to separation.

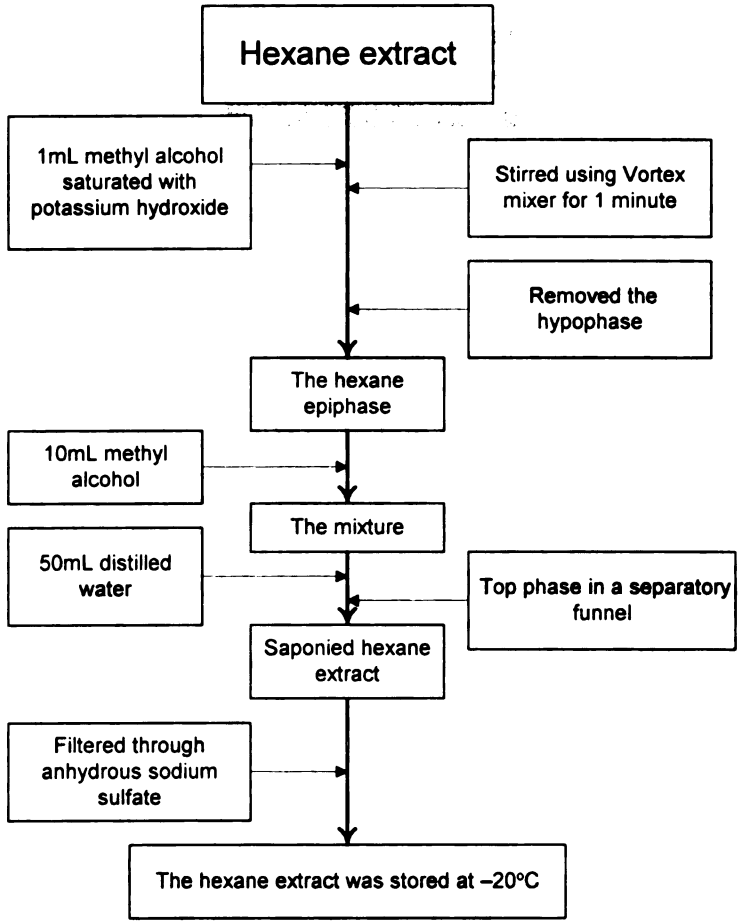


Figure 25. Flow diagram of saponification of the extract samples

(3) Separation

The lycopene hexane extract was chromatographed on glass columns (2cm OD × 13cm L) packed with an adsorbent combination of Hyflo Super Cel and MgO magnesia (1:1 by weight) mixture. Figure 27 shows a flow diagram of the lycopene separation procedure. A 0.5 cm thick layer of anhydrous sodium sulfate was placed above the absorbent. A 1 cm thick layer of glass fiber was placed on the bottom of the absorbent. The inlet port of the packed column was connected to a 125 mL pyrex separatory funnel. The outlet port of the packed column was equipped with a valve to control the flow of the solution. It was also connected to a 125 mL pyrex filter flask to collect the solution through the column. A continuous vacuum was generated using a tap water pump aspirator to filter the extract. A schematic diagram of the lycopene separation system is shown in Figure 26. The absorbent in the glass column was conditioned with 50 mL of hexane under vacuum. After conditioning, the hexane extract was introduced into a 125 mL separatory funnel connected to the inlet port of the column.
50 mL of hexane was then added to the same separatory funnel. The hexane extract was eluted using 50 mL of hexane.

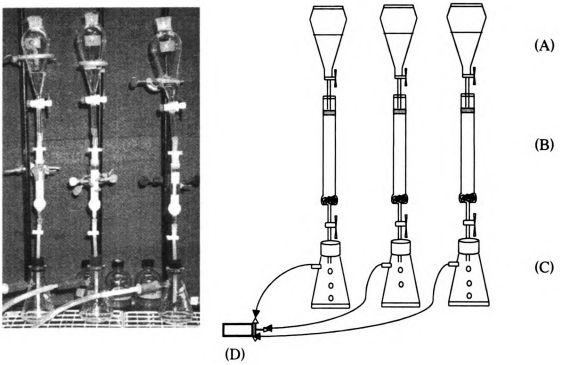


Figure 26. A schematic diagram of the lycopene separation system. (A) sample and solvent supply system (125mL) (B) glass columns (2cm OD × 13cm L) packed with adsorbent mixture of Hyflo Super Cel and MgO magnesia (1:1 by weight) (C) collection flasks (125mL) (D) vacuum source

The small band of yellow color was slowly separated from the main pink band at the top of the column. The column was then washed using 40 mL of a 3% acetone solution in hexane, causing the small yellow band (carotenes) to separate and move down the column ahead of the pink lycopene band that was slowly diffusing downward. The eluted material containing the yellow band was discarded. The lycopene was washed from the column with a solution of 50 mL of 10% acetone and 5% methanol in hexane. The eluted lycopene solution was transferred to a separatory funnel, and washed with 50

mL of distilled water to remove the acetone and methyl alcohol. The hexane extract was then filtered through anhydrous sodium sulfate on a fritted funnel to remove water, and made up to a volume of 50 mL with hexane. The concentration of lycopene in the hexane solution was determined using a UV-Visible Spectrophotometer (Model U-300, Hitachi Ltd.).

(4) Identification

The total lycopene content in the hexane solution was quantified using a Hitachi Model U-300 spectrophotometer. The absorbance of the lycopene was measured at a wavelength of 472.5nm. The purified lycopene was identified by comparison to the standard spectral lycopene (Britton, 1983).

The total lycopene content of the tomato fruit was calculated by substitution into the following equation:

$$\mu g \text{ lycopene/gram of tomato} = \frac{A_\lambda \times V_{total} \times 10^6}{E^{1\%}_{1cm} \times 100 \times W_t} \qquad (32)$$

where, A_λ is the measured absorbance, V_{total} is the total sample volume (mL), W_t is the tomato sample weight (g), $E^{1\%}_{1cm}$, specific extinction coefficient (SEC) of a lycopene is the extinction at a given wavelength and in a stated solvent of a 1% solution (i.e. 1 g lycopene/100mL solution) in a 1cm light-path sepectrophotometer cuvette. $E^{1\%}_{1cm}$ of lycopene is 3450 at λ=472.5 nm at hexane solvent (Davies, 1965)

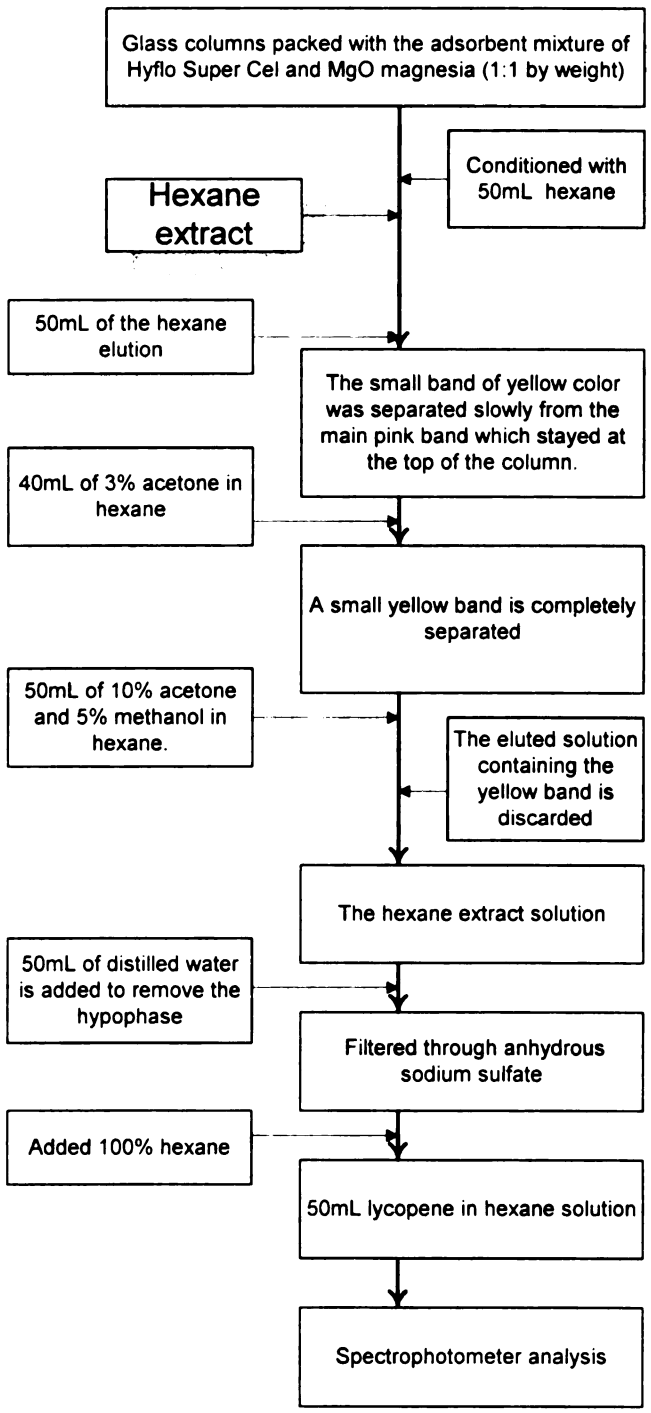


Figure 27. Flow diagram of the method used to determine the lycopene content in tomatoes

RESULTS AND DISCUSSION

Chlorophyll content

Change in the total chlorophyll content in the tomatoes at the respective temperatures was monitored over a period of 25 days (Figures 28, 29, and 30). The initial chlorophyll content in mature green tomatoes was 4.63, 3.81, and 4.18 μg/g in the pericarp, the gel, and the placenta tissue. The average chlorophyll content was 13.4μg/g in 'Walter' tomatoes (Watada, 1976a) and 13.8μg/g in 'San Marzona' tomatoes (Edwards, 1967) at the first green fruit maturity level. This would result in the level of total chlorophyll observed at the initial stage of mature green tomatoes being lower than the level of total chlorophyll based on the previous studies. Possible explanations for the lower level of observed total chlorophyll include the different tomato varieties and harvested maturity conditions, and the application of the several chlorophyll extraction analysis techniques.

Most of the chlorophyll was lost after the breaker stage. Sink (1974) determined the chlorophyll content of normal (a fourth generation of *Fireball* × *Cornell*) tomato fruits and *rin* fruits 30 days after pollination. Total chlorophyll content of the normal and *rin* fruits were 9.43μg/g and 7.38μg/g, respectively. The *rin* mutant fruits contained the lower amount of chlorophyll a and the highest amount of chlorophyll b, causing the lower a:b ratio (1.65) when compared to normal fruits.

Storage at 10°C delayed pigment development, when compared to the higher temperatures. Chlorophyll content in the green tomatoes at 20 °C decreased rapidly for 6 days and was not detected at 14 days. The tomatoes stored at 10 °C showed that the total

chlorophyll content decreased for 21 days, which means that the ripening process was delayed by the low temperature.

Sozzi (1999) observed the chlorophyll content of the controls decreased from 20μg/g at 0 day to 2μg/g at 6 day at 20°C. Tomatoes stored in air showed an immediate decline in chlorophyll and a steady increase in total carotenoids and lycopene contents. Wu (1972) also reported the chlorophyll content in control tomatoes under normal atmospheric conditions at 13°C decreased from 50μg/g to less than 5μg/g after 15 days.

Total chlorophyll content in the pericarp tissue of the green tomatoes with once-a-day 1-MCP treatment was shown to decrease slowly and remained similar to that at the 'green' color stage for 25 days, as compared to the controls without 1-MCP treatment, and the green tomatoes with a single 1-MCP treatment (Figure 28). The total chlorophyll content in the pericarp tissue of the green tomatoes at 20 °C was 2.71μg/g at 14 days using a once-a-day 1-MCP treatment, compared to the controls where chlorophyll was not detected at 14 days at 20°C.

The total chlorophyll content in the pericarp tissue of the green tomatoes was 0.81μg/g at 14 days at 10°C with no 1-MCP treatment. Total chlorophyll content in the pericarp tissue at 10°C using a once-a-day 1-MCP treatment was 2.32μg/g at 25 days. Thus, chlorophyll loss from tomatoes treated with once-a-day 1-MCP, and stored at 10°C, was slower than single 1-MCP treated application or storage temperature, or combination of both. Total chlorophyll content of the green tomatoes was 4.63, 3.81, and 4.18μg/g in the pericarp, gel, and placenta tissues at day 0. The total chlorophyll content in the pericarp, gel, and placenta tissues of green tomatoes with no 1-MCP treatment at 20°C rapidly decreased to 1.64, 0.72, and 1.01μg/g at 3 days (Figure 30).

Total chlorophyll content in the pericarp tissues was relatively higher than those in the other tomato tissues during the storage period. The internal gel and placenta tissues of green tomatoes have higher chlorophyll degradation rates than the pericarp tissue. 1-MCP treatment had a greater influence on color development of the pericarp tissue. Internal gel tissue had slightly lower chlorophyll content than the pericarp and the placenta.

Combination of 1-MCP treatment with low storage temperature showed very effective in delaying chlorophyll loss as well as the treatment with controlled atmosphere storage. In controlled-atmosphere storage with low oxygen or elevated carbon dioxide levels (Sozzi, 1999), tomatoes stored in 20% CO_2 was more effective in delaying chlorophyll degradation (12μg/g at 6 day) than the fruit continuously stored in air. Wu (1972) observed the total chlorophyll content of 'green wrap' tomato fruits as a function of storage at 13°C, after three sub-atmosphere pressure treatments. The loss of chlorophyll in the tomato fruits was delayed by storage at sub-atmospheric pressure. Tomato fruit treated at a sub-atmosphere pressure of 102mmHg did not ripen, and had a chlorophyll content of 40μg/g at 100 days storage.

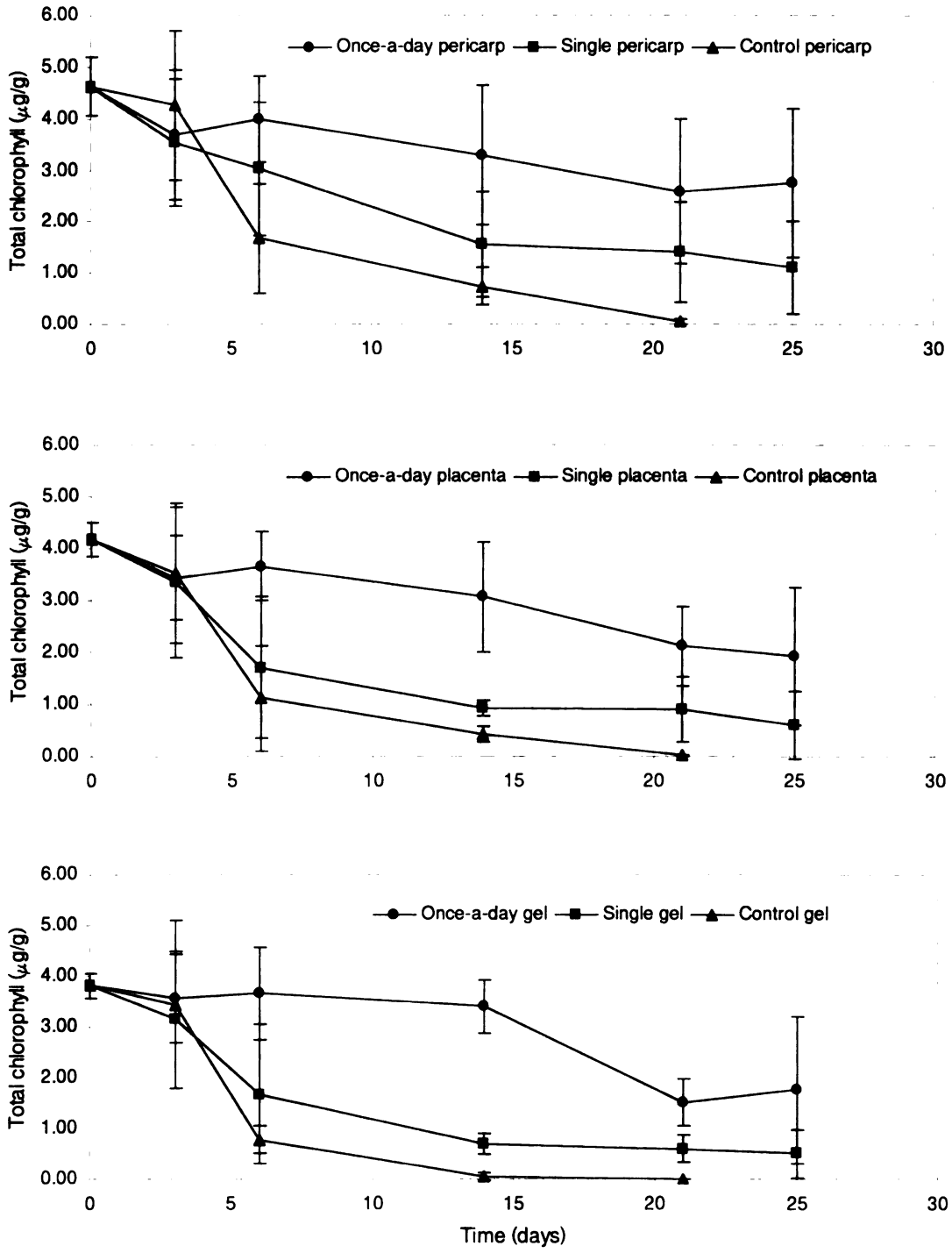


Figure 28. Changes in total chlorophyll content of green tomatoes in storage at 10°C

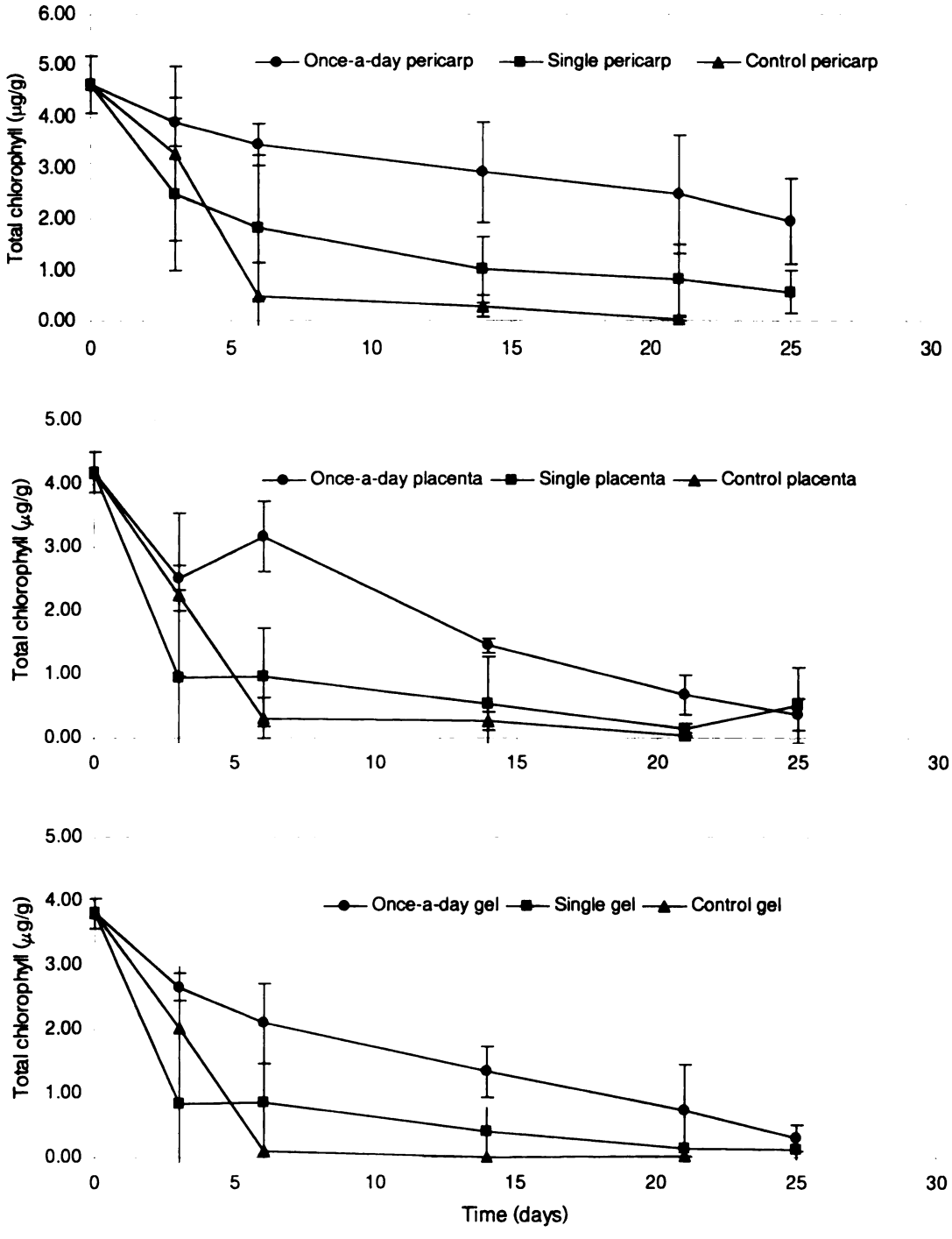


Figure 29. Changes in total chlorophyll content of green tomatoes in storage at 15°C

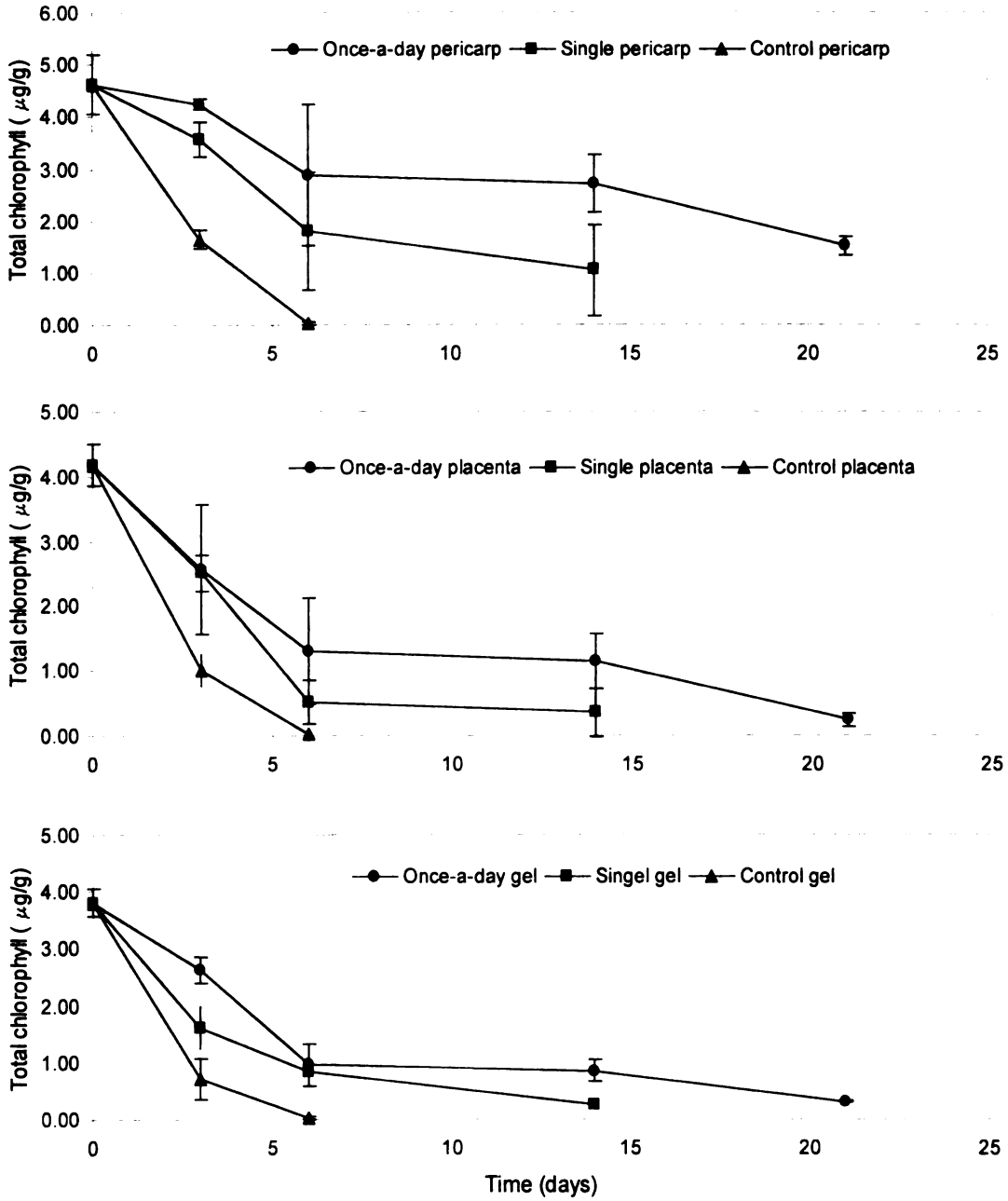


Figure 30. Changes in total chlorophyll content of green tomatoes in storage at 20°C

Lycopene content

Two main carotenoids were separated from the hexane extract and identified by spectrophotometric analysis (Figure 31). The spectral curve of the purified tomato lycopene showed maximum absorption at 470 nm, which is a major peak strongly indicating predominance of the lycopene (red color), with two minor peaks at 440 and 500 nm. The spectral for lycopene is similar to that published by Britton (1983).

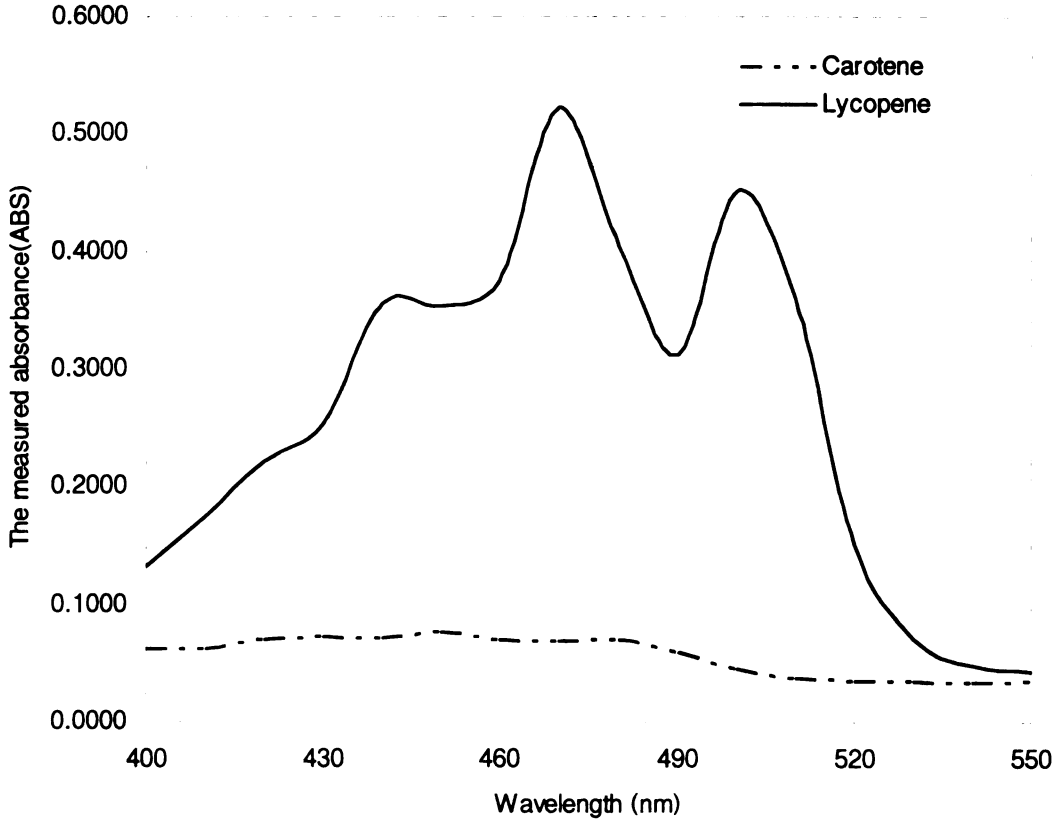


Figure 31. A spectrophotometeric profile of the purified lycopene extracts

Changes in lycopene content in 1-MCP treated and non-treated tomatoes was observed during storage at 10, 15, and 20°C (Figures 32, 33, and 34). The initial lycopene content in ripening tomatoes was 4.25 and 4.30μg/g in the pericarp and the internal tissues.

Observed lycopene level in tomatoes was within the range reported by the lycopene pigment content of the full ripened tomato varied between 12.1μg/g in 'Urbara' tomatoes and 198.6μg/g in 'San Marzano' tomatoes (Edwards, 1967). The lycopene in the 'San Marzano' tomatoes changed from 0μg/g in the fully green tomatoes to 180.6μg/g in the fully ripened tomatoes (Edwards, 1967).

The lycopene content in the pericarp tissue rapidly increased during 22 days of ripening, and reached 23.28μg/g in the fully ripened fruit at 20°C, with no 1-MCP treatment. The lycopene content at 22 days in the pericarp and internal tissues at 20°C, with 1-MCP treatment once a day, was 5.93μg/g and 12.39μg/g, compared to the controls with values of 23.28μg/g and 22.30μg/g. This difference is attributed to the 1-MCP treatment, which inhibited lycopene synthesis. In addition, the lycopene concentration in the pericarp tissue of 1-MCP treated tomatoes was lower than that in the internal tissue. It can be assumed that the pericarp tissue was affected more by the 1-MCP treatment due to direct exposure of 1-MCP gas at the fruit surface.

The pericarp and internal tissues also showed a slow increase in the lycopene content at 10°C, and had values of 9.21μg/g and 11.57μg/g at 29 days with no 1-MCP treatment. The lycopene content of pericarp and internal tissues on pink stage tomatoes at 10°C, under once-a-day 1-MCP treatment, was 5.74μg/g and 7.04μg/g at 29 days, respectively.

The relation between lycopene content (%) and chlorophyll content (%) was also determined for a single and once-a-day treatment of 1-MCP (Figure 35). Once-a-day 1-MCP treatment at 20°C showed the loss of chlorophyll much faster than the formation of lycopene when compared to the control fruit. In contrast, a single 1-MCP treatment did show the same pattern as the results of the control fruit. The relation between lycopene and chlorophyll at 10°C represented no significant difference for 1-MCP treatments.

1-MCP applied as a single exposure or multiple tissues plays an important role as an ethylene inhibitor, which delays tomato color change in storage. Lycopene synthesis in the tomatoes at 10°C was slower than at 20°C. Based on the results, low temperature storage may significantly affect the formation of lycopene and the loss of chlorophyll. It is clear that the lower the storage temperature, the longer it takes for lycopene synthesis, with the exception being storage at chill injury conditions. These results were similar to that of studies by Mencarelli (1988) using tomato fruit slices under storage at low temperature and under controlled atmospheres (CA) to slow the rate of lycopene synthesis.

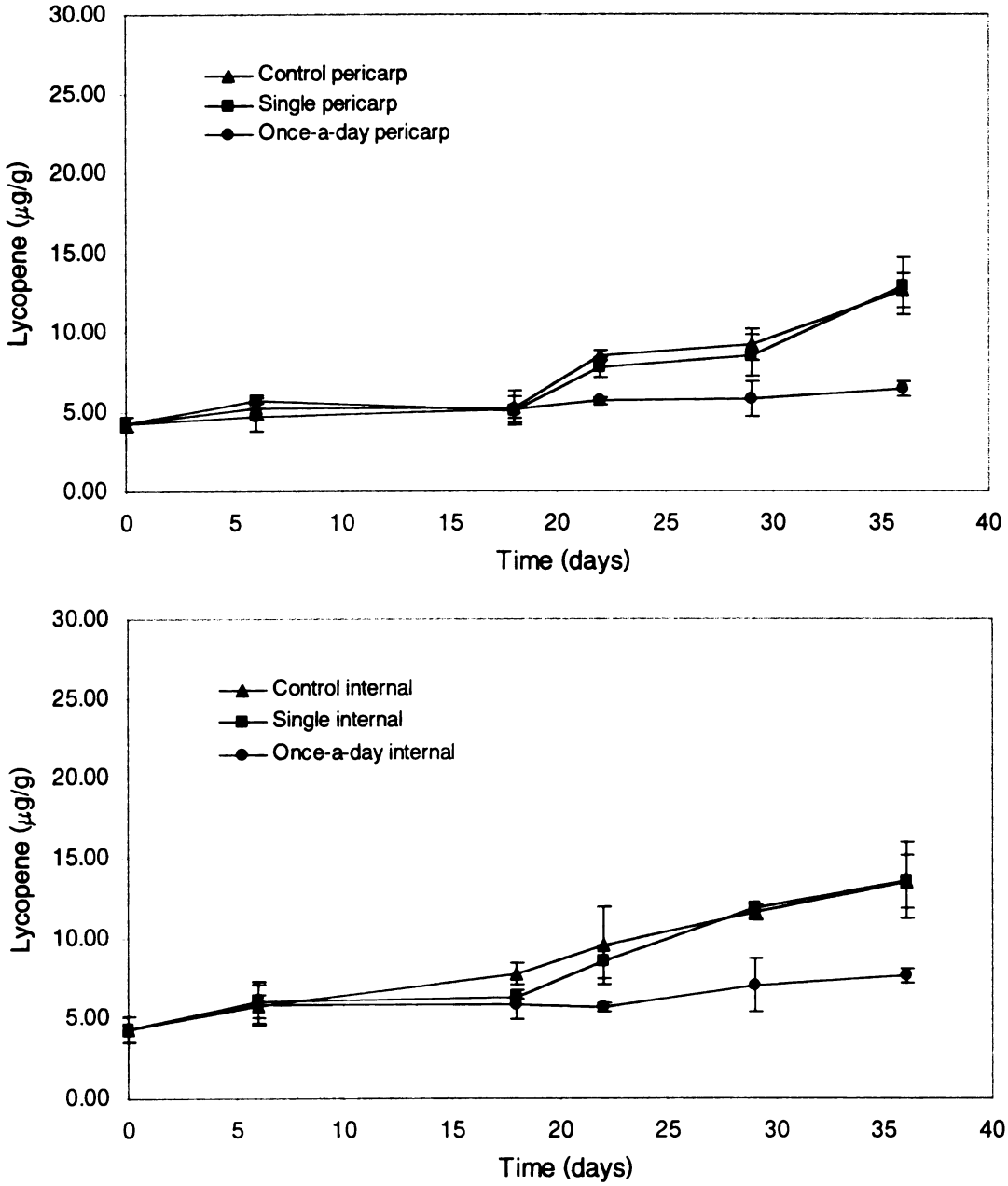


Figure 32. Formation of lycopene in pink tomatoes in storage at 10°C

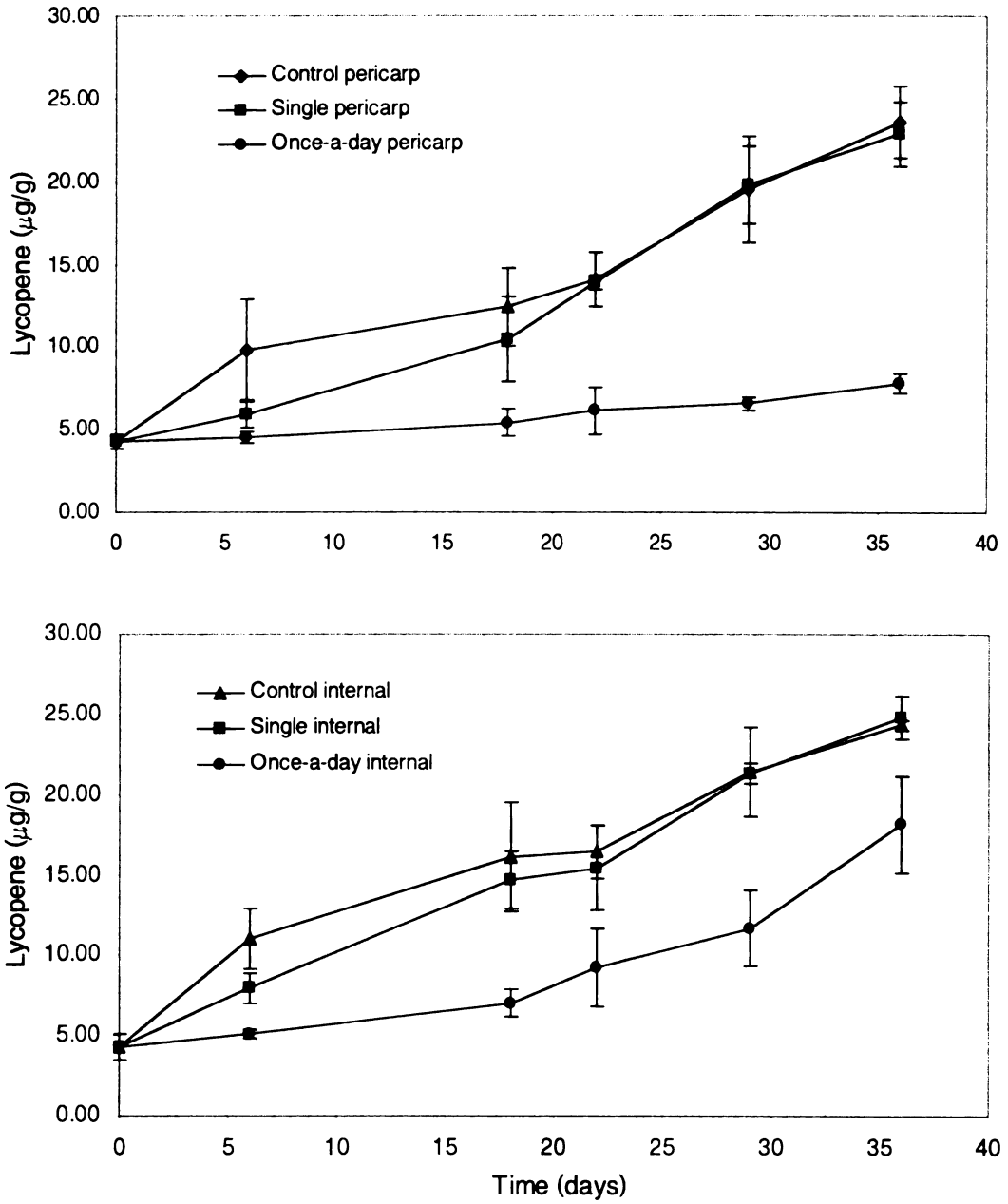


Figure 33. Formation of lycopene in pink tomatoes in storage at 15°C

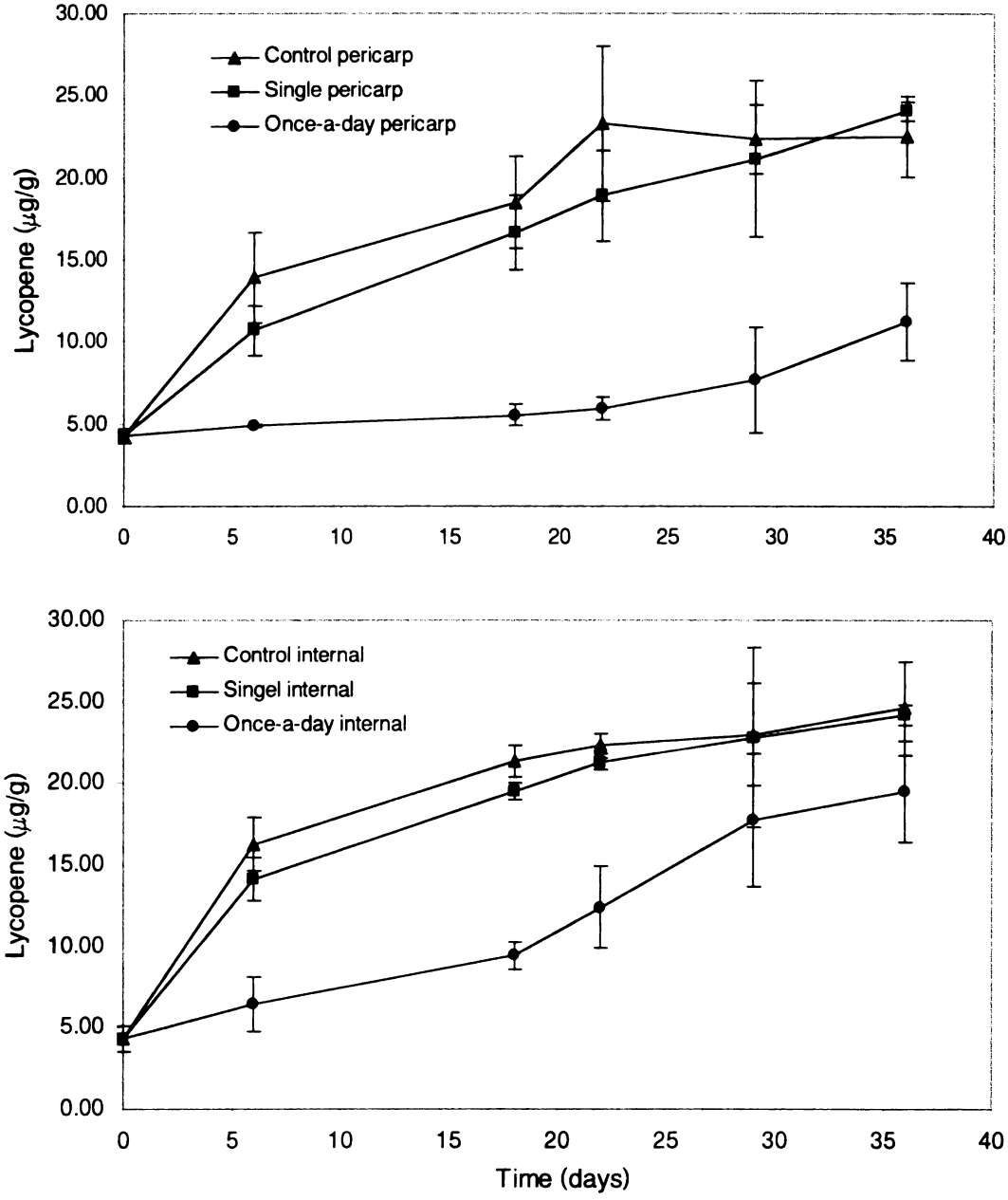


Figure 34. Formation of lycopene in pink tomatoes in storage at 20°C

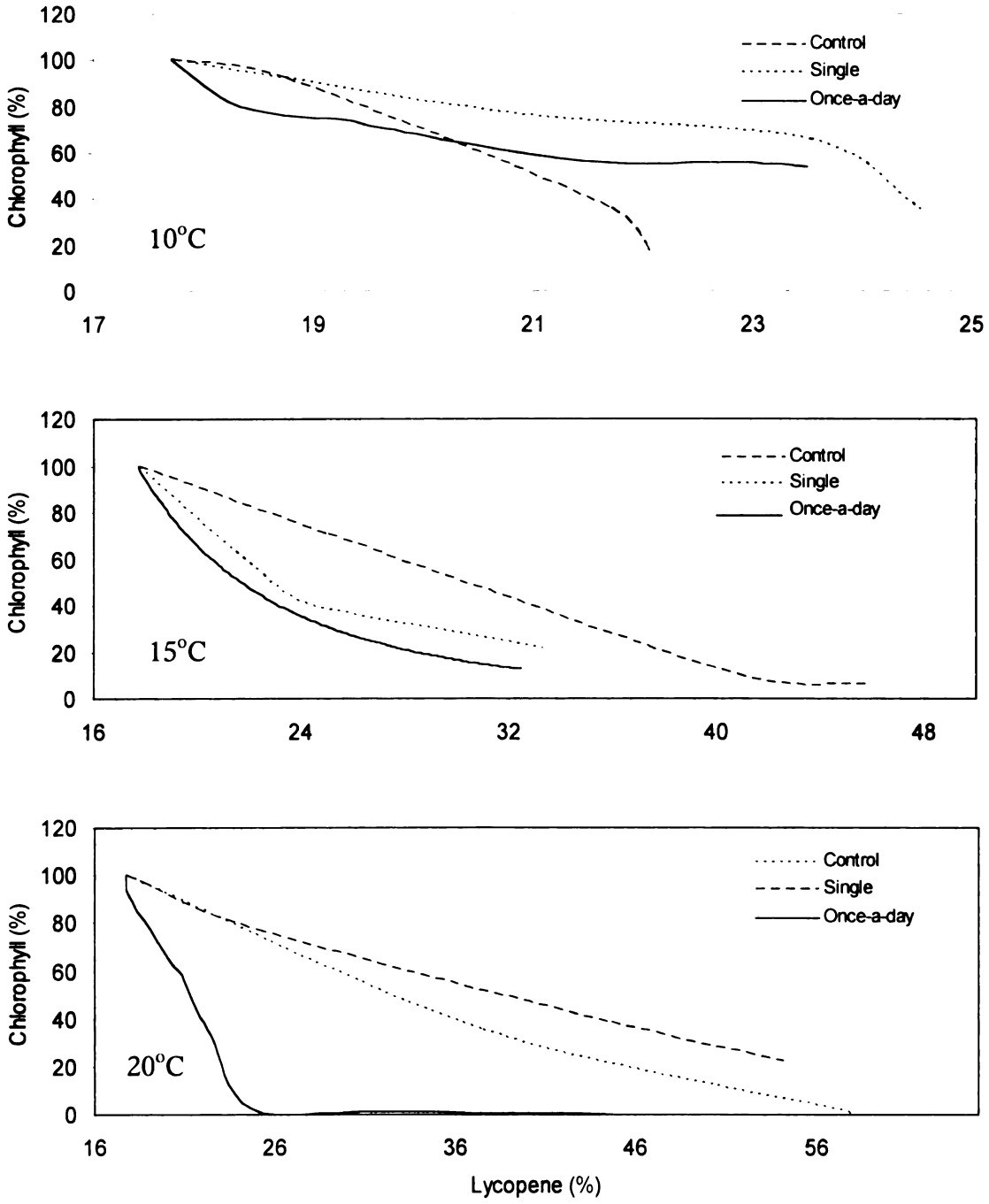


Figure 35. Relation between lycopene and chlorophyll in the pericarp tissue for the various treatments.

CONCLUSION

1-MCP delayed degradation of chlorophyll and production of lycopene. Combination of 1-MCP treatment with low storage temperature was very effective in delaying color changes in tomatoes compared to 1-MCP treatments or a low storage temperature. Low storage temperature slows ripening of tomatoes. 1-MCP also directly affects the ethylene inhibitor sites related to pigment changes in tomatoes.

APPENDIX B

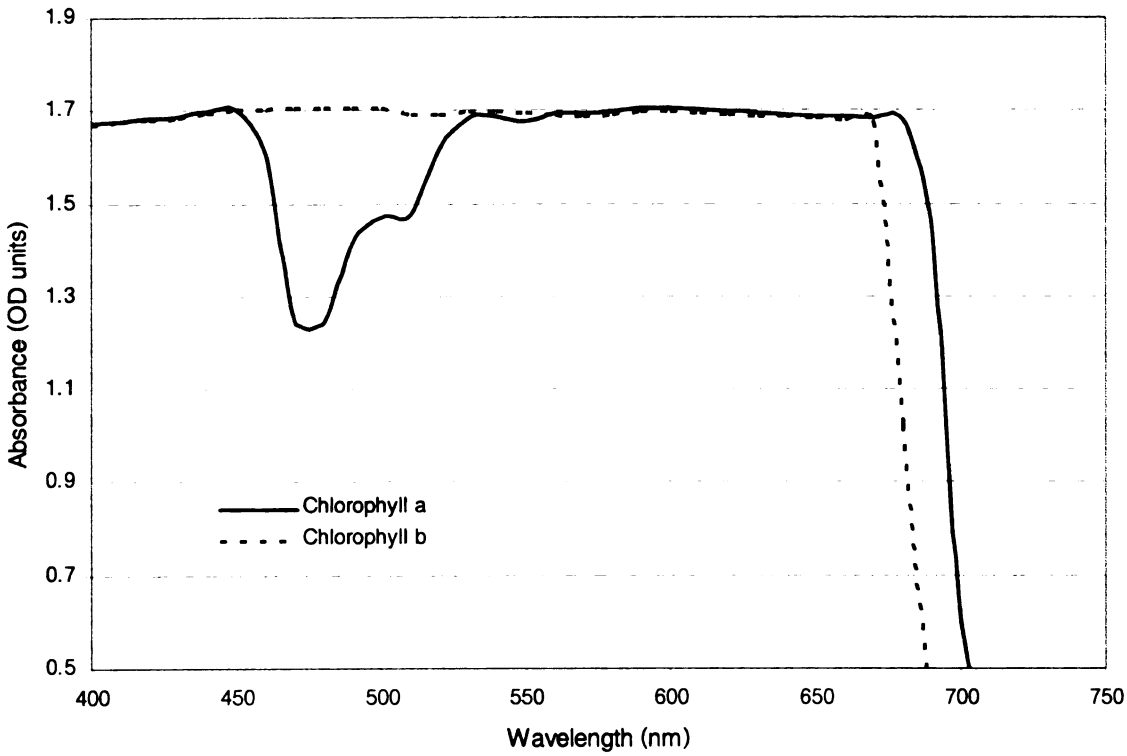


Figure 36. A spectrophotometeric profile of the standard chlorophylls *a* and *b* from a spinach source (Sigma Chemical Co., St. Louis, MO).

SECTION V: SORPTION STUDY OF 1-METHYLCYCLOPROPENE (1-MCP) BY SELECTED ADSORBING AGENTS USING INVERSE GAS CHROMATOGRAPHY

ABSTRACT

Sorption isotherms of 1-MCP in silica gel, Tenax-TA, and activated clay were determined using inverse gas chromatography at 50, 60, 70, and 80°C. The sorption isotherms, which were developed at very low sorbate concentration, followed Henry's law, and behaved according to binding site theory. Specific retention volumes for 1-MCP on the adsorbing agents were calculated. Silica gel had a much higher number of binding sites, compared to Tenax-TA and activated clay agents. Thermodynamic parameters, namely free energy, entropy, and enthalpy were also obtained as a function of temperature for all adsorbing agents. Silica gel and 1-MCP had the lowest Gibbs free energy, whereas the enthalpy value of sorption for Tenax-TA had a greater binding energy with a smaller number of binding sites. The entropy values for sorption for the 1-MCP/adsorbing agents were not affected by the 1-MCP concentrations and temperature.

INTRODUCTION

During postharvest storage, ethylene induces physiological changes in plants including, accelerating abscission, ripening of fruits, leaf abscission, and fading of flowers (Serek, 1995b). Managing the ethylene concentration is important to control the quality of produce during postharvest storage. To limit or remove ethylene, absorbing agents have been suggested during postharvest storage (Shorter, 1992). Most commercial applications that remove ethylene from the package headspace require adsorbing agents either in sachets or integrated into the packaging materials (Zagory, 1995). Instead of removing ethylene by absorption, 1-MCP offers a novel alternative, since it can inhibit the ethylene action. Therefore, by properly delivering 1-MCP into the package, it is possible to prevent or retard undesirable postharvest ethylene effects. A system that can deliver small quantities of 1-MCP vapor into the headspace of a package containing fresh produce is desirable. The aim of this work was to study the sorption behavior of 1-MCP by adsorbing agents. The sorption properties of 1-MCP adsorbing agents can be determined using inverse gas chromatography (IGC). Inverse gas chromatography is a powerful tool that can rapidly and efficiently describe sorption behaviors (Kontominas, 1994). Determination of the thermodynamic parameters by IGC results in a complete description of the sorption/desorption process, which can be useful in a 1-MCP delivery system.

The objectives of this study were: (a) to determine sorption isotherms for 1-MCP in silica gel, Tenax-TA, and an activated clay at several temperatures; (b) to evaluate the

specific retention volume of 1-MCP for each absorbing agent; and (c) to obtain the thermodynamic parameters associated with the sorption processes.

MATERIALS AND METHODS

Materials

1-methylcyclopropene (1-MCP) was obtained from Floralife, Inc., (Walterboro, SC) as EthylBloc® powder containing 0.14% active ingredient. 1-Butene (99.9% pure) was purchased from AGA Specialty Gas Inc., (Maumee, OH) for quantitative analysis of 1-MCP. Silica gel, grade 634-60A, 100-200 mesh was obtained from Aldrich Chemical Co. (Milwaukee, WI), while Tenax-TA, 60-80 mesh was purchased from Alltech Associates, Inc., (Deerfield, IL). Activated clay, a modified montmorillonite mineral (I-28E) was obtained from Nanocor, Inc., (Arlington Heights, IL). Activated carbon, 20-40 granular mesh was purchased from Aldrich Chemical Co. (Milwaukee, WI). Activated carbon, 80-100 granular mesh was purchased from Alltech Associates, Inc., (Deerfield, IL). α-cyclodextrin as a molecular encapsulation agent was purchased from Cerestar Co., (Hammond, IN).

Preparation of chromatographic column

In the IGC technique, the solid phase is packed inside the chromatographic column. Thus, the adsorbing agents used were packed in 300mm × 6.4mm O.D. stainless steel chromatographic columns and secured with glass fiber stoppers placed at both ends of the column. The amount of adsorbing agent used was determined by weighing the difference in the column before and after packing. Characteristics of the adsorbing agents and weights used as molecular encapsulation agents are shown in Table

7. The packed columns were pre-conditioned at 150°C under a stream of Helium gas for 24 hours before the chromatographic studies were conducted. Figure 37 shows the packing materials, and the stainless steel columns used for the stationary IGC phase.

Table 7. Parameters of the selected adsorbing agents used as the stationary phase of the IGC system

Agents	Characteristic	Weight of adsorbing agent in the column Grams	1-MCP retention time at 80°C min
Silica gel	100-200 mesh, 60A, white color, granular	3.60	13.28
Tenax-TA	60-80 mesh, grayish white color, granular	1.93	3.71
Activated clay	I-28E, Powder, brownish-white color	1.68	1.33
Activated carbon*	20-40mesh, black color, granular	1.01, 0.74, 0.57, 0.21	N/A
α-cyclodextrin*	white color, Powder	1.74, 0.45	N/A

*N/A = no peak was observed from the GC integrator after 4 hrs.

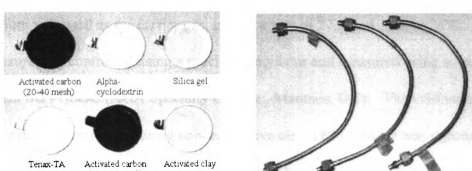


Figure 37. The packing materials and the stainless steel columns used for the stationary phase

Preparation of 1-MCP gas and standard calibration

Aliquots of 1-MCP as EthylBloc® powder were weighed and placed in a 250 mL glass serum vial sealed with aluminum top-hole caps and PTFE/Silicone 20 mm septa (Supelco Inc, Bellefonte, PA). 1-MCP was released from the EthylBloc® as gas by addition of 60 mL of distilled water. Concentration of 1-MCP was measured by sampling 200 μL of the headspace volume using a gas tight microsyringe (Hamilton Co., Reno, NV) relative to a certified pure 1-butene gas. The standard calibration solutions were prepared using a serial dilution procedure using 0.05, 0.10, 0.15, and 0.20 μg concentrations of a certified pure 1-butene gas.

Inverse gas chromatography system

A Hewlett-Parkard gas chromatograph Model 5830A (Avondale, PA) equipped with a flame ionization detector, was used for IGC measurements. Once the column was packed with a particular adsorbing agent, it was installed in the gas chromatograph (GC). Helium was used as the carrier gas, at a flow rate of 27.27 mL/min at 24°C. The inlet pressure was controlled using a precision regulator and measured using a pressure gauge, model SGVGSGC (AGA Specialty Gas Inc., Maumee, OH). The void volume of the column was determined using non-interactive air. 100 μL of air was injected into the column using a gas-tight syringe. This low volume of gas approached infinite dilution concentration inside the column. The operation conditions of the gas chromatograph were set as follows: oven temperatures, 50, 60, 70, and 80°C; inlet port: 150°C; and flame ionization detector: 250°C.

Adsorption isotherms

The adsorption measurements were made using an elution chromatography procedure. In this method, small quantities of sorbate of approximately $3x10^{-7}$ g in 0.5mL were injected at the column inlet port. By analyzing the corresponding elution peak, sorption parameters could be calculated (section II, page 29).

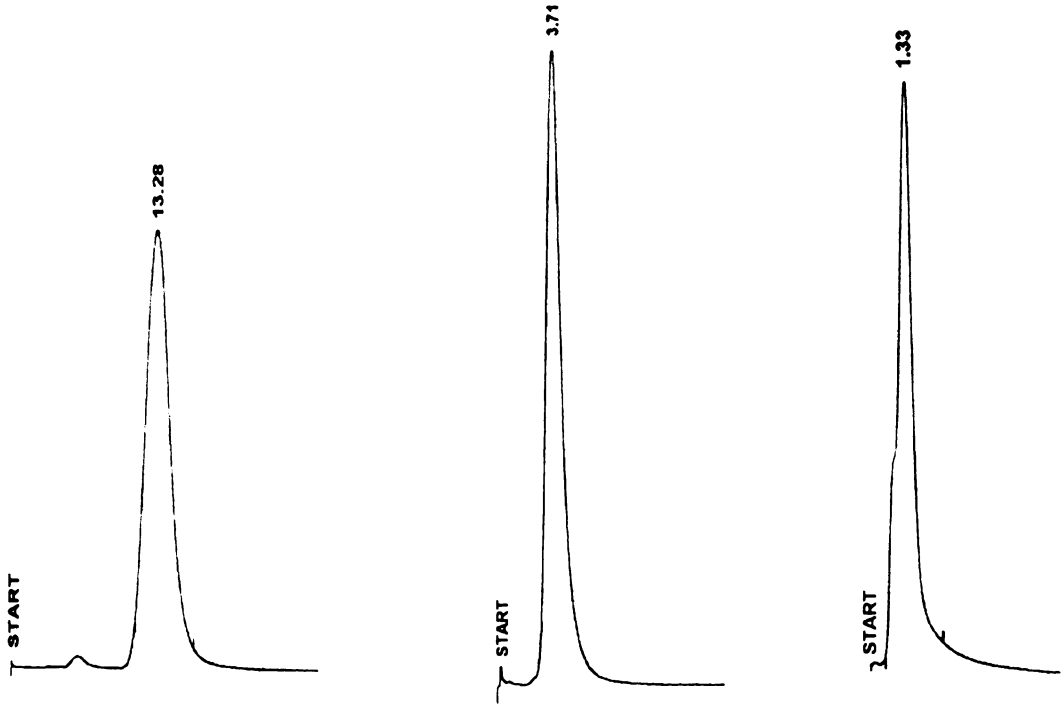
Other parameters

Specific retention volume and selected thermodynamic parameters were calculated for each adsorbent (Kalaouzis, 1992). The specific retention volume (V^o_g) defined as the net retention volume (V_N) at 273 K per unit weight of adsorbing agent (section II, page 32). The specific retention volume (V^o_g) was calculated using Equation 11. The larger the V^o_g, the stronger the attraction between the sorbate and the stationary phase. V^o_g is expressed in mL (STP) per gram, of stationary phase.

The following thermodynamic parameters were obtained using the equations in Section II, free energy of sorption, ΔG_s, which measures the adsorbing agent's affinity for 1-MCP; enthalpy of the sorption process, ΔH_s; and entropy, ΔS_s, which characterizes the degree of disorder of 1-MCP associated with the stationary phase as compared to 1-MCP in the gas phase.

RESULTS AND DISCUSSION

The adsorption of 1-MCP by silica gel, Tenax-TA, and activated clay at 80°C is shown in Figure 38. The amount injected for silica gel, Tenax-TA, and activated clay was 9, 6, and 4 nmole, respectively.



(a) Silica gel (b) Tenax – TA (c) Activated Clay

Figure 38. Adsorption of 1-MCP on (a) Silica gel, (b) Tenax-TA, and (c) Activated clay by inverse gas chromatography

The peak profiles are represented as typical chromatograms using the inverse gas chromatography elution technique. Smaller amounts of 1-MCP resulted in longer retention times than large amounts. Peak shapes and retention times were dependent on the amount of 1-MCP injected.

Chromatogram peaks of 1-MCP adsorption were not observed for the adsorbing agents of activated carbon and α-cyclodextrin. It is probably because 1-MCP has too strong an affinity for these adsorbing agents. Activated carbon has a high affinity to adsorb organic molecules of liquids or vapors because of strong interactions on the carbon surface (Bansal, 1988). Meanwhile, Bagreev (2000) studied the adsorption of hydrogen sulfide on activated carbons (unmodified and catalytic with introduced basic nitrogen group) using inverse gas chromatography at infinite dilution. The adsorption of hydrogen sulfide on noncatalytic and catalytic carbons was governed by Henry's law. López-Garzón (1993) used inverse gas chromatography at infinite dilution for studying the surface characteristics of several porous active carbons. The thermodynamic parameters of adsorption of n-alkanes were determined from the net retention volume.

Sorption isotherms

Sorption isotherms of 1-MCP adsorption versus 1-MCP partial pressure were determined at 50, 60, 70, and 80°C for the three adsorbing agents. The results for silica gel, Tenax-TA, and activated clay are presented in Figures 39, 40, and 41.

These studies show that inverse gas chromatography is a useful method for determining the sorption behavior of 1-MCP gas at an infinite dilution concentration. The results were obtained from the profile peaks of 1-MCP interaction with the adsorbing agents, silica gel, Tenax-TA, and activated clay.

Sorption isotherms of 1-MCP and their respective adsorbing agents were linear, that is, ruled by Henry's law. They were used to predict the amount of 1-MCP gas

adsorbed by the adsorbing agents (mole/g) at 23°C. Kontominas (1994) presented nonlinear isotherms for a series of n-alkanes on polystyrene in the finite concentration region. Demertzis (1987) obtained sorption isotherms of vinylidine chloride on vinylidene chloride copolmers (Vdc-VC and Vdc-AcN). Gavara (1997) determined the sorption isotherms for four food aroma components on three polymeric materials. The isotherms for both copolymers and three polymeric materials were linear in the low and high vapour pressure range.

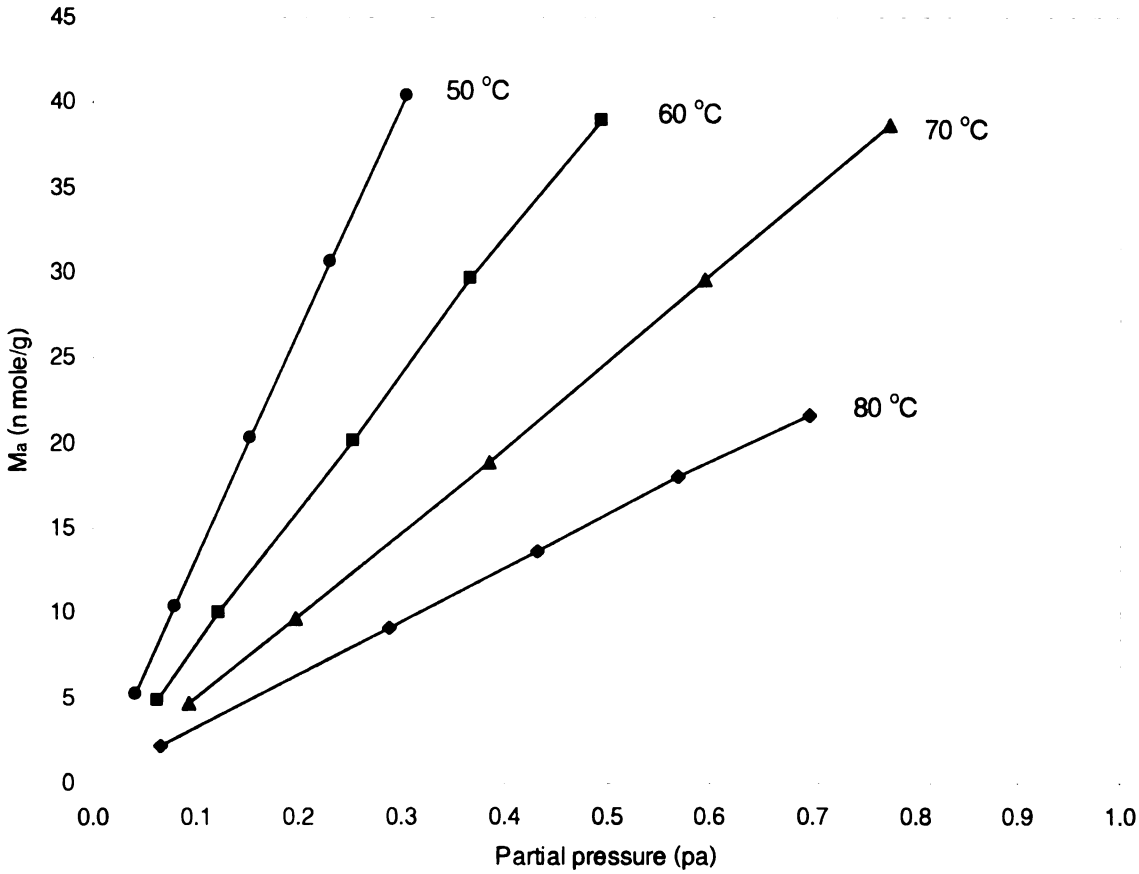


Figure 39. Sorption isotherms of 1-MCP on silica gel at 50, 60, 70, and 80°C

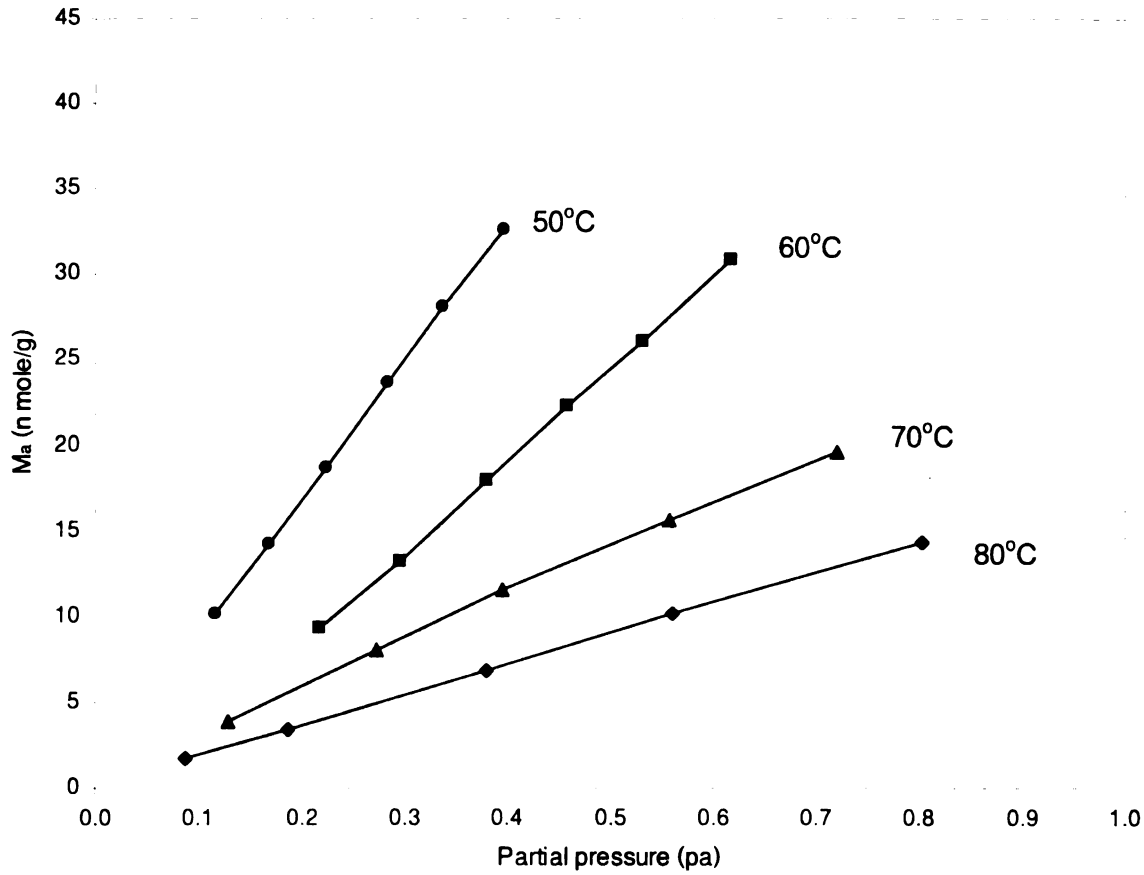


Figure 40. Sorption isotherms of 1-MCP on Tenax-TA at 50, 60, 70, and 80°C

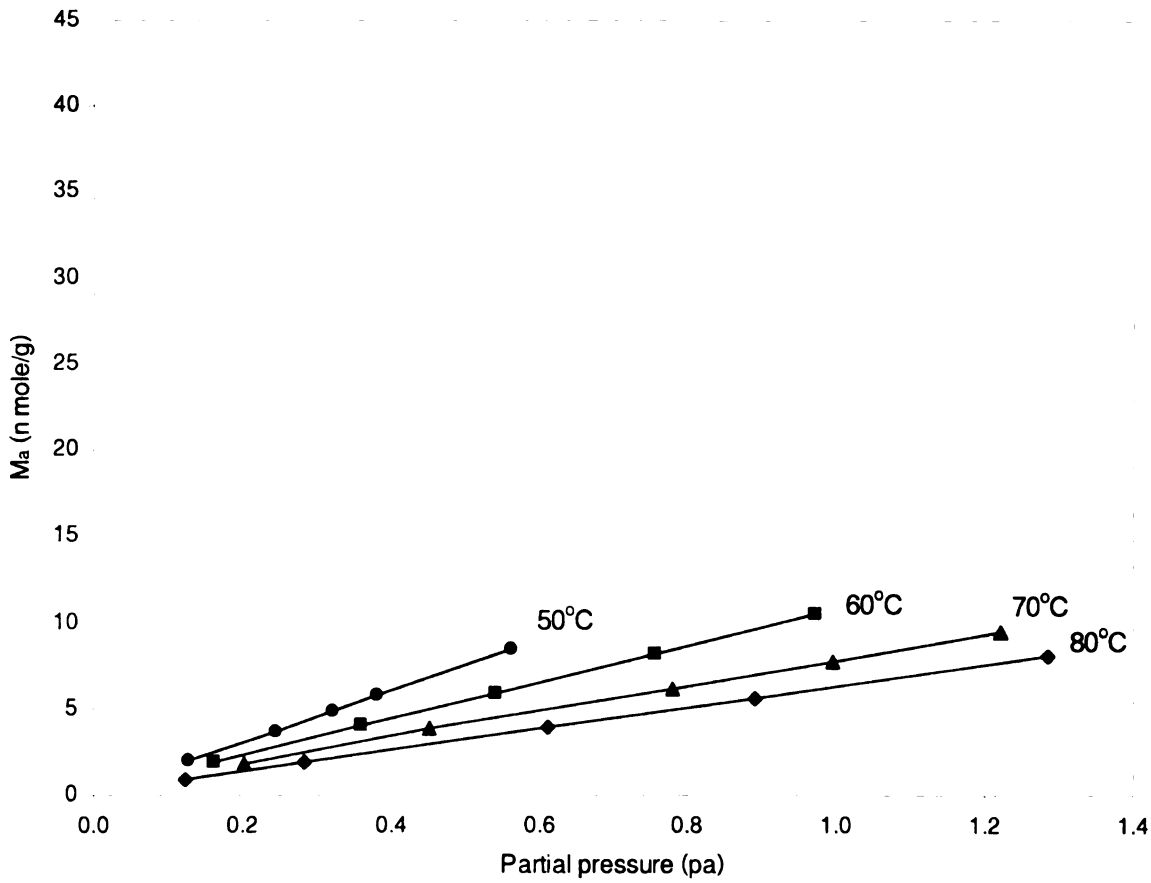


Figure 41. Sorption isotherms of 1-MCP on activated clay at 50, 60, 70, and 80°C

Linear plots were derived from a least square fit of the log constant value (LnK_s) versus the reciprocal of the absolute temperature (1000/T, K) for each adsorbing agent as shown in Figure 42. The linear equations were as follows: Y=5.5356X – 12.244 (silica gel); Y=6.0074X – 14.159 (Tenax-TA); Y=3.4438X – 7.9804 (activated clay), where X is the 1000/T (°K) and Y is the $-Log(K_s)$.

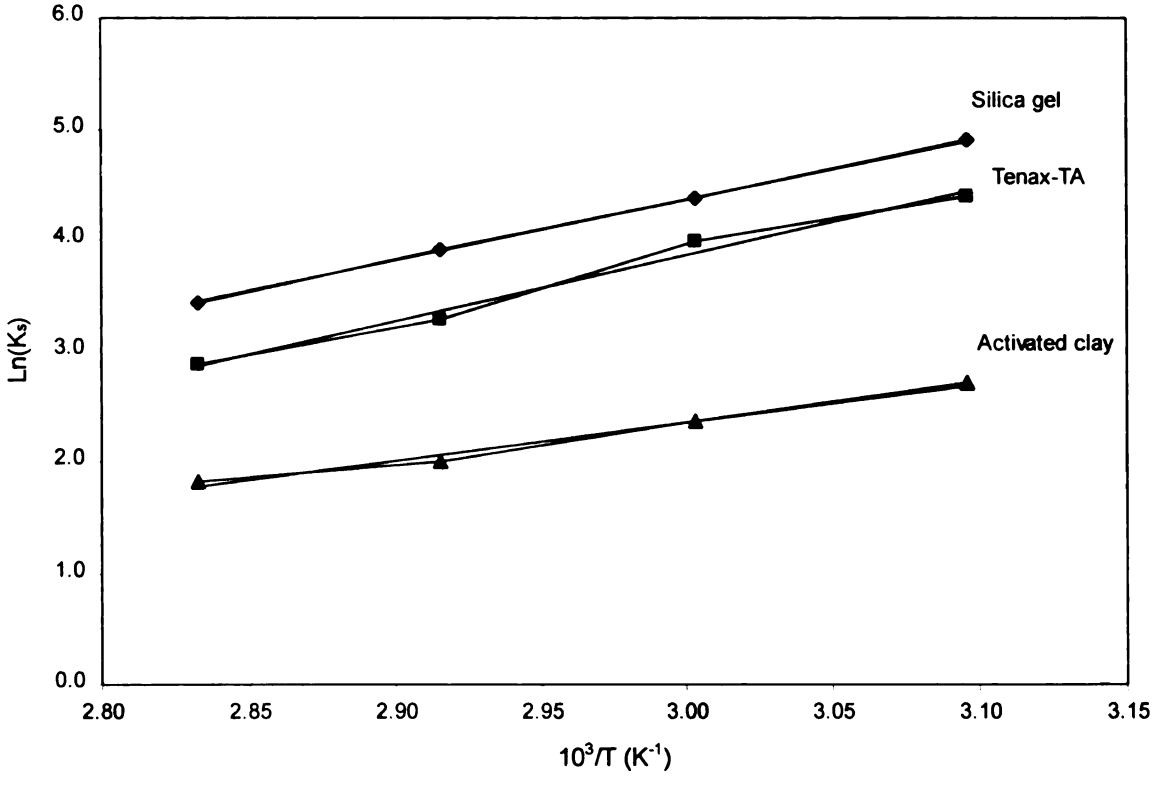


Figure 42. Linear plot of the log constant value (LnK_s) versus the reciprocal of the absolute temperature (1000/T, K)

As shown in Figures 39, 40, and 41, the sorption isotherms were linear functions of the partial pressure of 1-MCP, which indicates that they follow Henry's law,

$$M_a = K_s \cdot p \qquad (33)$$

where M_a is the concentration of 1-MCP adsorbed, K_s is the Henry's law solubility coefficient, and p is the partial pressure of the 1-MCP vapor entering the GC detector.

An increase in temperature decreased the amount of 1-MCP absorbed on silica gel. Similar results were obtained for the other two adsorbing agents. All correlation coefficients for the isotherms were higher than 0.98. K_s values for 1-MCP and each adsorbing agent are summarized in Table 8.

Table 8. Solubility coefficients (K_s) of 1-MCP as a function of temperature for each adsorbing agent

Temperature °C	K_s N mole/g Pa		
	Silica gel	Tenax-TA	Activated clay
50	133.91	80.85	14.90
60	78.99	54.10	10.57
70	49.80	26.52	7.41
80	30.97	17.69	6.14

Silica gel had a much higher Henry's law constant (K_s) than Tenax-TA and activated clay. This implies that the amount of 1-MCP adsorbed on the silica gel, at the corresponding partial vapor pressure of 1-MCP, was greater than that for Tenax-TA and activated clay. Equation 33 was used to estimate the sorption isotherms of 1-MCP at 23°C as shown in Figure 43. K_s values of at 23°C were 640 nmole/g·Pa for silica gel, 460 nmole/g·Pa for Tenax-TA, and 40 nmole/g·Pa for activated clay.

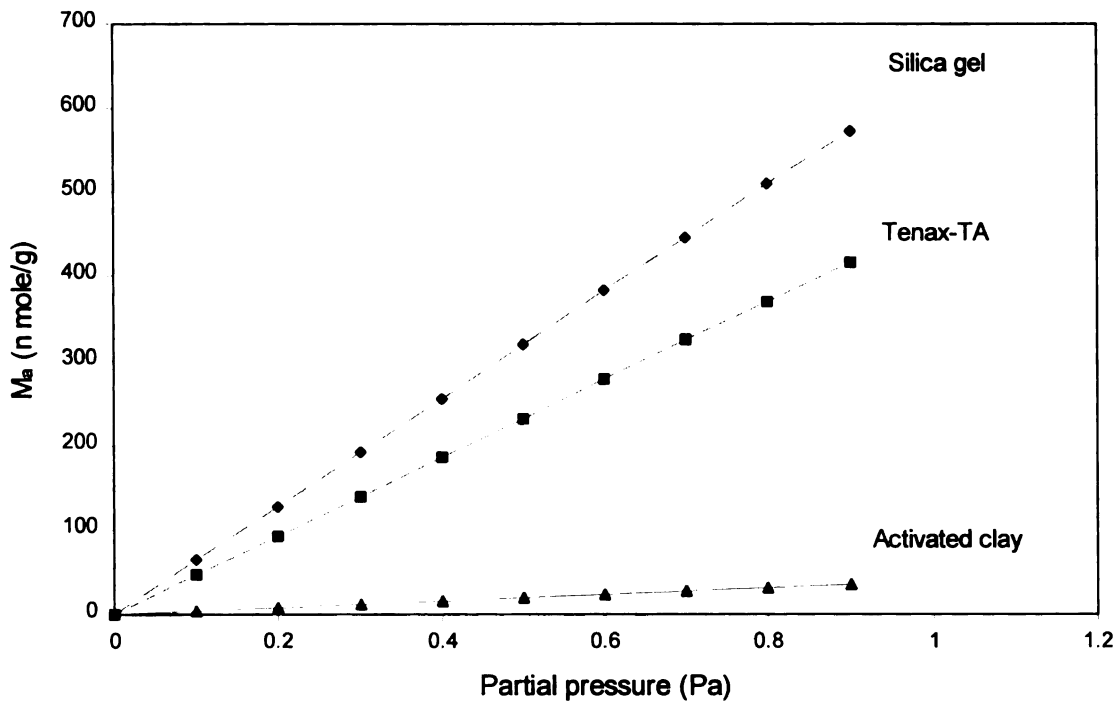


Figure 43. Calculated sorption isotherms of 1-MCP on each adsorbing agent at 23°C

Specific retention volume (V^{o}_{g}) values

Specific retention volumes (V^{o}_{g}) for each adsorbing agent at 50, 60, 70, and 80°C are presented in Figures 44, 45, and 46. Table 9 shows that the specific retention volume of 1-MCP gas for the adsorbing agents depends on the temperature. The specific retention volume of silica gel was higher than those for Tenax-TA and activated clay. These values indicate that 1-MCP gas has a stronger binding force with the silica gel than the other adsorbing agents. V^{o}_{g} was not affected by the amount of 1-MCP injected. This assumes that 1-MCP levels are relatively low and are not saturating the available number of binding sites in the respective stationary phases. The specific retention volume for all adsorbing agents increased with decreasing temperature because at lower temperature, lower kinetic energy was necessary to bind 1-MCP to the adsorbing agents' binding sites. Therefore, more binding sites could be occupied.

Table 9. Specific retention volumes (V^{o}_{g}) of 1-MCP with each adsorbing agent as a function of temperature

Temperature °C	Specific retention volume, V^{o}_{g} mL/g		
	Silica gel	Tenax-TA	Activated clay
50	265±0.84	156±0.22	25±0.28
60	161±1.66	91±0.15	17±0.19
70	99±0.62	52±0.16	12±0.31
80	64±0.03	32±0.05	9±0.18

Data are the average of 1-MCP uptakes of 1, 2, 3, 4, 5, and 7nmole/g at infinite dilution.

Silica gel was found to strongly interact with 1-MCP compared to Tenax-TA and activated clay, based on the specific retention volume (V^o_g) for 1-MCP and their adsorbing agents, and on the thermodynamic interactions of adsorption of 1-MCP molecules, based on binding site theory. Specific retention volume of 1-MCP gas for silica gel, Tenax-TA, and activated clay at 50°C was approximately 265, 156, and 25mL/g, respectively.

Values of water for unplasticized PVC and unplasticized P(VdC-VC) at 50°C were approximately 24 and 18mL/g (Kalaouzis, 1992). V^o_g of VCM for resin E and resin S of PVC polymers at 55°C for studying the effect of polyvinyl chloride (PVC) polymer structure, monomer level and temperature on vinyl chloride monomer (VCM) migration were approximately 12 and 0.13 mL/g, respectively (Apostolopoulos, 1988a). The VCM specific retention volume for resins was dependent on the concentration and temperature. The results showed that specific retention volume of 1-MCP gas for adsorbing agents represented relatively higher value, compared to those of water and VCM on PVC polymer.

Figure 47 shows a straight line for Log V^o_g against 1/T between 50 and 80°C for 1-MCP and the respective adsorbing agents. Delarue (2000) observed retention behavior using a linear plot of Log V^o_g vs 1/T of aroma molecules on carbohydrate matrixes such as starches or maltodextrins, and evaluated the enthalpy of adsorption of these molecules using IGC. Gauthier (1998) studied the physicochemical modifications of cellulose using acyl chlorides having 5 and 16 carbon atoms. He found a linear relation between Log V^o_g vs 1/T between 50 and 140°C for n-octane on a C_{16}-CE IGC column. The slope of the linear line was used to determine the differential enthalpy adsorption value.

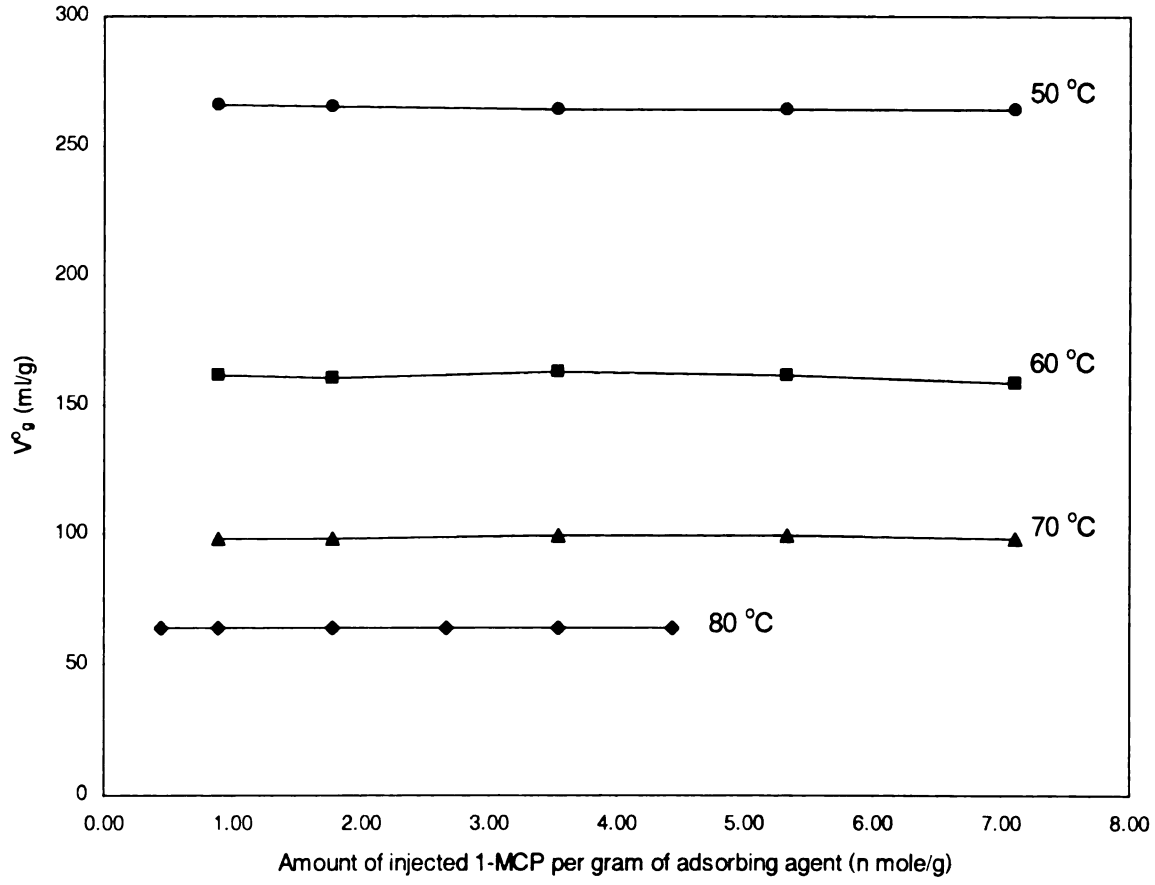


Figure 44. Specific retention volume (V^o_g) for silica gel as a function of 1-MCP concentration at 50, 60, 70, and 80°C

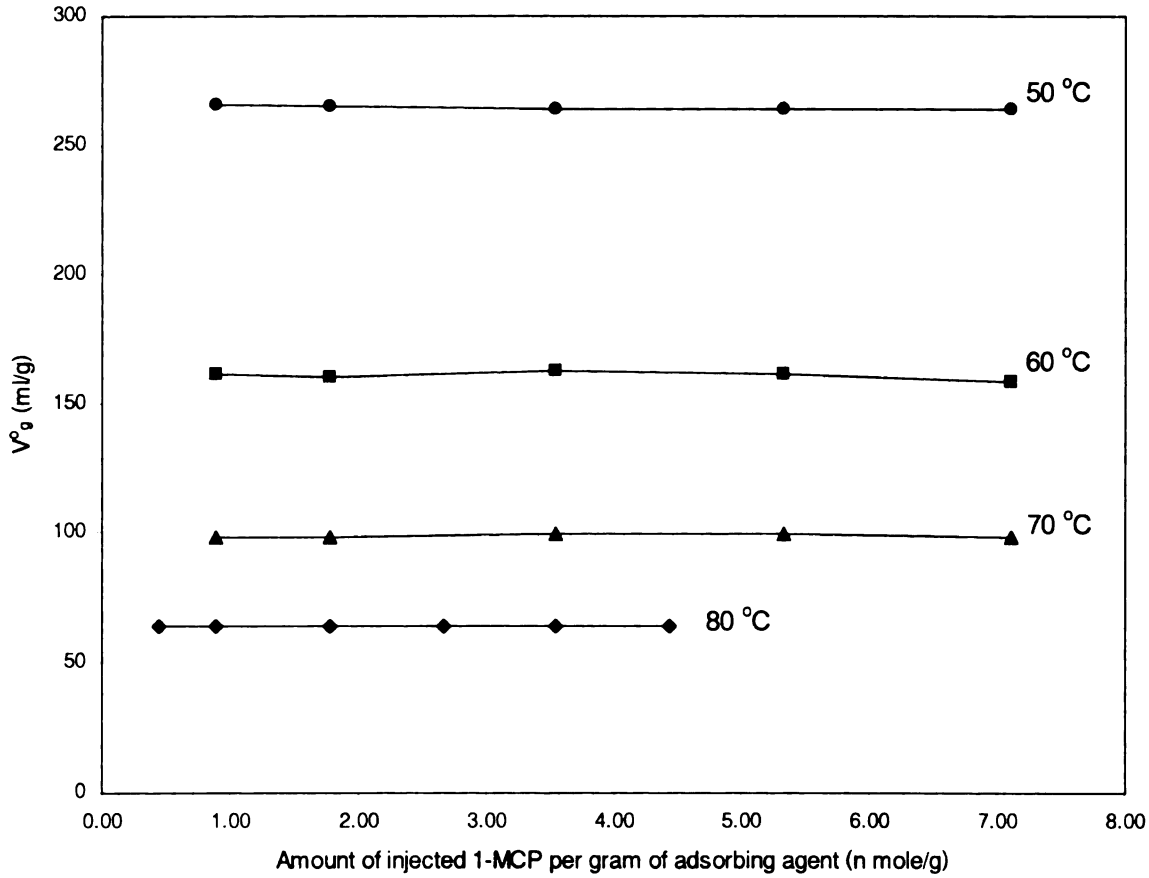


Figure 44. Specific retention volume (V^o_g) for silica gel as a function of 1-MCP concentration at 50, 60, 70, and 80°C

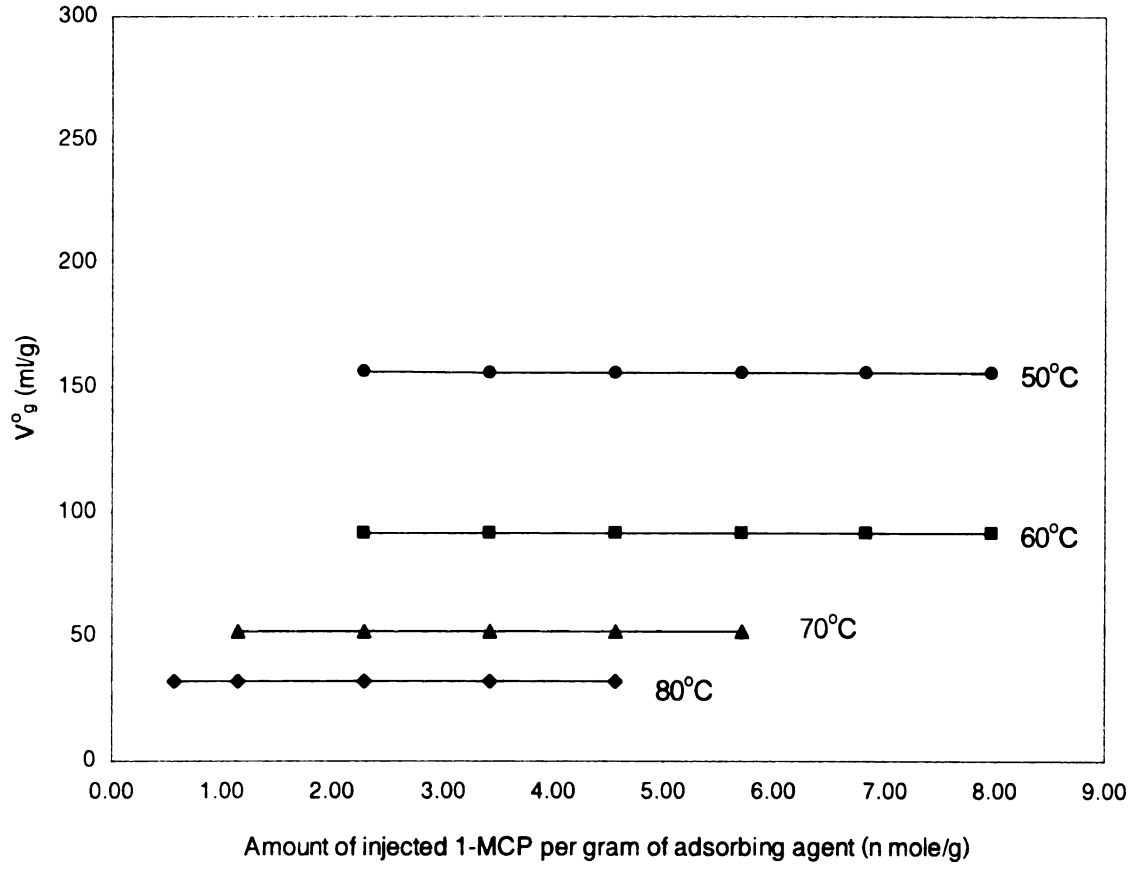


Figure 45. Specific retention volume (V^{o}_{g}) for Tenax-TA as a function of 1-MCP concentration at 50, 60, 70, and 80°C

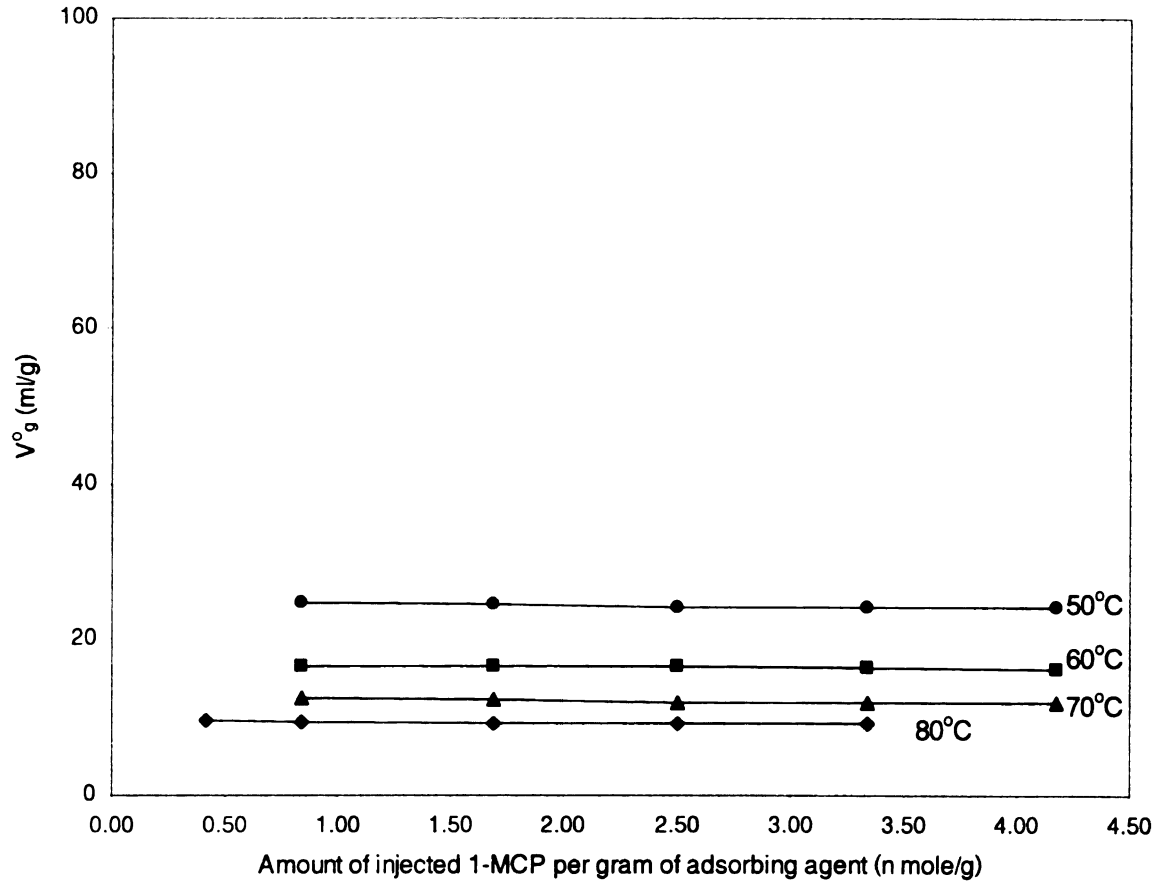


Figure 46. Specific retention volume (V^{o}_{g}) for activated clay as a function of 1-MCP concentration at 50, 60, 70, and 80°C

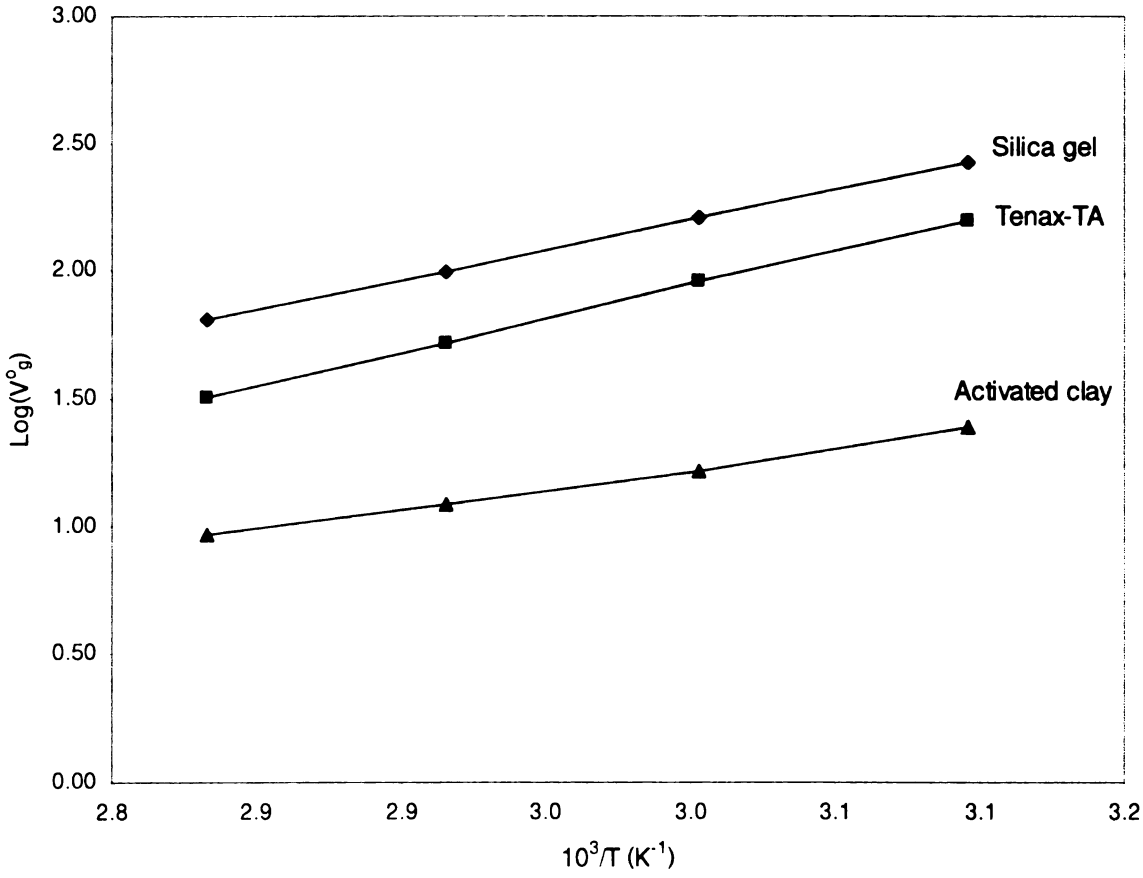


Figure 47. Log specific retention volume (V^{o}_{g}) for 1-MCP as a function of temperature

Thermodynamic parameters

Gibbs's free energy, entropy, and enthalpy at the four temperatures are shown in Tables 10 and 11. For these adsorbents, Data are from the average values of 1-MCP uptake at 1, 2, 3, 4, 5, and 7 nmole/g at infinite dilution.

Table 10. Gibbs's free energy values of 1-MCP at several temperatures

Temperature °C	$-\Delta G_s$ (kcal/mole)		
	Silica gel	Tenax-TA	Activated clay
50	3.36±0.02	2.46±0.01	1.60±0.07
60	3.16±0.04	2.20±0.01	1.41±0.08
70	2.94±0.05	1.91±0.02	1.27±0.02
80	2.74±0.01	1.64±0.01	1.13±0.01

ΔG_s values decrease with decreasing temperature as shown in Figures 48, 49, and 50. Low temperature favors the adsorption process. Gibbs's free energy values for all adsorbing agents were negative indicating that a spontaneous bonding process occurred between 1-MCP and the adsorbing agents. Silica gel had higher absolute values of ΔG_s as compared to Tenax-TA and activated clay. This indicates that silica gel is more interactive than the other adsorbing agents tested. Gibbs's free energy value for 1-MCP on silica gel at 50°C was approximately -3.36 kcal/mole at infinite dilution.

Gibbs's free energy of water vapor for PVC-plasticizer mixtures and p(Vdc-VC)-plasticizer mixtures at 50°C had the values of approximately -3.04 and -2.99 kcal/mole, respectively (Kalaouzis, 1992). These values are similar to the free energy results for 1-MCP on silica gel. However, ΔG_s values for adsorption of vinylidiene chloride on VdC-VC and VdC-AcN copolymers was approximately -1.57 and -1.63 kcal/mole,

respectively. Values increased with decrease in monomer concentration and temperature, indicating a more favorable monomer-polymer interaction at low temperatures and monomer concentrations (Demertzis, 1987). This pattern is similar to the free energy value of vinyl chloride monomer on unplasticized resins E and S of the PVC polymers as reported by Apostrolopoulos (1988a).

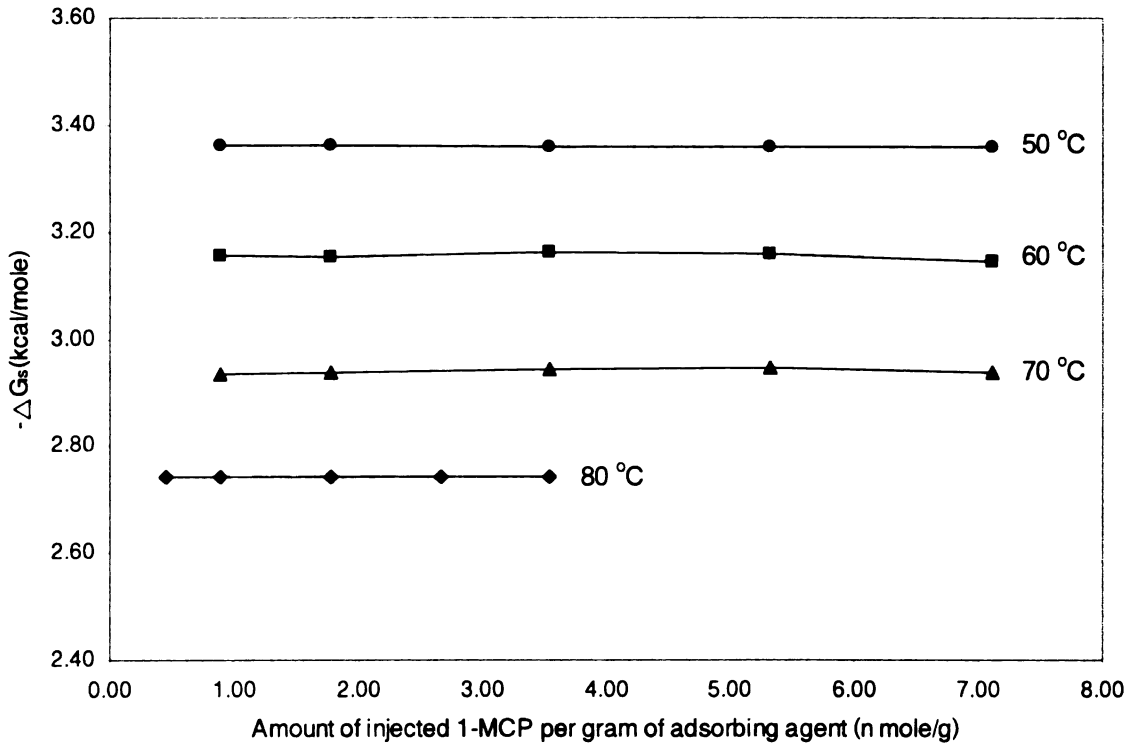


Figure 48. Gibbs free energy of sorption (ΔG_s) for silica gel as a function of 1-MCP concentration and temperature.

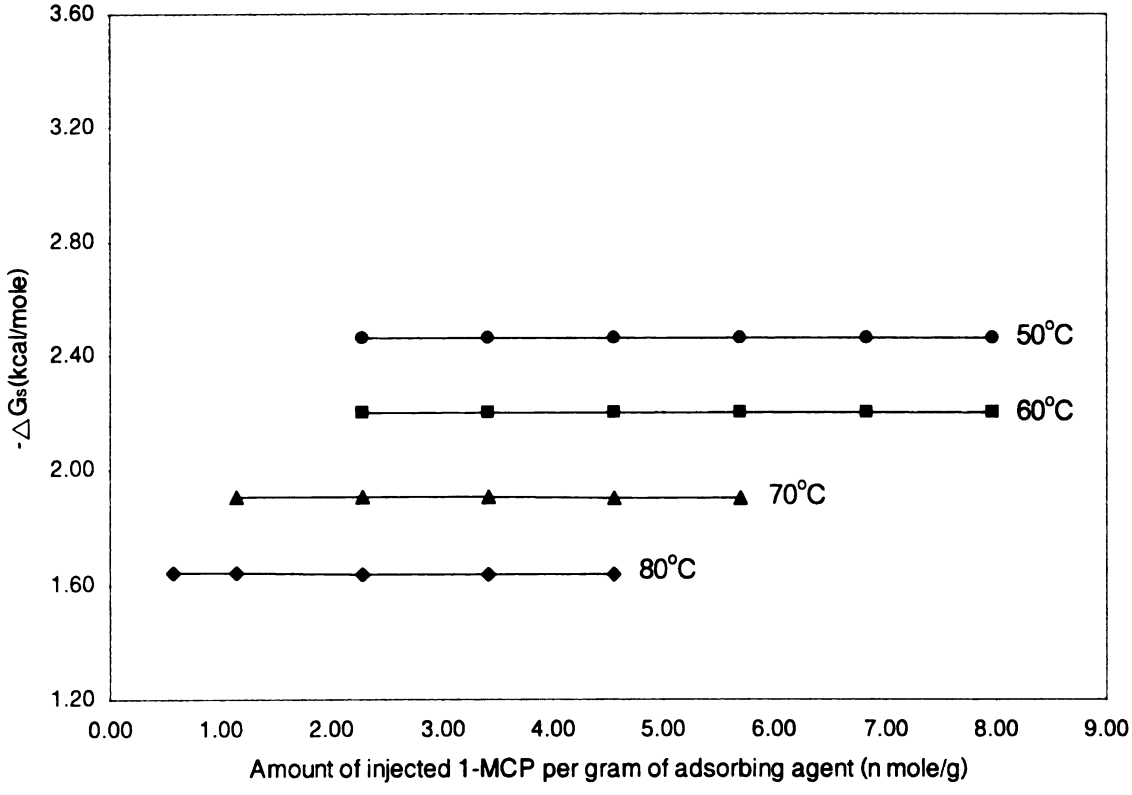


Figure 49. Gibbs free energy of sorption (ΔG_s) for Tenax-TA as a function of 1-MCP concentration and temperature.

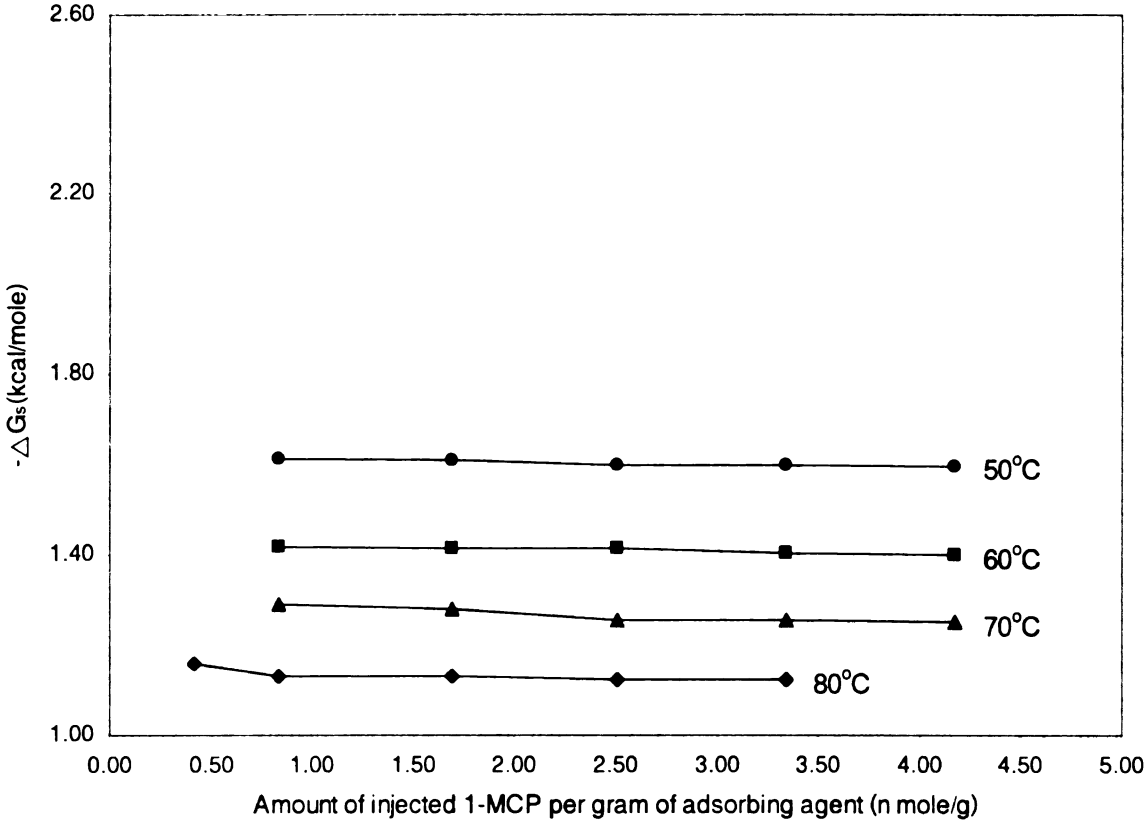


Figure 50. Gibbs free energy of sorption (ΔG_s) for activated clay as a function of 1-MCP concentration and temperature.

As expected, the entropy values were negative. Tenax-TA had higher absolute entropy values that silica gel and activated clay (Figures 51, 52, and 53). This indicates that 1-MCP is probably bonded more orderly to Tenax-TA than to the other two adsorbing agents. A more regular binding pattern may indicate that Tenax-TA has a more regular molecular architectural structure than the other two adsorbing agents.

Table 11. Entropy (ΔS_s) and enthalpy (ΔH_s) values of 1-MCP at selected temperatures

Temperature °C	$-\Delta S_s$ (cal/mole K)		
	Silica gel	Tenax-TA	Activated clay
50	18.69±0.07	25.07±0.02	14.96±0.05
60	18.74±0.08	25.09±0.01	15.09±0.03
70	18.83±0.09	25.22±0.01	15.07±0.07
80	18.85±0.07	25.26±0.01	15.02±0.05
$-\Delta H_s$ (kcal/mole)	9.40±0.03	10.56±0.01	6.44±0.01

Entropy values (ΔS_s) appear to be independent of temperature as shown in Table 11. In addition, ΔS_s values for the 1-MCP/adsorbing agents were not affected by the 1-MCP concentrations at infinite dilution. Within this temperature range, the spatial structures of the adsorbing agents are not basically altered. This indication is in good agreement with results reported by Kalaouzis (1992), Riganakos (1989), and Apostolopoulos (1988a). However, the ΔS_s values of freeze-dried coffee as a function of water content and temperature varied depending on the temperature and water content. Temperature dependence of the ΔS_s values could be related to the higher kinetic energy of water molecules at higher temperature.

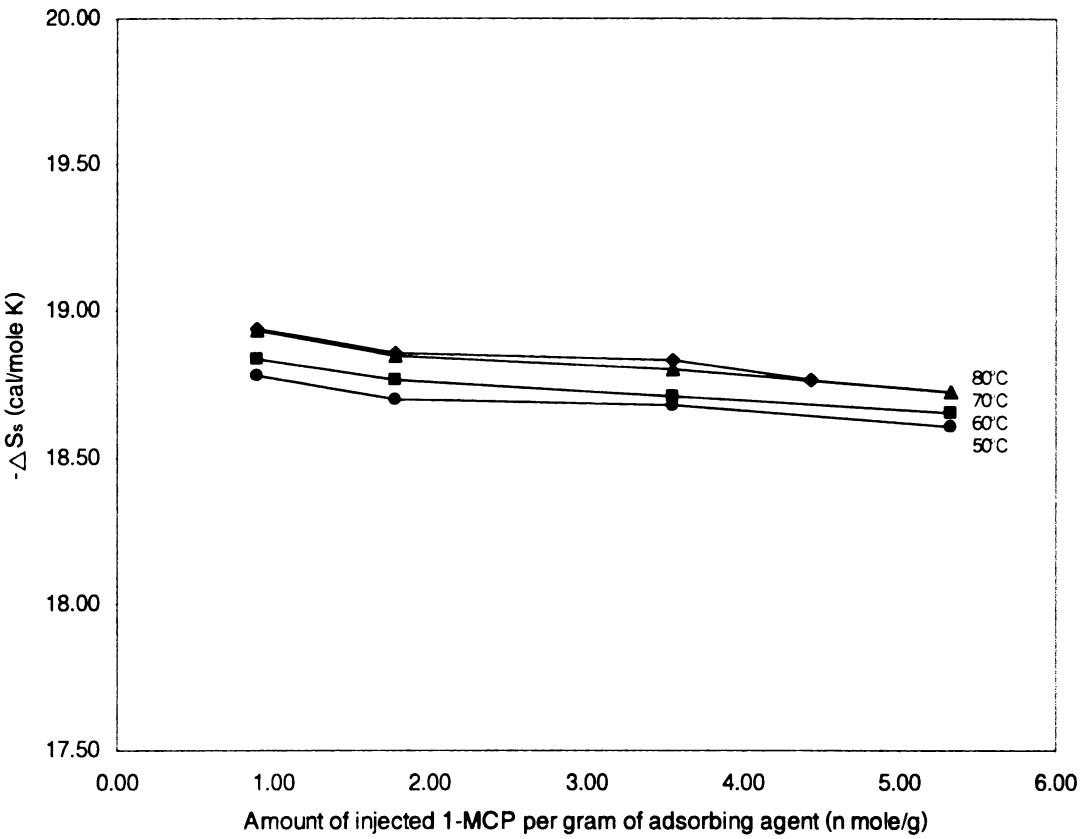


Figure 51. Entropy of sorption (ΔS_s) for silica gel as a function of 1-MCP concentration and temperature.

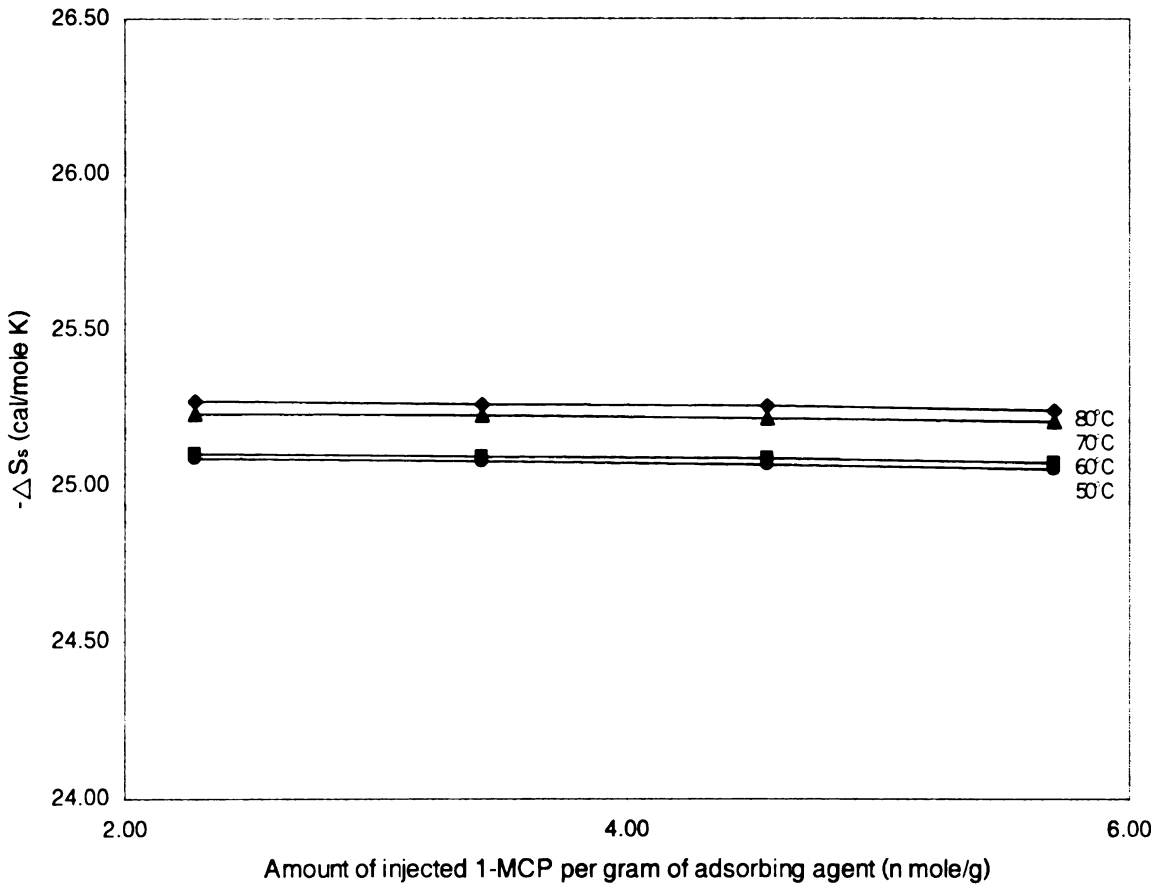


Figure 52. Entropy of sorption (ΔS_s) for Tenax-TA as a function of 1-MCP concentration and temperature.

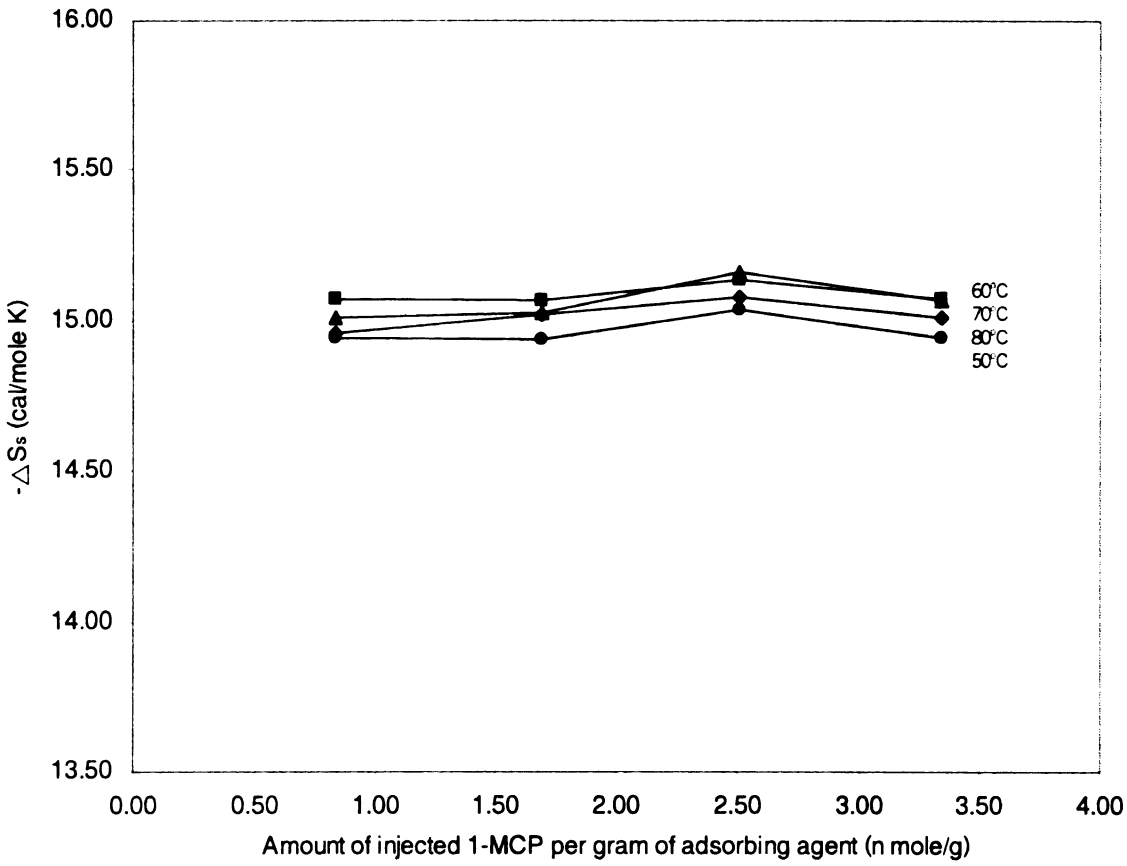


Figure 53. Entropy of sorption (ΔS_s) for activated clay as a function of 1-MCP concentration and temperature

Enthalpy values of (ΔH_s) for all the adsorbing agents were also negative (see Table 11), which indicates that an exothermic interaction took place, between 1-MCP and the adsorbing agents. Tenax-TA had the lowest enthalpy values as shown in Figure 54. This means that Tenax-TA not only adsorbs 1-MCP more regularly, but also does it more exothermically than silica gel despite the fact that silica gel has more adsorbing capacity for 1-MCP than that of Tenax-TA.

In the case of the enthalpy of the VCM-PVC system for the structure of the polymer (Apostolopoulos, 1988a), resin S of the PVC polymers showed more negative ΔH_s values than resin E. This suggests that resin S must sorb VCM more strongly than resin E. The ΔH_s values of sorption directly relate to the energy interaction between sorbed water molecules and sorption sites in the food material (Riganakos, 1989).

Such thermodynamic interactions between 1-MCP and the adsorbing agents may have an important role with respect to the release of 1-MCP molecules from the surface phase of the adsorbing agent into the headspace phase of the produce. Factors such as heat, moisture, and other interactive molecules can influence release of 1-MCP molecules from adsorbing agents.

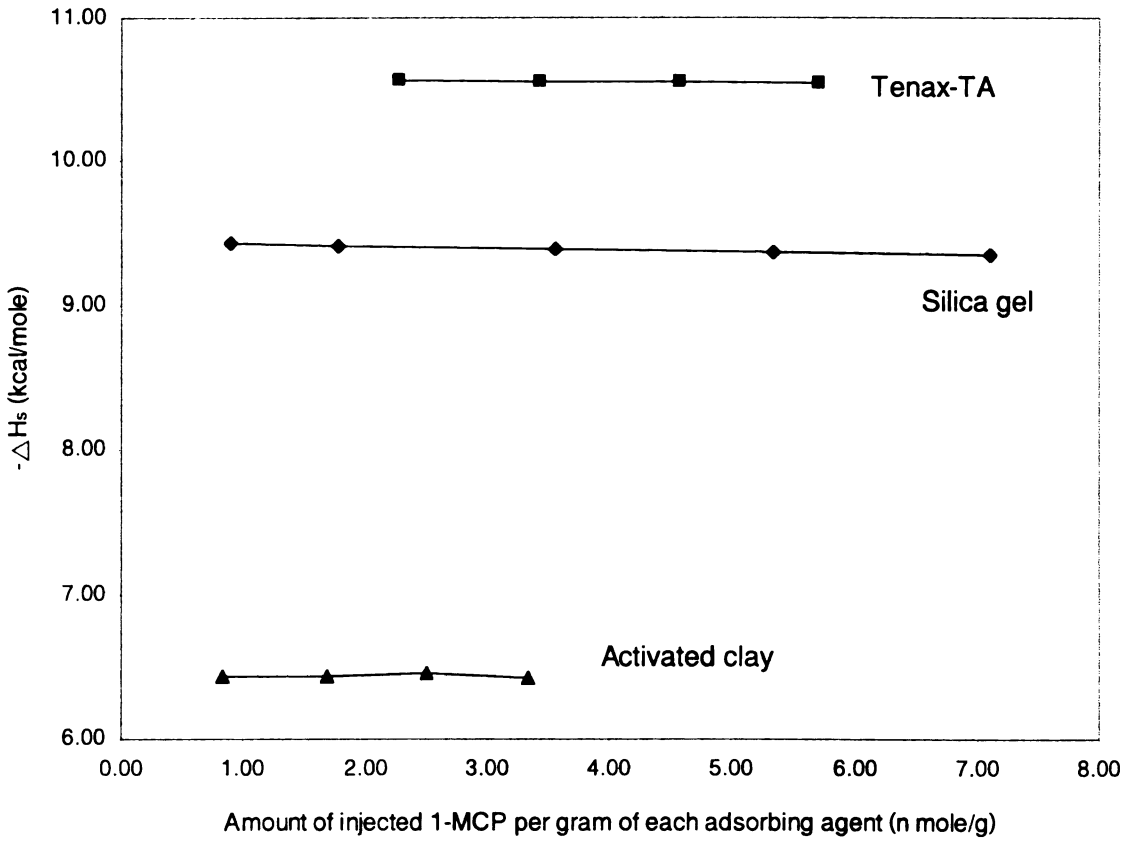


Figure 54. Enthalpy of sorption (ΔH_s) for each adsorbent as a function of 1-MCP concentration

CONCLUSION

Experimentation showed that IGC can be a useful method to study the interaction relationship between 1-MCP molecules and the surface of an adsorbing agent. IGC was used to obtain 1-MCP sorption patterns for selected adsorbing agents. The values determined can be used to predict the amount of 1-MCP sorbed by a specific adsorbing agent. Thermodynamic adsorption parameters were especially useful in characterizing the specific interactions of 1-MCP on the adsorbing agents.

However, IGC cannot be used to measure the sorption of 1-MCP on activated carbon due to its strong affinity which resulted in no measurable 1-MCP in the infinite concentration. Activated clay was not as effective in adsorbing 1-MCP as silica gel, or Tenax-TA. While silica gel adsorbed more 1-MCP than Tenax-TA, its adsorption pattern was less orderly and lower in enthalpy.

The relationship between 1-MCP and adsorbing agents must be known in order to estimate the amount of 1-MCP needed under different environmental conditions, other gases, and moisture levels. All must be considered in designing a 1-MCP delivery system. The interaction of another sorbate with sorbed 1-MCP on the surface of the adsorbing agent can also be evaluated by defining the sorbate-sorbate interaction relationship using IGC.

These time study results can be used to estimate the release rate of 1-MCP in a packaging system. The information obtained in this work could be useful in designing a device to control release of 1-MCP in modified atmosphere packages.

APPENDIX C

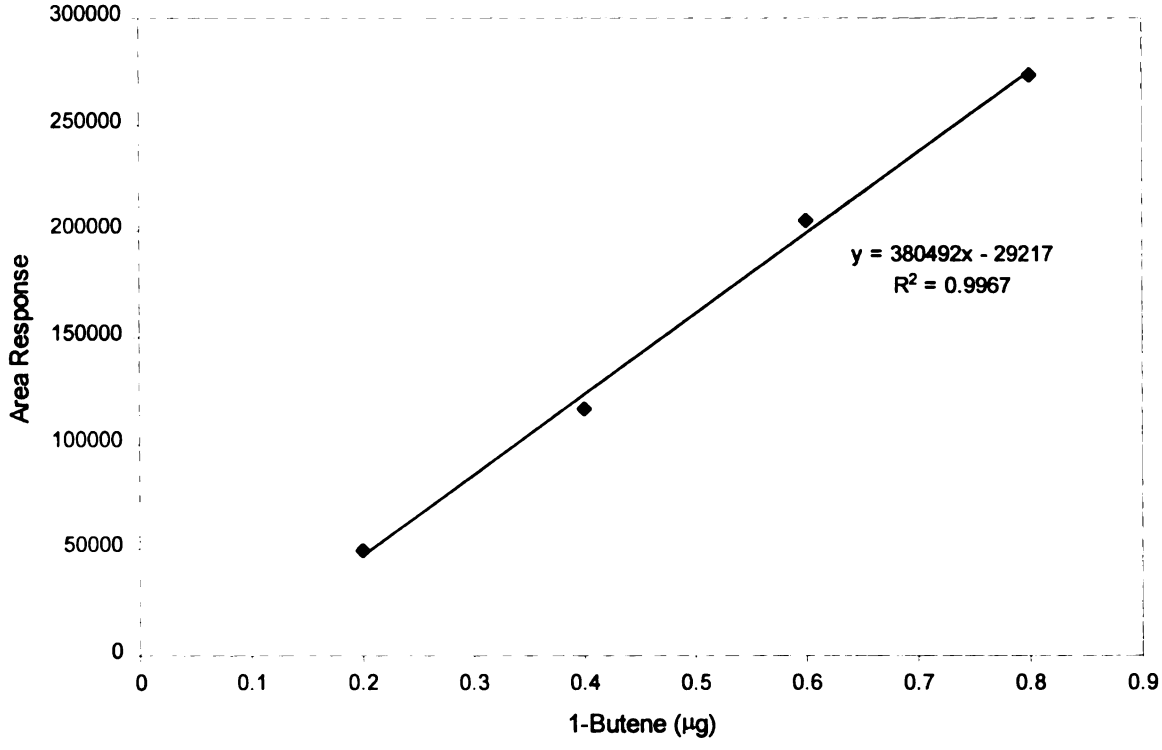


Figure 55. Standard calibration curve of 1-butene on silica gel packing column.

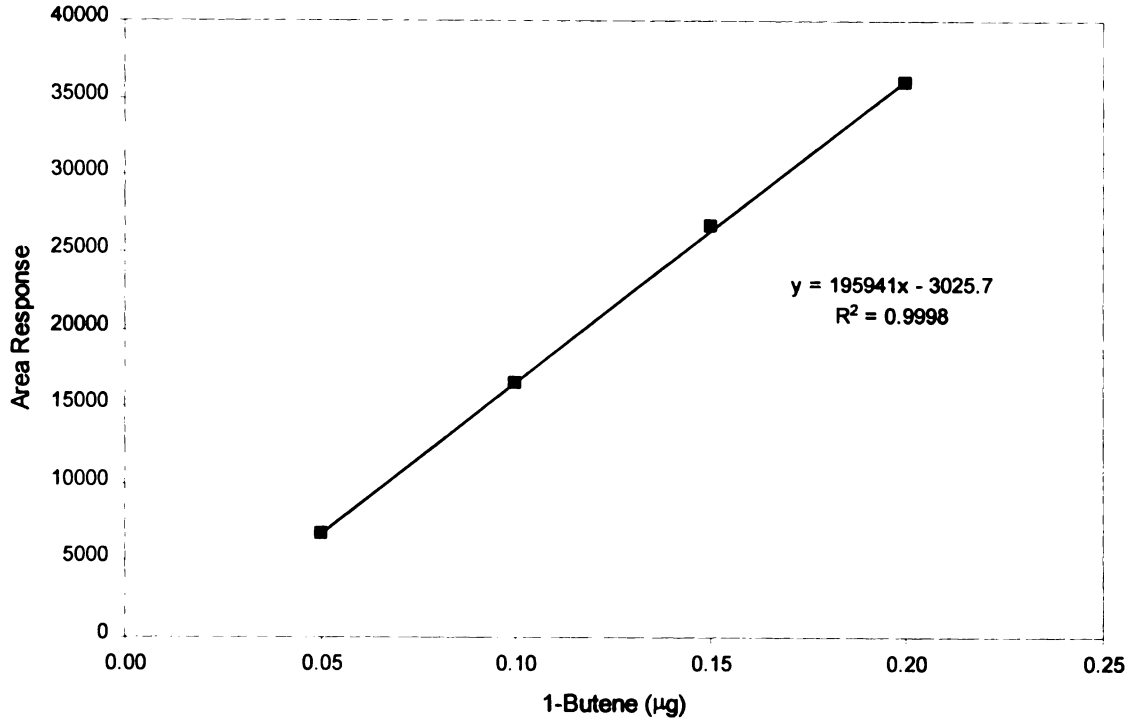


Figure 56. Standard calibration curve of 1-butene on Tenax-TA packing column.

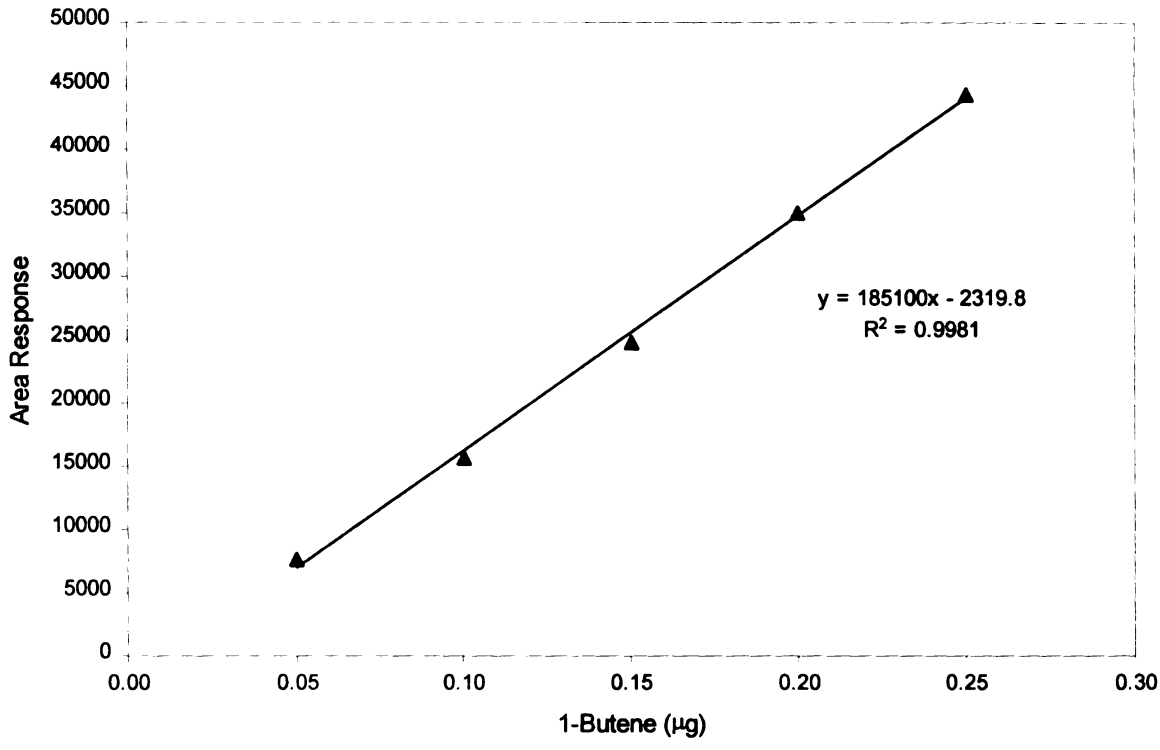
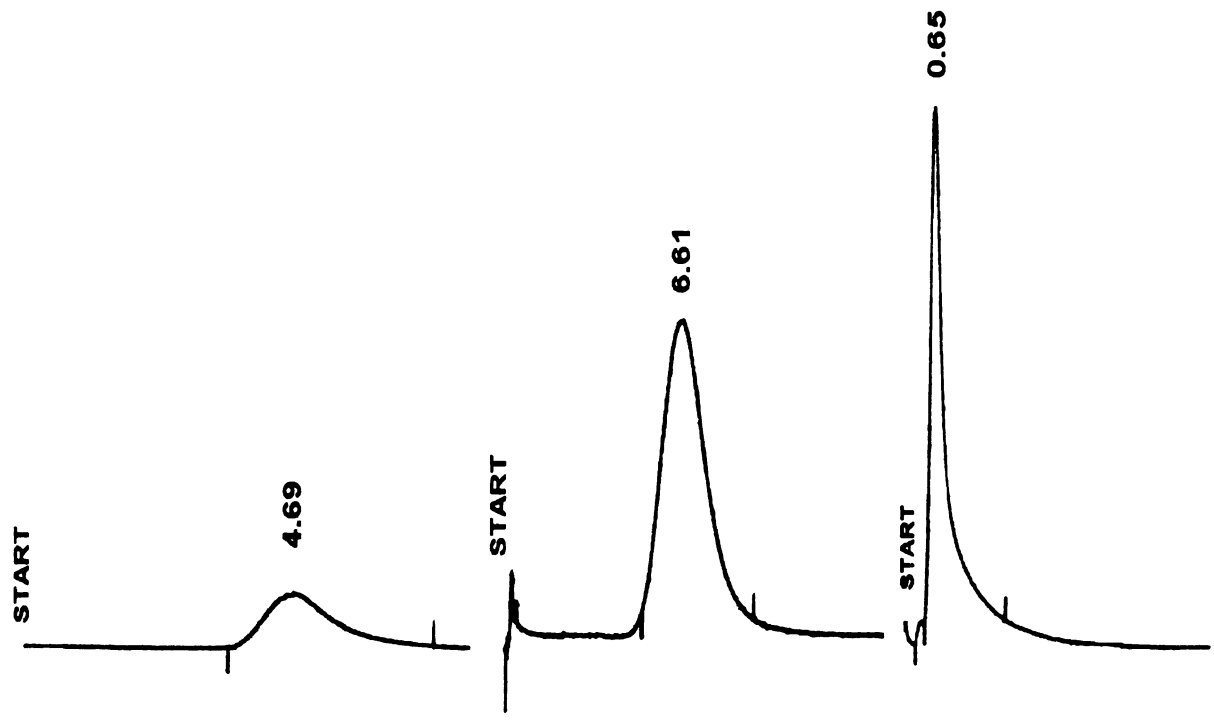


Figure 57. Standard calibration curve of 1-butene on activated clay packing column



(a) Silica gel (b) Tenax – TA (c) Activated Clay

Figure 58. Chromatographic peaks 1-butene on silica gel (a), Tenax-TA (b), and activated clay (c) stationary phase column for 1-MCP standard calibration curve: Silica gel (0.8μg, 100°C), Tenax-TA (0.2μg, 60°C), Activated Clay (0.1μg, 80°C).

SECTION VI: ADSORPTION AND RELEASE OF 1-METHYL CYCLOPROPENE (1-MCP) FROM SELECTED ADSORBING AGENTS

ABSTRACT

The partitioning of 1-MCP between the gas/polymer matrix was determined for several adsorbing agents, and in sachet materials to estimate the adsorption potential of 1-MCP in dry air at 23°C. The 1-MCP release study was performed using a closed system under two different environmental conditions, dry air (0%RH) and 90%RH. Sachets made from Tyvek®, paper, LDPE, and PVA materials were fabricated to contain silica gel and activated carbon. The silica gel sachet had a relatively higher release rate at 90%RH, as compared to the release rate at the dry air condition. The activated carbon sachet did not release 1-MCP at either testing condition. Activated carbon had a very strong affinity for 1-MCP. The permeability coefficients of 1-MCP and water in polyethylene and polyvinyl acetate films were determined using a quasi-isostatic method. LDPE sachets containing silica gel had a similar 1-MCP release rate under both dry and 90%RH conditions due to its good water barrier properties. PVA sachets containing silica gel had slow release of 1-MCP. The amount of 1-MCP released from PVA sachets containing silica gel at 90%RH was larger than the amount of 1-MCP released at the dry air condition in a closed system.

INTRODUCTION

Among developing technologies used to prolong the storage life of fresh produce, 1-MCP is a novel compound which can inhibit the ethylene action on fresh produce (Serek, 1994; Jiang, 1999; Abdi, 1998; Ku, 1999). Controlled exposure of 1-MCP gas at the surface of fresh produce can delay ripening and senescence.

To be used commercially with ripening produce during postharvest storage, 1-MCP gas must have a delivery system. Two patents related to 1-MCP for inhibiting the ethylene response in the plants in order to extend their shelf life and developing the delivery methods for the convenient and safe application with molecular encapsulation agents such as cyclodextrin were issued in 1996 (Blankenship and Sisler, 1996) and 2000 (Daly, 2000). A 1-MCP trapping system using a α-cyclodextrin component was marketed under the trade name EthylBloc®. This commercial product requires a gas preparation procedure with solvent in order to release the 1-MCP gas.

Use of an inert matrix material to release 1-MCP may have application for fresh produce without the 1-MCP gas preparation step. In addition, use of a sachet may be able to provide slow release of 1-MCP gas in a fresh produce package.

The proposed release mechanism of 1-MCP involves three stages. First, the 1-MCP molecules are released into the free volume within the matrix of the adsorbing agents in the sachet pouch. Second, 1-MCP molecules pass through the film microstructure into the headspace containing the fresh produce. Third, the 1-MCP molecules penetrate the surface of the fresh produce.

Release systems associated with drugs (Shao, 2001), pesticides (Fernandez-Perez, 1999), herbicides (Zhu, 2001), flavor (Greenblatt, 1993), food ingredients (Meyers, 1998), and antioxidants (Miltz, 1988) for use in the pharmaceutical, agricultural, and food industries have been studied. The rate of release from these release systems depends on the pH of the medium, the geometry, the size of the system structure, and environmental conditions such as temperature, humidity, and air flow.

The present study focuses on 1-MCP release using a delivery system that consists of a sachet containing 1-MCP sorbed onto selected adsorbing agents. The objectives of this study were: (a) to determine the partition coefficient of 1-MCP in adsorbing agents and pouch materials; (b) to evaluate the sachet materials regarding their water and 1-MCP gas barrier properties; (c) to determine the release of 1-MCP from the sachets containing the adsorbing agents.

MATERIALS AND METHODS

Materials

The selected materials used as adsorbing agents in these studies were silica gel, grade 634-60A, 100-200 mesh (Aldrich Chemical Co., Milwaukee, WI), Tenax-TA, 60-80 mesh (Alltech Associates, Inc., Deerfield, IL), activated clay (a modified montmorillonite mineral, I-28E, Nanocor, Inc., Arlington Heights, IL), activated carbon, 20-40 mesh (Aldrich Chemical Co., Milwaukee, WI), and activated carbon, 80-100 mesh (Alltech Associates, Inc., Deerfield, IL). The molecular encapsulation agent used was α-cyclodextrin (6 glucose monomers, Cerestar Co., Hammond, IN).

1-MCP was supplied by Biotechnologies for Horticulture, IL. EthylBloc® is a commercial product. In its powder it has about 0.14% active ingredient by weight.

Sachet materials for 1-MCP controlling release system

The materials used to fabricate the sachet pouches were filter paper (Walgreen Co. Deerfield, IL), Tyvek® (Style 1073B, Dupont Co. Wilmington, DE), low density polyethylene (LDPE, Dow Chemical Co., Midland, MI), and polyvinyl acetate (PVA, Dow Chemical Co., Midland, MI). The average thickness of the test materials was measured using the Model 549 micrometer from Testing Machines, Inc., (Amityville, L.I., NY) and summarized in Table 12.

Table 12. The average thickness of the test materials used to fabricate the sachet.

Materials	Thickness[a] (mil)
Filter paper	5.4±0.06
Tyvek® (1073B)	5.1±0.11
LDPE	1.3±0.06
PVA	1.3±0.10

[a] Mean values of three replicate results

Water vapor transmission rate of the paper and Tyvek® sachet materials

The water vapor transmission rates of the filter paper and Tyvek® materials were determined using the dish method (ASTM Standard method E96-00). Three replicates were tested at each of the following conditions, 25°C, 60%RH and 36.9°C, 89%RH using a controlled environmental chamber (Hotpack Co., Philadelphia, PA). Approximately 20 grams of desiccant (anhydrous calcium sulfate, size 8 mesh, W.A. Hammond Drierite Co. Xenia, OH) were placed into the bottom of an aluminum dish (1.75cm depth). The sample material was cut into a circular piece (8.5cm diameter) and placed on top of the test dish containing desiccant. A mask (9cm diameter) containing a small round hole (2.5cm diameter) was then pasted onto the specimen's top surface. The edges of the mouth area in the test dishes were sealed using a melted paraffin wax, and the 2.5cm specimen area was exposed to the environmental conditions previously described. The test dishes with desiccant were weighed using an analytical balance and placed in the appropriate chambers. To determine the moisture sorption of the sample materials, a control film specimen was treated in an identical manner (without desiccant) as the other samples. The control and sample dish sets were reweighed at predetermined intervals.

The water vapor transmission (WVT) and permeance rate were calculated based on the slope of the graph of weight gain as a function of time using equations 34 and 35.

$$\text{WVTR} = \frac{SL}{A} \tag{34}$$

$$\text{Permeability} = \frac{WVT \cdot \ell}{\Delta P} = \frac{WVT \cdot \ell}{S_a \cdot (R_1 - R_2)} \tag{35}$$

where SL=slope of the straight line for the weight gain versus time graph, g/min., A= test area ($0.0005 m^2$), ℓ= thickness (m), ΔP = vapor pressure difference (mmHg), S_a = saturated vapor pressure at 25°C (23.756mmHg) or 36.9°C (46.336mmHg), R_1 = relative humidity at the test chamber (60%RH or 89%RH), R_2 = relative humidity at the vapor sink (0%RH).

Water vapor transmission rate of the LDPE and PVA sachet materials

The water vapor transmission rates of the LDPE and PVA materials were measured using a Permatran-W® 3/31 apparatus (Mocon Inc, Minneapolis, Minnesota) in accordance with ASTM F1249. The test film materials were evaluated at the test temperatures of 23±0.5°C and 32±0.5°C, at a relative humidity of 90±3%.

Permeation measurements of 1-MCP gas for sachet materials

1-MCP gas permeation experiments were carried out at 24±1°C, based on the quasi-isostatic method. A schematic diagram of the permeation test apparatus is presented in Figure 59. A constant concentration stream of 1-MCP gas was supplied through a 4 L glass reservoir using a gas circulation rotary pump (Universal Electric Co., Cowosso, MI) and then passed into the permeation cell. A constant concentration of permeant vapor was then flowed through the center cell chamber at a continuous flow rate of 45 to 50 mL/min. The unit was connected to a flow meter. The 1-MCP gas that passed through the films accumulated in the bottom chamber, and monitored as a function of time. The permeation cell was comprised of two aluminum disc-shaped plates and a hollow center ring. Both cell chambers were equipped with a sampling port. The center ring contained an inlet and outlet port to accommodate the carrier gas stream. The upper and lower cell chambers each had a volume of 50 cm^3; the volume of the center cavity was approximately 50 cm^3. The test films were placed in the cell so that the center ring effectively isolated the upper and lower cell chambers. Hermetic isolation of the chambers from each other and from the atmosphere was achieved by the compression of overlapping Viton 0-rings on the film specimen. Viton is a fluorocarbon elastomer that is resistant to attack from most organic vapors. The concentration of 1-MCP gas in the lower cell chambers was determined by gas chromatography using flame ionization detection. At predetermined time intervals, 0.3 mL of headspace gas was extracted from the upper and lower cell chambers with a gas-tight syringe and injected directly into the gas chromatograph.

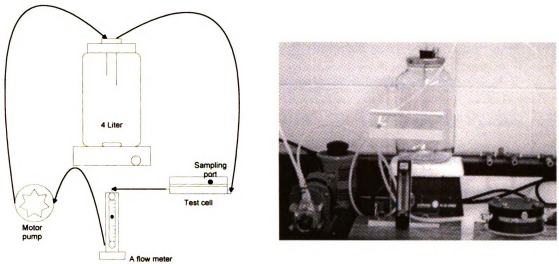


Figure 59. A schematic diagram of the 1-MCP permeation test system.

GC instrumentation for 1-MCP gas release analysis

The concentration of 1-MCP gas was quantified using a Hewlett-Packard Model 5890A gas chromatograph equipped with dual flame ionization detectors and a fused silica capillary SPB-5 nonpolar column (60m × 0.32mm ID) (Supelco Inc., Bellefonte, PA). 1-MCP gas was injected through the injection port of a thermal desorption unit and into an empty glass tube. This allowed a sample to be thermally transferred, with carrier gas flow into a capillary column in the gas chromatograph. The GC conditions were as follows. The column temperature was 80°C for 10 minutes. Helium was used as a carrier gas, at a flow rate of 2.2 mL/min. Injection and detector temperatures were set at

150 and 300°C. Standard calibration solutions were prepared using a serial dilution procedure with 1-butene gas (99.9% AGA Specialty Gas, Maumee, OH).

Partition coefficient

Partition coefficient studies were carried out using glass cells, 15 mL glass vials were sealed with screw top hole caps and PTFE/Silicone septums (Supelco Inc, Bellefonte, PA). The glass cells were purged using dry air (a compressed air, BOC gases, Ann Arbor, MI) prior to adding the test materials at 23°C. The test material in Petri dishes was conditioned in an open oven at 95°C for 24 hours, and cooled at room temperature. Samples of each test material of about 0.5 g were transferred to the glass vials which were sealed immediately. Glass vials containing 3 mm glass beads were used as the controls for a non-interactive agent. A 250mL 1-MCP stock gas was generated by mixing about 1 gram of an EthylBloc® powder and 30 mL distilled water in a glass vial, and sealing with PTFE/silicone septa and aluminum crimp caps (Fisher scientific, Pittsburgh, PA) and holding at 23°C for 10 hours. 500 μL aliquots of 1-MCP gas were extracted through the stock cell septum and immediately transferred into the 15 mL sealed vials containing a known amount of test material. After 1 hour, the 1-MCP concentration in the headspace of the glass cell was determined by extracting a 40 μL sample with a syringe through the septum of the 15 mL vial, and quantified using GC.

The 1-MCP partition coefficient between the test materials and air was calculated as follows:

a) Concentration of 1-MCP in the headspace (mass of 1-MCP/air mass)

$$C_g = \frac{(R_s \times k \times V_n)}{W_a \times V_i} \quad (36)$$

where R_s is the detector area response, k is the calibration factor from a standard calibration curve (g/A.U.), V_n is net vial volume (mL) representing the volume (a glass vial volume – the test material volume), V_i is an injection volume (mL), and W_a is the air mass (g) in the headspace.

b) Concentration of 1-MCP in test materials (mass of 1-MCP/mass of test material)

$$C_s = \frac{I_M - (C_g \times W_a)}{W_s} \quad (37)$$

where I_M is an amount of 1-MCP injected (g) and W_s is the test material mass (g).

c) Partition coefficient ($K_{s/g}$)

$$K_{s/g} = \frac{C_s}{C_g} \quad (38)$$

Adsorption efficiency

About 0.4 g of adsorbing agents was conditioned at 95°C for 24 hours to activate the binding sites on the surface. After cooling for two hours, samples of each test material were placed into the 15 mL empty glass vials (Supelco Inc, Bellefonte, PA). The vials were flushed with dry air and sealed immediately using screw caps and PTFE/silicon covered septa (Supelco Inc, Bellefonte, PA). A known amount of 1-MCP was transferred through the septa and exposed to the surface of the adsorbing agents in a dry air condition.

After exposing 1-MCP gas to the surface of the adsorbing agents, 0.5 mL of distilled water was added to the glass vial to obtain a high humidity environmental condition. After one hour, 40 μL of gas in the headspace was extracted from the sealed glass cell using a 100 μL gas tight syringe (Hamilton Co., Reno, NY).

The gas sample was injected into the gas chromatograph to quantitatively determine the amount of 1-MCP at the dry air and high humidity conditions, respectively. Each sample analysis was performed in three replicates.

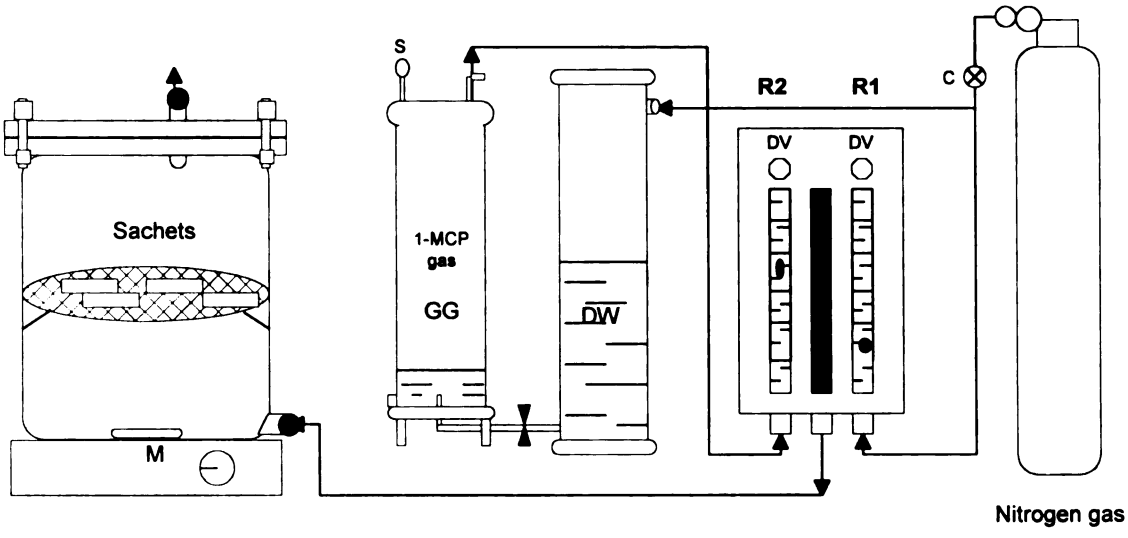
Release of 1-MCP gas through the sachets with adsorbing agents

Adsorbing agents were packaged in 3 cm × 4 cm test pouches, fabricated from the four test materials. Each adsorbing agent was conditioned at 80°C for 24 hours and placed into the sachets after cooling. Tyvek® (Style 1073B) and LDPE materials were sealed using an impulse heat sealer (Sencorp Systems Inc.) at 241.3 KPa with a 0.7 heating time and a 0.5 cooling time. PVA was sealed using an impulse heat sealer at 241.3 KPa with a 1 heating time and a 0.4 cooling time. Filter paper was sealed using casein based white glue (Elmer's Inc., Columbus, OH). For controls, each sachet material was sealed in the absence of adsorbing agents.

Dynamic 1-MCP gas trapping system for Tyvek® and filter paper sachets

The Tyvek® and paper sachets containing a known amount of silica gel (approximately 1 gram) and activated carbon (approximately 0.5 grams) were placed into a gas trapping glass cell and purged under dried nitrogen at a flow rate of 15 mL/min for 4 hours prior to exposing 1-MCP gas to the adsorbing agents in the sachets. A dynamic 1-MCP trapping system was developed, which is described below. As shown in Figures 60 and 61, the gas trapping glass cell was designed to allow the adsorbing agents to trap 1-MCP gas by the adsorbing agents at constant temperature. The glass cell was equipped with one metal-link shelf with a stainless stand, to allow exposure of 1-MCP gas uniformly onto the surface of the sachets. The glass cell was also equipped with a gas inlet and outlet port to provide continuous flow of the dried nitrogen gas stream and

1-MCP gas. Trapping 1-MCP gas onto the adsorbent agents was carried out at 23±1°C for 24hrs. A constant concentration of 1-MCP vapor was flowed continually through the test glass cell. When water reached the maximum level in the 1-MCP generator cell, the control valves at the gas inlet and outlet ports were closed. This allowed exposure of the adsorbing agents to 1-MCP gas for 24 hours.



1-MCP Trapping System

R1:Rotometer for dried gas
DV:Needle valve
C:Gas control valve
M: magnetic bar

R2:Rotometer for 1-MCP gas
DW:Distilled water tank
S:Sampling port
GG:1-MCP gas generating container

Figure 60. A schematic diagram of the 1-MCP gas trapping system used for sachets containing adsorbing agents.

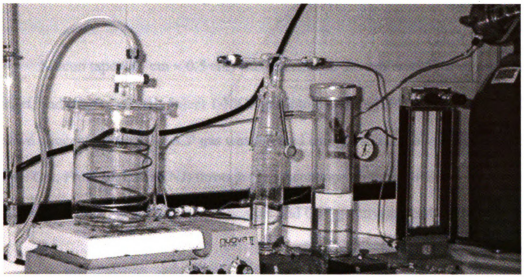


Figure 61. 1-MCP gas trapping system for the sachets containing adsorbing agents.

1-MCP generator and dynamic trapping system

A 1-MCP gas generator glass flask was interfaced to a water container, which supported a constant concentration of 1-MCP. A constant concentration of 1-MCP gas flowed continually through the adsorbent agent in the glass column by water pressure. The gas generator system was interfaced to a flow meter and needle valve. The 1-MCP generator and gas trapping apparatus are shown in Figure 61.

1-MCP (3 grams) as EthylBloc® powder, Floralife, Inc., (Walterboro, SC), 0.14% active ingredient, was weighed, and placed into a 320 mL glass flask holding 80 mL distilled water. After mixing, the solution was stored in a glass flask for 6 hours at room temperature to develop the appropriate concentration of 1-MCP gas.

Direct injection of 1-MCP gas for LDPE and PVA materials

Silicon tape (0.5 cm × 0.5 cm) was attached to the center surface of each sealed sachet, and used to directly inject 1-MCP gas into the sachet (Figure 62). 6 mL of a known concentration of 1-MCP gas was injected using a 10 mL polypropylene syringe (B-D. Co., Franklin Lakes, NJ) through the silicon tape on the LDPE and PVA sachets containing adsorbing agents, 0.5 g silica gel and 0.35 g activated carbon 20/40.

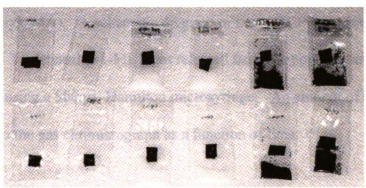


Figure 62. The sachets containing adsorbing agents ready for direct injection of 1-MCP.

Controlled release of 1-MCP gas from the adsorbing agents

After reaching equilibration at a constant concentration of 1-MCP gas, the 1-MCP gas generator system was closed using the gas control valve in the outlet port of the glass flask. A known amount of the 1-MCP saturated adsorbing agent was then packed in a 3 cm × 4 cm test sachet. The paper sachet was formed using glue to seal 3 sides. The Tyvek® sachet was sealed using an impulse heat sealer (Sencorp Systems Inc., Hyannis,

MA) at 241.3KPa with a 0.8 heating time and a 0.6 cooling time. A test sachet was positioned using stainless steel wire in an environmentally controlled glass cell which was tightly closed with a glass cover. Release conditions were selected to maintain the packaged sample in dried air (0% RH), and 90% RH using salt solution in a closed system with no air flow at 23°C. A saturated salt solution was composed of 370 g/L potassium nitrate in distilled water. The salt solution was prepared using the procedure described in ASTM E-104 (1999) to obtain a relative humidity over 90%. Relative humidity in the glass cell was then monitored using a hygrometer (Hygrodynamics Inc., Silver Springs, MA). A schematic diagram of the 1-MCP gas release system is shown in Figure 63. To determine 1-MCP gas released from the pouches, an aliquot was withdrawn using a 500 μL Hamilton microsyringe. An amount of this gas was injected directly into the gas chromatograph as a function of time.

Figure 63. A schematic diagram of the closed system for 1-MCP release study at controlled humidity conditions. (SS: Salt solution, H: sachet holder, P: sachet sample)

The concentration of 1-MCP released from the pouch was determined in duplicate for each time interval. The 1-MCP level in the closed system was calculated by substitution into the following equation:

$$\text{1-MCP level } (\mu g/g) = \frac{R_s \times C.F \times V_t}{V_i \times W \times 1000} \qquad (39)$$

where V_i is the injection volume (0.3mL), V_t is the total headspace volume (380mL at 90%RH and 480mL at dried condition), C.F. is the calibration factor from the external standard calibration curve (ng/A.U.), R_s is the detector response value for the sample obtained using GC, and W is the amount of adsorbing agent (g)

RESULTS AND DISCUSSION

Partition coefficient

Partition coefficient ($K_{s/g}$) values (Table 13) were determined as the ratio of the 1-MCP in the dried air headspace, to the concentration of the 1-MCP in the solid matrix.

Table 13. Partition coefficients of 1-MCP gas between solid matrix and air in a closed system at 23°C.

Solid matrix	C_{air} ($\times 10^{-6}$g/g)	C_{solid} ($\times 10^{-6}$g/g)	Partition coefficient ($K_{s/g}$)
Silica gel	704.21±24.43	637.67±4.51	0.92±0.01
Tenax-TA	714.60±16.00	649.98±6.33	0.91±0.03
Activated clay	5582.52±68.76	204.33±4.45	0.04±0.01
Activated carbon (20/40mesh)	52.79±12.52	698.69±2.45	13.78±3.45
Activated carbon (80/100mesh)	52.94±13.54	609.03±5.91	13.61±3.40
α-cyclodextrin (6 glucose monomers)	7753.63±113.31	10.68±10.10	≈0

The air density at 23°C used to calculate the partition coefficient values was 0.001176 g/cm^3. The results were carried out in triplicate at a 1-MCP concentration of 142μg. The partition coefficient values of the activated carbons were 13.78 (20/40mesh) and 13.61 (80/100mesh), respectively. These values indicate that the activated carbon has a very strong affinity for the 1-MCP molecules at 23°C. 1-MCP molecules also have good affinity for silica gel and Tenax-TA, but less than the activated carbons. Partition coefficients in silica gel and Tenax-TA were close to 1.00. The partition coefficient indicates the attraction of 1-MCP to the matrix. The affinity of 1-MCP for the matrix is

low when the partition coefficient is close to zero. These data are in good agreement with the results reported in chapter 5, which dealt with the interactions between 1-MCP and activated clay, and cyclodextrin materials. Low adsorption of 1-MCP on the cyclodextrin was expected (at dry condition) because 1-MCP is trapped through encapsulation by mixing with an aqueous solution (Bhandari, 1999).

The partition coefficient values for 1-MCP and the respective solid matrix at 23°C in shown in Figure 64. The results indicate that the partition coefficient values at a 1-MCP concentration of 142μg were not significant different from values at 86, 165, and 235μg of 1-MCP. These data demonstrate that the partition coefficients were not significantly affected by the concentration of 1-MCP in dried air. Biran (1979b) found that partition coefficient values of vinyl chloride were fairly constant for vinyl chloride concentrations between the headspace and tested food constituents. However, Halek (1988) reported that partition coefficient values were dependent on the concentration in a substrate between a cookie (or film) and air in a closed vial for each solvent. Kieckbusch (1979) measured the partition coefficients of acetates in sucrose solutions at different temperatures and concentrations. A sharp increase in the partition coefficient values with increasing sucrose concentration was observed.

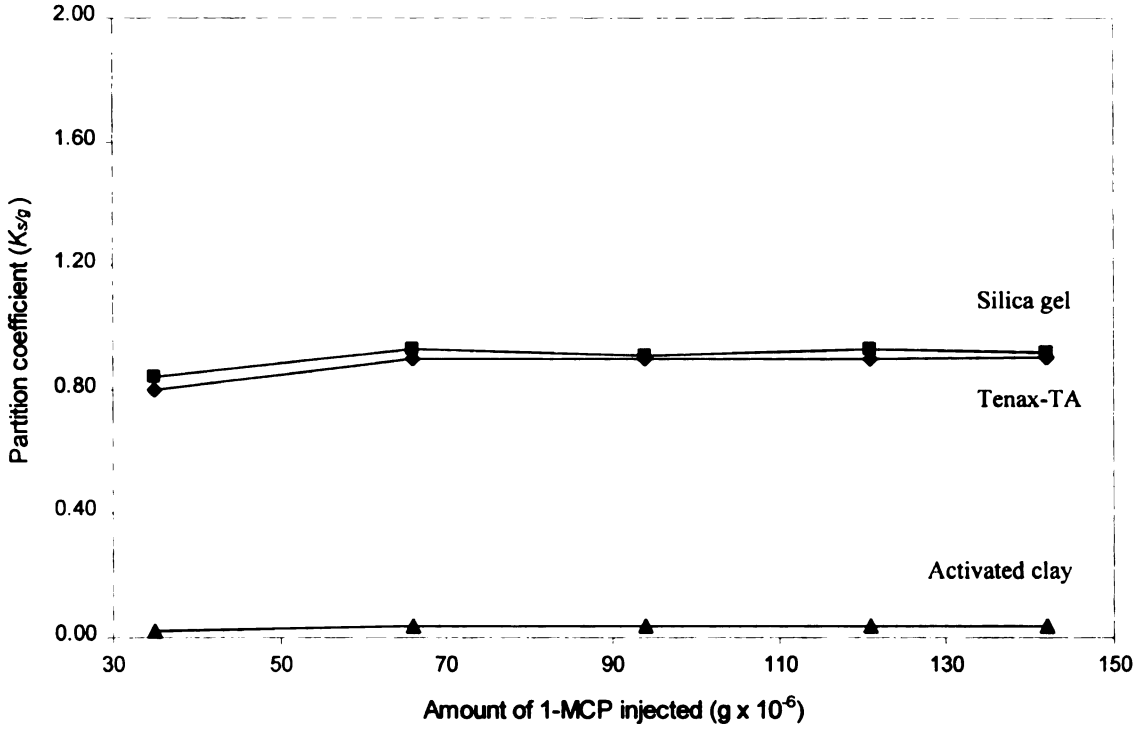


Figure 64. Partition coefficient of 1-MCP gas between solid matrix and air in a closed system as a function of the amount of 1-MCP injected at 23°C.

Comparison of 1-MCP gas partition coefficient values for sachet material and air in a closed system is shown in Table 14.

Table 14. Partition coefficient values of 1-MCP gas between sachet material and air in a closed system at 23°C.

Sachet materials	C_{air} ($\times 10^{-6}$g/g)	C_{solid} ($\times 10^{-6}$g/g)	Partition coefficient ($K_{g/s}$)
Filter paper†	473.98±3.64	2.34±0.98	≈0
Tyvek®†	433.47±15.65	6.40±0.44	0.01±0.001
LDPE‡	643.31±18.01	5.13±1.41	0.01±0.002
PVA‡	493.30±8.43	21.45±0.69	0.04±0.001

† and ‡ Values represent the average of triple tests at 1-MCP concentrations of 8.11μg and 11.62μg.

The partition coefficient of the PVA film/air system had a relatively higher value in comparison to LDPE film or Tyvek®. These values indicate some affinity for 1-MCP by the PVA film. However, the partition coefficient values of paper, Tyvek®, and LDPE were close to zero respectively, and thus no adsorption occurred. Halek (1988) indicated that isotatic polypropylene film had a higher affinity for hexane and toluene, and a lower affinity for oxygenated compounds in the film and air system. Gavara (1996) reported that polystyrene film had high partition coefficient values for toluene in water/polystyrene systems.

Partition coefficients can be affected by interaction of the 1-MCP sorbate, the solid matrix, and the packaging film, including polarity, molecular structure, solubility, and active adsorption sites in the matrix. Biran (1979b) reported that the chemical nature of the sorbent, the sorbate concentrations, and temperature are important factors affecting the affinity of the migrant for the contacting phase.

Permeability of 1-MCP gas and water vapor through the sachet materials

Water vapor and 1-MCP gas permeability effect the selection of sachet materials by impacting the slow release of the 1-MCP molecules from the adsorbing agents. Results for the water vapor permeability of the respective sachet materials are presented in Table 15. These data showed the average of three samples for Filter paper and Tyvek®, and the average of two samples for LDPE and PVA. Each value is expressed in SI units.

Table 15. Water permeability of sachet materials used in the 1-MCP release system.

Materials	Transmission rate	Permeability coefficient	Conditions
Filter paper	2.77±0.24†	5.25±0.46§	25°C, 60%RH
	9.41±0.17†	6.16±0.11§	37°C, 89%RH
Tyvek® (1073B)	2.01±0.28†	3.80±0.52§	25°C, 60%RH
	8.93±0.29†	5.84±0.19§	37°C, 89%RH
LDPE	0.26±0.01‡	0.85±0.03*	23°C, 90%RH
	0.63±0.03‡	1.22±0.05*	32°C, 90%RH
PVA	2.13±0.04‡	6.96±0.09*	23°C, 90%RH
	4.46±0.03‡	8.60±0.25*	32°C, 90%RH

† and ‡ Transmission rate values represent in SI units of $\times kg/m^2 \cdot day$ and $\times 10^{-2} kg/m^2 \cdot day$, respectively.
§ and * Permeability coefficient values represent in SI units of $\times 10^{-11} kg \cdot m/Pa \cdot s \cdot m^2$ and $\times 10^{-14} kg \cdot m/Pa \cdot s \cdot m^2$, respectively.

Filter paper and Tyvek® materials exhibited relatively high permeability values, due to their porous structures. Thus, moisture from the fresh produce could easily reach the adsorbing agent through the sachet material. LDPE film showed good water vapor barrier properties, compared to the filter paper and Tyvek® materials. The permeability of water vapor through PVA film was approximately 10 times greater than the permeability through LDPE film. The materials also exhibited an increase in

permeability of water vapor with an increase in temperature from 23°C to 32°C. The results shown are similar to those for Tyvek® as reported by Peelmaster packaging Co. Niles, Illinois (Peelmaster Co., 2002) and data for LDPE published by Piergiovanni (1995).

The transmission curves of 1-MCP molecules through LDPE and PVA films at 23°C are presented graphically in Figures 71 and 72. The typical transmission profile curve for the PVA film includes a large lag time while the LDPE film exhibits 1-MCP permeation early in the permeability test period. For the first 3 hours, no permeation through the PVA film was detectable. Following this initial induction period, a significant increase in the transmission was observed, after 9 hours steady state was obtained. The straight line portion of the curve was used to approximate the steady state portion by performing a linear regression analysis.

Table 16. 1-MCP gas permeability of selected sachet materials

Materials	Transmission rate	Permeability coefficient	Diffusion coefficient	1-MCP conc. (ng/mL)
LDPE	0.78±0.03†	2.94±0.29‡	1.33±0.09§	25
PVA	0.45±0.03†	3.47±0.25‡	5.98±0.22*	10

† Transmission rate values represent in SI units of $\times 10^{-6}$ kg/m^2·day.
‡ Permeability coefficient values represent in SI units of $\times 10^{-16}$ kg·m/Pa·s·m^2.
§ and * Diffusion coefficient values represent in SI units of $\times 10^{-12}$ m^2/sec and $\times 10^{-14}$ m^2/sec, respectively.
The values were performed at 23°C, 43%RH condition.

1-MCP permeability coefficient values for LDPE and PVA films were determined using a quasi-isostatic test method. Constant 1-MCP gas concentration flow of 25ng/mL for LDPE film and 10ng/mL for PVA were used throughout the test period. The permeability coefficient values of LDPE and PVA films at a given 1-MCP concentration

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(Table 16) were determined to be 2.94×10^{-16} kg·m/Pa·s·m^2 and 3.47×10^{-16} kg·m/Pa·s·m^2, respectively. These results showed that the water vapor permeability coefficients for LDPE and PVA films was approximately 200 times greater than the values for 1-MCP permeability coefficient through LDPE and PVA films at 23°C. This may impact the release of 1-MCP from the sachet pouch. Moreover, the diffusion coefficient of 1-MCP for the LDPE film was approximately 22 times greater than that for the PVA film. Thus, 1-MCP molecules can move faster through the LDPE structure, compared to the PVA structure. The lower solubility coefficient of 1-MCP for the LDPE film than that for the PVA film might be expected, because the permeability depends on both the solubility and the rate at which permeant diffuses through the film (equation 20 in Chapter 2). It represents the relative higher affinity of 1-MCP molecules for the PVA structure. These results are consistent with the partitioning of 1-MCP between each film structure and air (Table 13). These results are comparable to the data from Zobel (1985) regarding the permeability of organic compounds through LDPE. The permeability of limonene and linalool through LDPE was 1.90×10^{-16} kg/m^2·day and 1.80×10^{-16} kg/m^2·day at 10.67 Pa vapor pressure, determined using a quasi-isostatic procedure. The diffusion coefficient of the 1-MCP gas through LDPE was higher than that for PVA. The diffusion coefficients were affected by several factors, including the film barrier properties and absorption properties of its molecular structure. Sadler (1991) mentioned that diffusion coefficients relating to absorption of flavor volatiles by LDPE depended upon the amount and volatility of the absorbed volatile.

1-MCP adsorbing efficiency

The efficiency of 1-MCP adsorption to the matrix agents was determined from the difference between the initial added 1-MCP, and that remaining in the headspace of the matrix material in a closed system at 23°C. Table 17 shows the adsorbing efficiencies for the different adsorbing agents.

Table 17. 1-MCP adsorbing efficiency of selected adsorbing agents in a closed system.

Solid matrixes	Average weight (grams)	Adsorbing efficiency (%)*	
		A dry air (0%RH)	After treating with distilled water
Silica gel	0.5023	88.57	70.56
Tenax-TA	0.4610	89.42	79.93
Activated carbon (20/40mesh)	0.5072	98.01	98.17
Activated carbon (80/100mesh)	0.4712	98.86	98.21

*Adsorbing efficiency is the amount of 1-MCP in solid matrix × 100/ amount of 1-MCP applied in a closed system. The values are the results of duplicate analyses.

Activated carbon (80/100 mesh) had an adsorbing efficiency of 98.86% in dried air, in a closed system. This result is in agreement with the partition coefficient values and that obtained in Chapter 5, comparing 1-MCP adsorbing efficiencies of silica gel, Tenax-TA, and activated clay. After treating with distilled water, silica gel showed a relatively lower adsorbing efficiency, compared to that under dry air. Activated carbon had a strong affinity for 1-MCP even when treated with distilled water. Smísek (1970) found that activated carbon had high affinity for organic molecules, and other non-polar substances because most forms of activated carbon are non-polar. Zhou (2002) measured the adsorption equilibria of nitrogen on silica gel over a large range of

temperatures. The transition of adsorption mechanisms from the sub- to the supercritical region is considerably different for the adsorption of gaseous nitrogen by activated carbon and silica gel.

Several studies were performed to determine the sorbate efficiency of the matrix agents. Zhu (2001) determined the encapsulation efficiency of a starch-g-poly(vinyl alcohol) matrix for 2,4,5-trichlorophenoxyacetic acid herbicide as influenced by the starch grafting ratio. Increasing the grafting ratio, increased the encapsulation efficiency (%) and consequently reduced the release rate of the herbicide. Jiang (2001) reported on the preparation of a myoglobin (Mb) loaded polymethacrylic acid (PMAA)/gelatin complex and the release characteristics of myoglobin in order to evaluate the polyanion/gelatin complexes as matrices. Mb entrapment efficiency in PMAA/gelatin decreased with increase in the Mb/PMAA ratio. Even with a large Mb/PMAA ratio, an entrapment efficiency of over 80% was achieved. Fernández-Pérez (1999) investigated the controlled release of diuron from an alginate-bentonite formulation. The encapsulation efficiency of diuron was higher than 98%.

1-MCP release

1-MCP release from the respective sachet materials containing either silica gel or activated carbon was studied. The cumulative release of 1-MCP gas from each sachet pouch containing silica gel and/or activated carbon at two different environmental conditions in dried air and 90% RH, is shown in Figures 65-67. The equilibrium

concentration of 1-MCP was approximately 22.40×10^{-6} g/mL for Tyvek® and paper sachets in a gas trapping glass cell. As shown in Figures 65 and 66, most of the 1-MCP gas was rapidly released (less than 1 hour) in a closed system. The release of 1-MCP through the porous surface of the Tyvek® and filter paper was significantly faster than that through the film structure. Tyvek® and filter paper sachet pouches, with no adsorbing agents, were used as controls. No 1-MCP molecules were released because these sachet materials have low affinity for 1-MCP molecules throughout a dynamic gas trapping system. However, silica gel and/or activated carbon have high affinity for 1-MCP molecules. Specifically, the Tyvek® and paper sachets containing activated carbon released no 1-MCP at both dry air and 90%RH conditions during the storage study. The Tyvek® and paper sachets containing silica gel released different amounts of 1-MCP between dry air and 90%RH conditions (Figures 65 and 66). This indicates that a certain amount of moisture absorbed through the sachet materials can enhance the desorption of the 1-MCP molecules from the active binding sites of silica gel. 1-MCP molecules may then permeate through the sachet material.

Approximately 122×10^{-6} g/mL of 1-MCP gas were directly supplied into each LDPE and PVA sachet containing silica gel, activated carbon, and control (without adsorbing agent). As shown in Figure 67, LDPE sachets released 1-MCP at about the same rate at 90% RH and dry air conditions, because LDPE has good barrier properties to water vapor and 1-MCP gas. The 1-MCP release rate through the films was mainly dependent on 1-MCP permeation through the film. However, PVA is a lower moisture barrier film than LDPE film. Thus, the moisture can easily get through the film, and help desorb 1-MCP from the silica gel. Therefore, the release rate of 1-MCP from the

PVA sachets containing the silica gel at 90%RH is greater than that at 0%RH in a closed system (Figure 70, C). Activated carbon did not release 1-MCP gas regardless of the sachet materials or storage conditions (Figure 65 and 67). Thus, water is not a strong release factor for activated carbon. Activated carbons have high adsorptive capacity for herbicides at low concentrations (Bosetto, 1992).

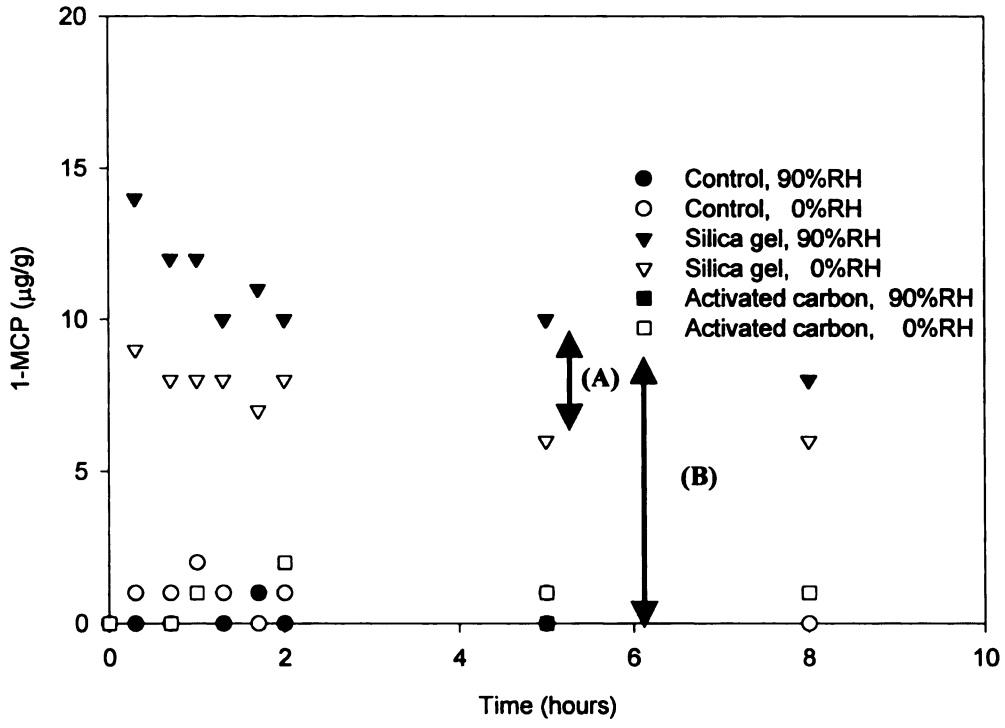


Figure 65. 1-MCP gas release from the Tyvek® sachets in a closed system at 23°C.

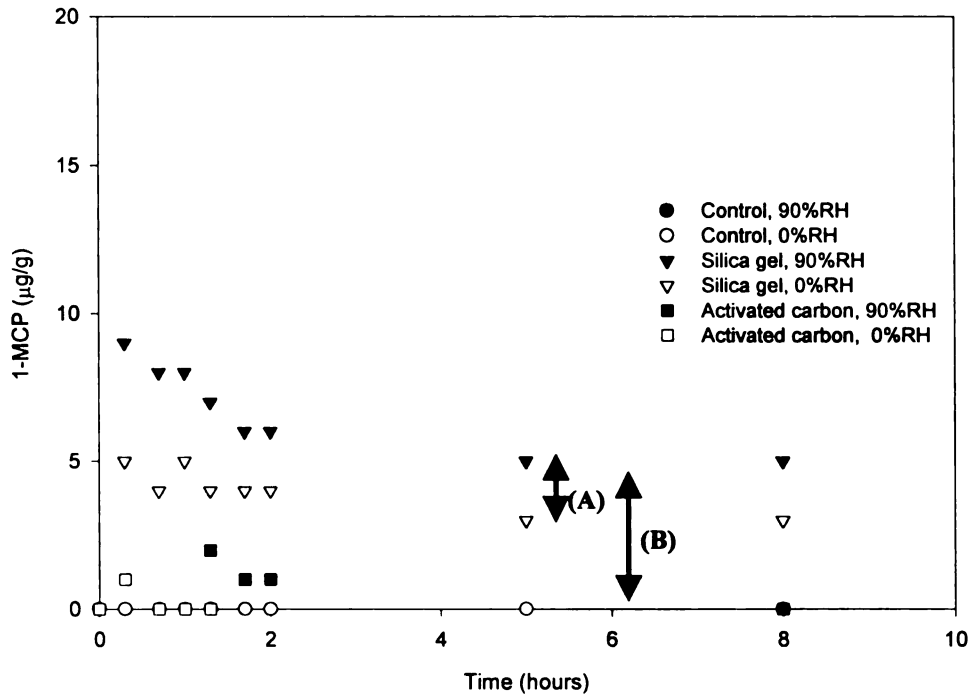


Figure 66. 1-MCP gas release from the paper sachets in a closed system at 23°C.

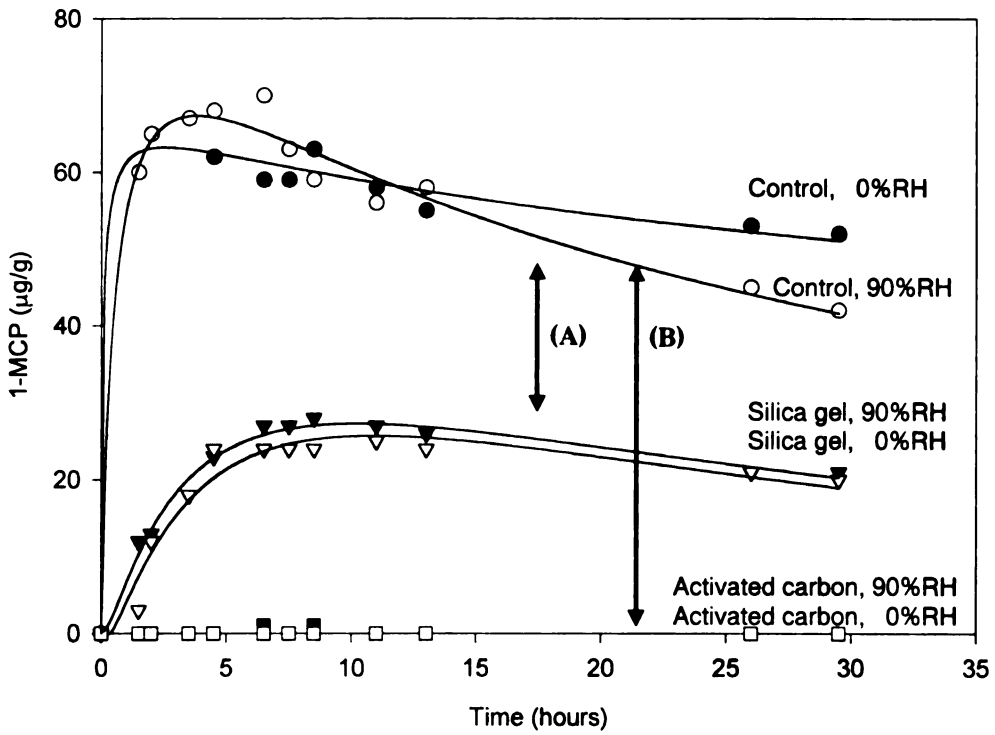


Figure 67. 1-MCP gas release from the LDPE sachets in a closed system at 23°C.

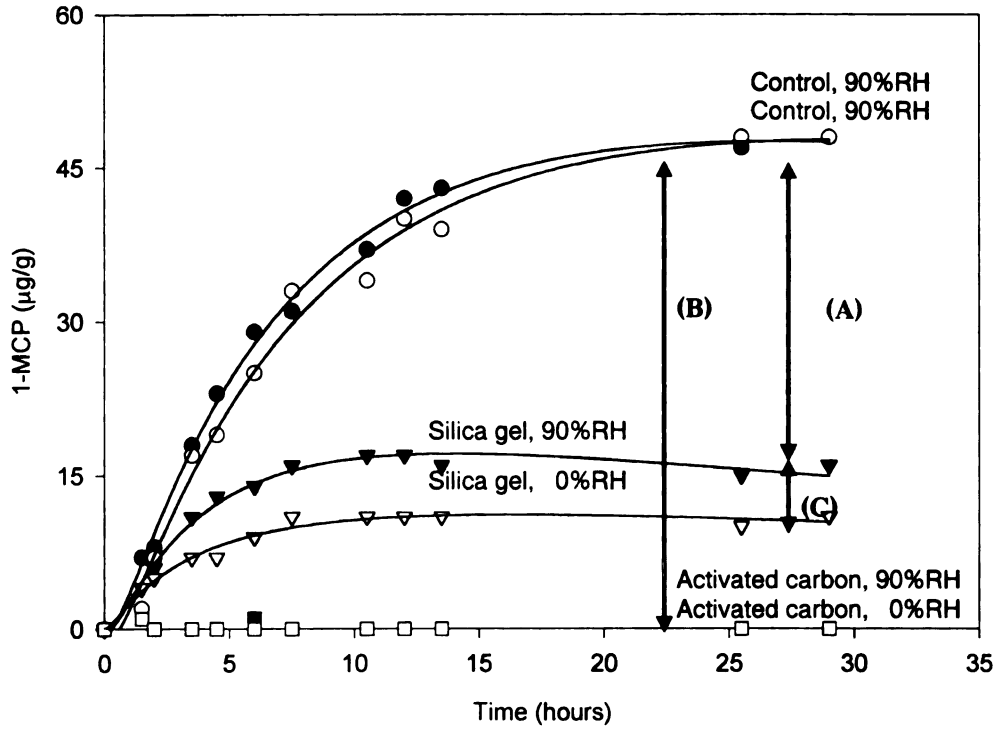


Figure 68. 1-MCP gas release from the PVA sachets in a closed system at 23°C.

CONCLUSION

Release of 1-MCP from a sachet can be influenced by a large number of factors such as, characteristics of the film materials, the adsorption abilities of the adsorbing agents, the concentration of sorbate, the size of the sachet and environmental factors. Sachets containing silica gel and activated clay can be adapted to trap 1-MCP using a dynamic adsorbing method for paper and Tyvek® sachets, and direct injection methods for LDPE and PVA sachets in a closed system.

Silica gel is suitable for binding and release of 1-MCP gas because the silica gel has good binding efficiency at a dry air, and relatively lower binding efficiency of 1-MCP at a high relative humidity condition.

1-MCP gas held by the adsorbing agent must release only under the environmental conditions in the presence of the fresh produce. 1-MCP gas held by the adsorbing agent may desorb into the headspace containing the fresh produce at high relative humidity because most fresh produce continues to lose moisture into the storage or package atmospheres through transpiration. Thus, it is important to change the binding efficiency of 1-MCP on the adsorbing agent, depending on the relative humidity during storage.

1-MCP release from filter paper and Tyvek® sachets was not affected by the sachet materials but largely depended on the sobate adsorbing ability of the adsorbing agents. The release rate of sachets made from LDPE and PVA were significantly influenced by the permeation rate of the film structures. PVA is more suitable for slow release of 1-MCP gas than LDPE because PVA has higher water vapor permeability and

the same lower permeability to 1-MCP gas, compared to LDPE. Therefore, PVA sachets containing silica gel have potential use for slow release of 1-MCP.

Activated carbon proved to be a very strong binding agent for 1-MCP due to its high adsorbing efficiency. Activated carbon did not release 1-MCP molecules during the test periods. However, multiple adsorbing agents, a combination of activated carbon with silica gel, may have good potential for 1-MCP delivery in long term storage.

From the experimental results using a closed system, the selection of intermediate water barrier films containing silica gel could be effective for slow release of 1-MCP from a sachet in an open environmental system.

APPENDIX D

The salt employed and its corresponding measured relative humidity is shown in Table 18.

Table 18. Salt solution and its corresponding relative humidity at 23°C

Headspace air condition	Relative humidity at 23°C	Source
Dried air gas	≅ 0%	AGA Gas Inc., Maumee, OH
Potassium nitrate	90±2%	EM Science, NJ

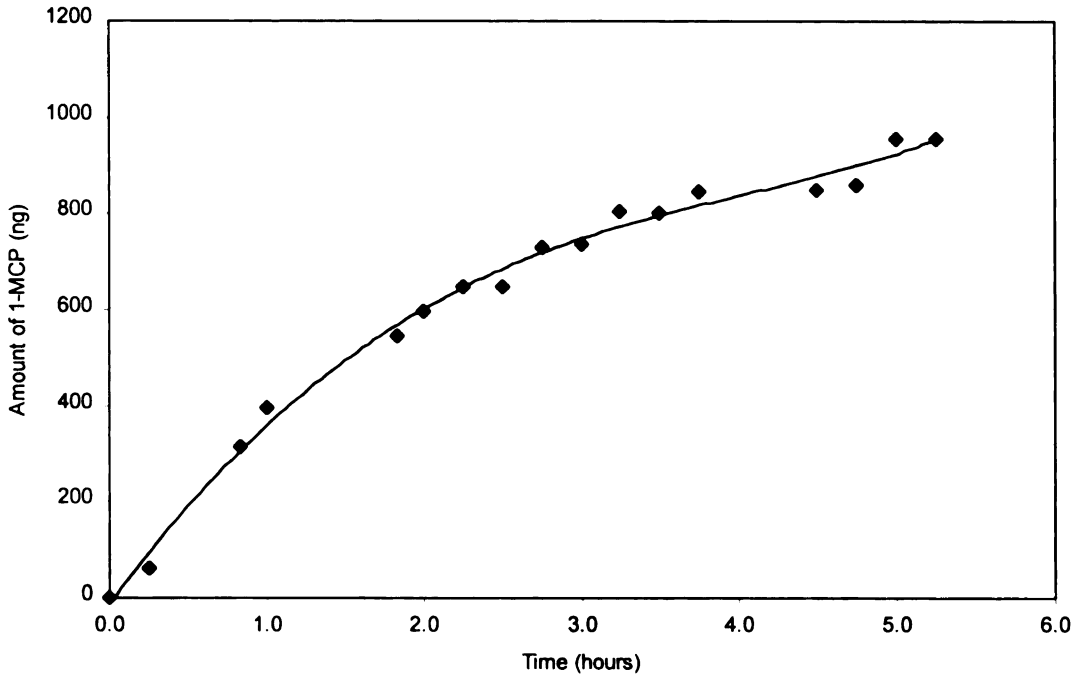


Figure 69. 1-MCP gas transmission of LDPE film structure at 23°C

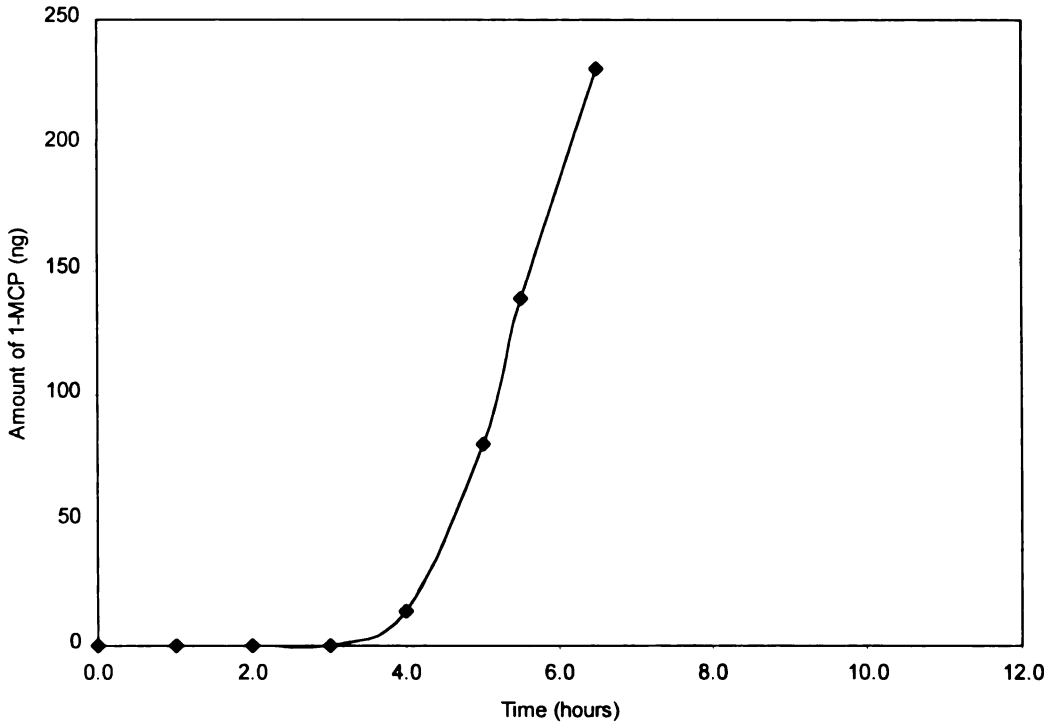
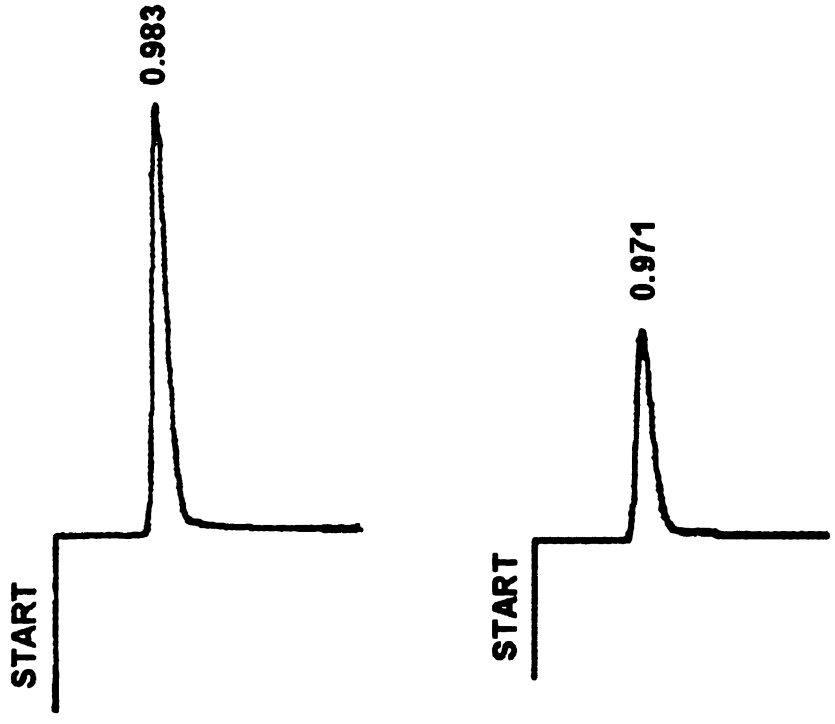


Figure 70. 1-MCP gas transmission of PVA film structure at 23°C



(a) 1-MCP (b) 1-Butene

Figure 71. Chromatographic peak shapes of 1-MCP and 1-Butene on SPB-5 nonpolar capillary column at 80°C for 1-MCP standard calibration curve: (a) 1-MCP (0.14μg), (b) 1-Butene (0.06μg).

NOMENCLATURE

a	Amount of sorbate adsorbed per unit mass of adsorbent (Eqn. 1)
A	Film surface area (Eqns. 16, 22, 23, 24, 25, 27)
A_a	Chart area bounded by the common curve along the peak height maxima; the gas holdup distance (cm^2) (Eqn. 2)
A_s	Calibration peak area (cm^2) on the recorder chart when N moles of the sorbate gas are injected into the column (Eqns. 2, 3)
A_λ	Absorbance (OD units) at a given wavelength (Eqns. 30, 32)
c	Concentration of sorbate (Eqns. 1, 17)
C_g	Concentration of 1-MCP in the headspace (mass of 1-MCP/air mass) (Eqns. 36, 37, 38)
C_i	Amount of injected ethylene (ppm, v/v) (Eqn. 29)
C_{ss}	Steady state concentration of permeant (Eqn. 25)
C_s	Concentration of 1-MCP in test materials (mass of 1-MCP/mass of test material) (Eqns. 37, 38)
D	Diffusion coefficient (Eqns. 20, 21, 22, 23, 26, 28)
ε_λ	SEC of the solution at wavelength λ (Eqn. 30)
$E^{1\%}_{1cm}$	Specific extinction coefficient (SEC) of a lycopene (Eqn. 32)
F	Flux in the permeation process (Eqns. 16, 18, 21)
f	Rate of carrier gas flow (Eqns. 25)
F	Flux (Eqns. 16, 18, 21)
F_c	Correct gas flow rate (mL/s) (Eqns. 4, 6, 7, 8, 10, 11)
F_r	Measured flow rate (mL/s) (Eqn. 7, 10)
h	Recorder pen deflection (peak height, cm) (Eqn. 3)
I_M	Amount of 1-MCP injected (Eqn. 37)
J	James and Martin compressibility factor (Eqns. 6, 7, 8, 9, 11)
k	Calibration factor from a standard calibration curve (Eqn. 36)
K	Kelvin degree temperature
K_d	Distribution constant (Eqns. 5, 13)
K_s	Henry's law solubility coefficient (or S) (Eqn. 33)
$K_{s/g}$	Partition coefficient (Eqn. 38)
ℓ	Film thickness (Eqns. 21, 22, 23, 24, 25, 26, 27, 28, 35)
l	Beam-path (1cm) in the measuring cuvette (Eqn. 30)
θ	Lag time (Eqn. 28)
m	Amount of 1-MCP expressed in moles (Eqns. 2, 3)
M_a	Uptake moles of sorbate gas per unit weight of adsorbent (mole/g) (Eqns. 2, 33)
p	Partial pressure of 1-MCP vapor (Eqns. 3, 33)
P	Permeability coefficient (Eqns. 20, 24, 25, 27)
p_i	Column inlet pressure (Eqn. 9)
p_o	Column outlet pressure (Eqns. 9, 10)
p_W	Partial pressure of water at the room temperature (Eqn. 10)
ΔP	Partial pressure gradient (Eqns. 24, 25, 27)
q	Chart speed (cm/min) (Eqn. 3)

Q	The amount of the permeant molecules permeated (Eqn. 16, 22, 23, 24, 27)
R	Universal gas constant (Eqns. 3, 13, 14)
R_s	Detector area response (Eqn. 36)
S	Solubility coefficient (or K_s) (Eqns. 17, 20)
T	Collecting time (hrs) of external ethylene produced in a tomato (Eqn. 29)
T_c	Column temperature (^oK) (Eqns. 3, 10, 11, 12, 13, 14, 15)
T_r	Room temperature (^oK) (Eqn. 10)
t	Unit time (Eqns. 16, 22, 23, 24)
t_r	Retention time of the 1-MCP (Eqns. 4, 6, 7, 11)
t_m	Retention time of an unsorbed compound (air) (Eqns. 6, 8, 11)
v	Corrected carrier gas flow rate (mL/min) (Eqn. 3)
V_c	Retained volume of adsorbate at concentration, C (Eqn. 1)
V_i	Injection volume (mL) (Eqn. 36)
V^o_g	Specific retention volume (Eqns. 11, 12, 14)
V_M	Volume of an unsorbed compound (air) peak (Eqns. 5, 6)
V^o_M	Corrected retention volume of a non-retained compound (Eqns. 6, 8, 11)
V_N	Net retention volume (Eqns. 6, 12)
V_n	Net vial volume (Eqn. 36)
V_R	Retention volume of sorbate peak (Eqns. 4, 5, 6)
V^o_R	Corrected retention volume of a retained sorbate (Eqns. 6, 7, 11)
V^*_R	Adjusted retention volume excluding the void volume (Eqns. 5, 6)
V_s	Volume of the stationary phase (Eqns. 5)
V_t	Total headspace volume (Eqn. 29)
V_{total}	Total sample volume (Eqn. 32),
W	Adsorbent weight in the column (Eqns. 1, 2, 11, 12)
W_a	Air mass (g) in headspace (Eqn. 36)
W_s	Test material mass (Eqn. 37)
W_t	Tomato weight (g) (Eqns. 29, 32)
ΔG_s	Free energy of sorption (kcal/mol) (Eqns. 13, 15)
ΔH_s	Enthalpy changes (kcal/mole) (Eqns. 14, 15)
ΔS_s	Entropy changes (kcal/^{o}K mole) (Eqn. 15)

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