EFFECT OF ATMOSPHERIC PRESSURE FLUCTUATIONS ON BULK GAS FLOW AND COMPOSITION OF FLAVOUR VOLATILES FROM BULKY PLANT TISSUES

By

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ABSTRACT

The occurrence of total gas pressure gradients, which act as a driving force for the mass transport of fluids, may be common phenomena in plant organs. A gas-exchange system was devised to characterize changes in CO_2 emission rates of greenhouse tomato fruit (*Lycopersicon esculentum* L. Dombito), greenhouse green bell pepper fruit (*Capsicum annum* L. Doria), slicing cucumber fruit (*Cucumis sativa* L. Straight Eight and Sweet Success) and jumbo yellow onion bulbs (*Allium cepa* L.) in response to the imposition of total gas pressure gradients. Cyclical variations in atmospheric pressure induced significantly higher rates of gas exchange in peppers and onions but not in tomatoes and cucumbers. Oxygen concentration significantly affected carbon dioxide efflux rates in onions subjected to variable pressures. Temperature had no significant effect on relative efflux rates in any of the plant organs used in this study. Duration of the interval between varying pressure treatments was an important factor in CO_2 emission rate in onions, tomatoes and cucumbers. The differing response of various commodities to varying pressure treatments was probably due to differences in routes of gas exchange as well as intercellular space volumes and internal structure.

To determine if variable pressure treatments had a metabolic effect on tissues, a dynamic head-space sampling technique was developed to collect and concentrate aroma volatiles for analysis by gas chromatography/mass spectrometry. Principal component analysis of pepper, onion and tomato volatiles revealed that variable pressure storage increased levels of compounds associated with oxidation compared to those stored under constant pressure. Data from peppers stored under 3% oxygen and variable pressures for 1 week indicated that compounds associated with off-flavours were lower than in peppers stored in air.

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LIST OF ABBREVIATIONS

GC, - Gas chromatograph, gas chromatography

GC-MS - Gas chromatography coupled with mass spectrometry

DCAP - Duration of the interval of constant pressure between 5 min variable pressure treatments.

HPLC - High performance liquid chromatography

NMR - Nuclear Magnetic Resonance

CA - Controlled atmosphere

MA - Modified atmosphere

ppm - Parts per million ($\mu L\ L^{-1}$)

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INTRODUCTION

Shortly after the turn of the century the marketing of fresh plant produce was revolutionized by the introduction of mechanical refrigeration. Huge profits were realized by reduction in postharvest losses, extension of the fresh produce season, and generation of new marketing opportunities far removed from the location of crop production.

Several decades later, refrigerated controlled atmosphere (CA) storage was developed for commercial use in the storage of tree fruit crops in North America and Western Europe. The primary goals of CA storage are to control the concentrations of oxygen, carbon dioxide, water vapour and ethylene in the atmosphere surrounding plant produce in order to slow the rates of deterioration and decay. Conventional CA storage, with O_2 levels of 2 to 5% and CO_2 levels of 2 to 5% with the remainder balanced by N_2, will maintain pome fruit quality longer than storage in air. Since the introduction of CA storage, the system has benefited from a number of refinements. Storage of apples in atmospheres containing 1.5% O_2 ("Ultra-low Oxygen CA") and the imposition of CA conditions in less than 6 days ("Rapid CA") have shown promise both on a research and a commercial scale (Little and Peggie, 1987).

"Modified atmospheres" (MA) is a term more commonly applied to the manipulation of the atmosphere surrounding the commodity by the introduction of a specific gas mixture into a sealed wrap or the use of specially designed gas permeable plastic films. MA differs from CA only in the precision of control over external gas partial pressures, CA being more precise than MA.

The reasons for the beneficial effects of CA and MA on fruits and vegetables are complex. Lowering the O_2 partial pressure around fruits and vegetables reduces their respiration rate in proportion to the O_2 concentration. However, a shift from aerobic to anaerobic respiration will occur if the O_2 level

falls below 1-3%, depending on the commodity (Solomos, 1982). Under such conditions, the glycolytic pathway replaces the Krebs cycle as the main source of energy for plant tissues. Instead of being oxidized, pyruvic acid is decarboxylated to form acetaldehyde, CO_2 and ultimately, ethanol, resulting in off-flavours and tissue breakdown (Kader, 1986). Although the O_2 concentration within a cell may be as low as 0.2% before anaerobic respiration occurs, the gradient of O_2 concentration from that cell to the external atmosphere requires that the commodity be maintained in an atmosphere containing substantially higher levels of O_2 (Solomos, 1982). The required O_2 concentration in the storage environment depends on the rate of O_2 consumption and the rate of gas diffusion through dermal and subdermal tissues of the specific cultivar of each fruit or vegetable.

Elevated CO_2 concentrations also reduce the respiration rate of fruits and vegetables, but above a level of about 20% (lower or higher depending on O_2 concentration) there is a danger of anaerobiosis in part of the plant organ due to inhibition of decarboxylation reactions of normal respiration. Storage of fresh plant tissues in elevated CO_2 atmospheres reportedly inhibits glycolysis and succinic dehydrogenase activity, reduces formation of citrate/isocitrate and α-ketoglutarate and may also have an uncoupling effect on oxidative phosphorylation (Kader, 1986).

Ethylene has been shown to damage various fruits and vegetables by accelerating senescence. Reduced O_2 levels can decrease the rate of ethylene production and reduce the sensitivity of horticultural produce to ethylene (Chen et al., 1981). Elevated CO_2 may reduce plant tissue response to ethylene presumably by competing with ethylene for binding sites on ethylene receptors (Veen, 1987) but it may also induce the production of ethylene in the presence of light (Kao and Yang, 1982). The presence of nonethylenic volatile

compounds in the storage atmosphere can inhibit regrowth in tubers or cause bleaching of green vegetables (Lougheed et al; 1987).

Exchange of CO_2, O_2, ethylene and volatile compounds between the outer atmosphere and internal tissues occurs along a concentration gradient by: gaseous diffusion through the dermal system and the intercellular system, an exchange of gases between the intercellular atmosphere and cell sap, and diffusion in solution within the cell to centers of metabolic activity. Diffusive movement of gases is not always adequate to prevent excessive CO_2 build-up or O_2 depletion in bulky plant organs or parts of the organs. Mass gas flow, on the other hand, would not be restricted to movement along concentration gradients. Although mass gas flow does not occur in bulky tissues under normal storage conditions, such gas movement may take place wherever temperature or pressure gradients exist.

Many of the recent CA experiments have revealed that small changes in the storage environment can have a significant response in terms of extending shelf-life for days or weeks, enhancing quality, and ameliorating or controlling CA injuries. To date, little research attention has focused on mass gas transfer as a means to alter internal gas compositions thus effecting an "ultra rapid CA". Consequently there is little information characterizing the effects of pressure-driven gas transfer between fruit and vegetable commodities and their surroundings. Information obtained by Jolliffe and Dyck (1988) on pressure-driven bulk gas flow and Corey and Tan (1990) on temperature-driven bulk gas flow implies that internal gas compositions of large plant organs could be altered at a much faster rate than through diffusion. In this light, characterization of factors which may affect rate of mass gas transfer is of both theoretical and practical interest. Identification of the more subtle metabolic effects of mass gas transfer may be useful in determining the benefit of mass gas transfer. The main

objectives of this study were: (1) to ascertain if variations in external atmospheric pressure induce bulk gas flow in onion, tomatoes, sweet peppers and cucumbers, (2) to determine the effects of factors such as temperature, oxygen concentration, and timing of atmospheric pressure variations on the induction of bulk flow in these plant tissues, and (3) to elucidate the short-term effects of variable pressure treatments on emissions of aroma compounds of onions, tomatoes, and sweet peppers. Onion, sweet pepper, tomato and cucumber were chosen for this study because these vegetables represent diverse morphological types with a variety of internal matrices and, therefore, more likely to differ in their response to varying atmospheric pressures.

LITERATURE REVIEW

A. PHYSIOLOGICAL GASES AND THEIR ROLE IN TISSUE FUNCTION

In plant tissues there are individual gaseous spaces which may be interconnected with one another forming a continuous air-space in the whole plant. The air spaces contain the main components of air, i.e., nitrogen and oxygen, and also carbon dioxide, the product of respiration as well as volatile products of metabolism. The proportions of these gases are different from normal air, however. The proportion of CO_2 has been found to be generally in the range of 3 to 6%, and in many cases, as high as 20 and 30% while the concentration of oxygen must be lower than ambient air (Phan, 1987). In an intact plant ethylene may occur at concentrations ranging from 100 to 1000 ppm. Although minute amounts of this gas may trigger an increase in respiration rate in a harvested tissue, in the attached organ such effects are suppressed, possibly due to the antagonistic action of a high internal concentration of CO_2 (Burg and Burg, 1965).

Upon detachment of the plant organ, there is an inflow of oxygen through the cut end of the pedicel and an outflow of CO_2 resulting in an abrupt increase in the respiration rate and an increase in all oxidative processes. Ethylene apparently escapes through the harvest wound and the internal concentration can fall as low as 0.5 to 1 ppm. The combined effects of an increase in the respiratory process and healing of the harvest wound allows ethylene to accumulate to preharvest levels in a short period of time (Phan, 1987).

Another consequence of harvest is the interruption of the water supply to bulky organs or leafy tissues. Vaporization, or the passage of water molecules from the liquid phase to the vapor phase continues after harvest creating a water deficit within plant tissues and a consequent loss of turgor. Postharvest

increases in respiratory activity result in a greater production of heat and an augmented loss of moisture. Resistance to water vapour loss is derived mainly from the cuticular layer (Burg and Burg, 1965; Ben-Yehoshua et al., 1985).

The Importance of Oxygen in Tissue Biochemistry and Physiology

Generally, reducing the external O_2 concentration will decrease respiration rate of vegetables. Evidence indicates that the substantial decrease in rate of oxygen uptake that occurs with decreasing external oxygen concentrations is connected with the enzymes involved in the respiratory machinery, rather than being an indirect effect of a decrease in the general metabolism of the tissue (Solomos, 1982). For any given external O_2 level in storage, the cells at the center of a bulky tissue will experience significantly lower O_2 levels than those at the surface if the effective diffusivity is low (Rajapakse et al., 1990). Under low-O_2 environments, like those encountered in CA or MA storage, this gradient could result in loss of quality.

Vegetable crops reacting positively to CA conditions, require a minimum of 1-3% O_2 in the storage atmosphere. Asparagus and potatoes require an external concentration of 10% O_2 otherwise inner tissues become anaerobic as internal oxygen levels fall below the respiratory minimum which may be as low as 0.2% O_2 (Kader, 1986). This may occur not only in low O_2 storage but also in piles of warm, poorly aerated vegetable material.

In an anaerobic environment pyruvic acid is no longer oxidized but is decarboxylated to form acetaldehyde, CO_2 and finally ethanol. Weichmann (1987) stated that such a change in metabolism is not the result of specific biochemical reactions of certain crops to a low O_2 environment but of the anatomy and morphology of those crops. Thus, certain varieties of apples respond well to ultra-low O_2 storage while other varieties, or even the same

variety in a different harvest year, will suffer adverse effects under such conditions (Sharples and Johnson, 1987).

The explanation for the reduction in respiratory metabolism as a reaction to low O_2 concentration (10-11%) has not yet been fully elucidated. Inhibition of cytochrome oxidase was ruled out when it was discovered that this enzyme has a very high affinity for oxygen, allowing it to function even when the intracellular O_2 concentration is only 0.01% (Drew, 1979). Solomos (1982) theorized that the storage atmosphere had to contain less than 2% O_2 to have any influence on the activity of cytochrome oxidase in apples. Any decrease in the rate of respiration in response to the decrease in external O_2 concentration must stem, therefore, from the diminution of the activity of oxidases other than cytochrome oxidase (Solomos, 1982).

An atmospheric concentration of less than 8% O_2 will reduce the rate of ethylene production in fruit and vegetable tissues (Dilley et al., 1982). Oxygen is required for the conversion of 1-aminocyclopropane-carboxylic acid to ethylene (Yang, 1985).

Growth and development of crops in storage greatly reduces their retail value and accelerates deterioration. Isenberg (1979) suggested that O_2 concentration affects endogenous growth regulators either promoting or inhibiting sprouting depending on the crop.

Reviews of the effects of reducing O_2 levels in the storage environment on chlorophyll retention, prevention of premature softening, maintenance of nutritive value and flavour retention in selected crops can be found in the literature (Kader, 1986; Weichmann, 1986; Kader et al, 1989).

The Importance of Carbon Dioxide in Tissue Biochemistry and Physiology

As previously mentioned, the internal CO_2 concentration in bulky tissues prior to harvest is much higher than that in the surrounding air and possibly suppresses the action of endogenous ethylene. Increasing the CO_2 concentration in the atmosphere surrounding the harvested tissue has much the same effect. Elevated levels of CO_2 prevent or delay many responses of fresh fruits and vegetables to ethylene presumably by competing with ethylene for binding sites on receptor molecules (Veen, 1987). However, elevated CO_2 levels can reduce, promote or have no effect on ethylene production rates by fruits, depending on the commodity and the CO_2 concentration (Kader, 1986).

Raising CO_2 concentration in the atmosphere to 6% can reduce respiration rate of bulky tissues possibly by preventing the oxidation of Krebs Cycle intermediates (Brecht, 1980; Kader, 1985). However, CO_2 concentrations as low as 2.5% can cause tissue damage in leafy commodities (Isenberg, 1979). In bulkier tissues, CO_2 levels can rise as high as 15% before ethanol and acetaldehyde begin to accumulate and tissue damage is evident (Frenkel, 1977; Kader, 1985; Lougheed, 1987). Short-term exposure to 4-15% CO_2 before storage at chilling temperatures has been shown to decrease subsequent chilling injury in okra (Ilker and Morris, 1975; Morris and Kader, 1977).

The effects of reduced O_2 elevated CO_2 on respiration rate are additive; 10% CO_2 in air reduces respiratory metabolism to about the same extent as lowering the O_2 concentration to 2% in the atmosphere (Kader, 1985). A mixed atmosphere of 2% O_2 and 10% CO_2 has approximately double the effect of either component alone (Kader, 1985).

High levels of CO_2 can have a beneficial or detrimental effect on appearance, flavour and nutritional qualities of fruit and vegetables. Treatments with 1-5% CO_2 were reported to retard softening and maintain high

concentrations of sugars and acids in peaches and nectarines (Anderson and Penny, 1975). Elevated levels of CO_2 have been found to reduce browning of cut surfaces of Brussels sprouts (Weichmann, 1983) and lettuce (Singh et al., 1972). The rate of starch-to-sugar conversion in potatoes can be slowed by storage in an atmosphere containing 5-20% CO_2 (Burton, 1974). Pitting injury was induced by 5% CO_2 in asparagus kept at 6 °C for 1 week (Lipton, 1965). Carbon dioxide levels above 10% cause off-flavours in sweet corn (Saltveit, 1985). General reviews of the effects of elevated levels of CO_2 in storage environments on sensory and nutritional quality of various fruits and vegetables can be found in the literature (Weichmann, 1986; Herner, 1987). In most of the research investigations, mixtures of high levels of CO_2 and low levels of O_2 were evaluated for their effect on plant tissues.

The Importance of Ethylene in Tissue Biochemistry and Physiology

Fruit have been characterized as climacteric or non-climacteric depending on their respiratory behavior during ripening (Burg and Burg, 1967). Ripening in climacteric fruit is associated with a large increase in respiration and ethylene production. The process is irreversible once autocatalytic ethylene production increases to a certain level (McGlasson, 1985). A climacteric-like respiratory increase can be induced in non-climacteric fruit by treating them with ethylene, ethane or ethanol. However, an increase in ethylene production does not accompany the increase in respiration which will rapidly subside once the hydrocarbon stimulus is removed (McGlasson, 1985).

As a fruit develops "competence" to ripen, ethylene perception by the plant stimulates respiration and turnover of macromolecules (Grierson, 1987). An enhanced production of enzymes in the ethylene biosynthesis pathway results in an autocatalytic burst of ethylene which leads to synthesis of mRNAs

responsible for a host of physiological changes in the mature fruit. Some of the responses to ethylene during ripening include: accumulation of the pigment lycopene, accelerated degradation of chlorophyll and starch, production of flavour compounds, synthesis of cell wall degrading enzymes such as polygalacturonases and fruit abscission from the parent plant.

The accumulation of ethylene in the storage environment can have a detrimental effect on many crops, reducing shelf-life by as much as 33% in as little as 2 days (Kader, 1985). Schouten (1985), Knee et al. (1985) and Lougheed et al. (1987) summarized the undesirable effects of ethylene upon vegetables. As little as 1-5 ppm ethylene can cause loss of chlorophyll in leafy vegetables such as celery, cabbage, broccoli, Brussels sprouts and in fruit-vegetables like cucumbers, peppers and tomatoes as well as leaf abscission in some cultivars of cabbage, Brussels sprouts and cauliflower (Kader, 1985).

The effects of ethylene on textural quality of some commodities can be observed only after cooking or processing. "Hardcore" in sweet potato can be induced by exposure to ethylene, resulting in a hard, inedible core in the cooked product (Watada, 1986). Short-term exposures to ethylene have been reported to increase spear toughness in asparagus (Haard et al., 1974) and cause softening in pickling cucumbers (Poenicke et al., 1977).

The development of bitter flavours in sweet potatoes, carrots, cabbage and Brussels sprouts has been attributed to the presence of ethylene (Chalutz et al., 1969; Kader, 1985; Hardenburg et al., 1986). Auxin-induced ethylene is thought to play a role in the production of isocoumarin, a compound responsible for the bitterness in carrots (Chalutz et al., 1969).

The Consequences of Water Loss

The most important change induced by harvest is the loss of turgidity due to a termination in the water supply to the harvested organ. With loss of turgor, transpiration rate is then lessened, affecting the protective cooling afforded by vaporization of water. Since biological reactions operate well only within a narrow range of temperatures, plant organs already subjected to warm temperatures during harvest are likely to suffer biochemical damage (Phan, 1987). While internal changes due to water loss affect the flavour, texture and nutrition of bulky plant tissues, the loss of the "fresh" appearance caused by wilting and withering of the outer tissues is a clear signal to the consumer that harvest and handling was less than optimum. Transpiration has been considered the major cause of postharvest losses and poor quality in leafy vegetables such as chard, lettuce, cabbage and spinach, and second in importance only to overmaturity at harvest, to losses of fruit-type vegetables such as eggplant, okra, snap bean, cucumber and sweet pepper (Kader, 1983). It is important to note that the relative contributions of plant structures (lenticels, stomata, cuticle, etc.) to transpiration varies among organ types. While O_2, CO_2 and ethylene diffuse mainly through air-filled stomata, lenticels, floral ends and stem scars, water vapour diffuses through the aqueous phase of the cuticle; the proportions may vary according to morphology (Burg and Burg, 1966; Burton, 1982; Ben-Yehoshua et al., 1985).

Cells that have lost their turgor are more susceptible to infection by pathogens. Storage of fruits and vegetables in a water-saturated atmosphere alleviates water stress, encourages wound healing and helps maintain the skin's resistance to pathogens (Ben-Yehoshua, 1987).

Ben-Yehoshua (1987) cited numerous reviews discussing the effects of water stress on phytohormones. Apparently water stress promotes activities

associated with senescence, such as a drop in endogenous levels of gibberellins and cytokinins, and a rise in the level of abscisic acid and ethylene.

In most retail storage facilities, diverse commodities are stored together under the same level of humidity. Since plant products vary greatly in their response to water loss, different strategies must be developed to control the humidity of the air immediately surrounding the product. Plastic films and coatings have been used successfully to control water loss in many commodities; however, excessive moisture in the pack atmosphere increases risk of pathological disorders (Geeson et al., 1985; Ben-Yehoshua, 1985).

B. DIFFUSIVE GAS FLOW IN BULKY PLANT TISSUES

Mechanism of Gas Exchange

The primary mechanism for exchange of metabolic gases between the interior and exterior of bulky plant organs is diffusion (Burg and Burg, 1965; Cameron and Yang, 1982; Ben-Yehoshua et al., 1985; Solomos, 1987) which can be defined as the net movement of gas molecules from one point to another because of the random kinetic activities or thermal motions of molecules. Metabolic rates and skin resistance to gas diffusion, as well as effective avenues for gas exchange such as gaseous pores, lenticels or stomata, calyx or pedicel openings, result in gas concentration gradients between the external atmosphere and the atmosphere just beneath the fruit skin (Burg and Burg, 1965). In most plant tissues, especially those containing parenchymatous tissues, a network of intercellular spaces forms a continuous air-space. Gas concentration gradients can also exist between these intercellular gas spaces and internal cells, the magnitude of which is influenced by apparent diffusivity of internal tissues, fruit size and rate of gas production or consumption (Burg and

Burg, 1965; Solomos, 1987; Rajapakse et al., 1989). Kader (1987) outlined the steps for gas exchange between a plant organ and its environment which are: 1) diffusion in the gas phase through the dermal system, 2) diffusion in the gas phase through the intercellular system, 3) exchange of gases between the intercellular atmosphere and the cellular solution, and 4) diffusion in solution within the cell to centers of O_2 consumption, or away from centers of CO_2 production.

The rate of gas exchange in bulky storage organs can be approximated by Fick's first law of diffusion (Burg and Burg, 1965; Cameron and Yang, 1982; Solomos, 1987). This law states that the flux of a gas in or out of a plant tissue depends on the concentration gradient across the barrier involved, the surface area of the barrier and the resistance of the barrier to diffusion. A simplified version of Fick's Law (Cameron and Yang, 1982) can be written as:

$$\frac{ds}{dt} = \frac{(C_{in}^t - C_{out}^t)A}{R}$$

where ds/dt is the rate of efflux (nL s^{-1}), R is the resistance coefficient (s cm^{-1}), A is the surface area of the tissue (cm^2), and C^t_{out} and C^t_{in} are the concentrations (nL/cm^3) outside and inside the tissue, respectively, at time t. Once the production (or consumption) rate of the gas by the organ and the concentrations of the gas in the internal and external atmospheres are determined, then resistance can be calculated from:

$$\mathrm{R} = \frac{\text{concentration gradient}}{\text{production rate}}$$

Although partial pressure gradients between the interior and exterior of plant organs may occur for individual gases, normally the total internal pressure of gases approximates atmospheric pressure for organs in a gaseous environment (Solomos, 1987).

Part of the difficulty in developing a model to study gas exchange in bulky tissues is the limited availability of information on resistances of dermal and paradermal tissues, gas transport pathways, tissue porosity and internal gas concentrations. The sheer diversity of cultivars and types of edible vegetable and fruit tissues makes the collection of such detailed gas exchange information a formidable task.

Application of the Principles of Gas Exchange

Determination of diffusivity of gases in bulky plant tissues is important for the development of controlled-atmosphere (CA) and modified (MA) atmosphere storage treatments which extend the shelf-life of many plant products by modifying respiration rate and metabolic changes. The terms 'controlled atmosphere' and 'modified atmosphere' mean that the atmospheric gas composition surrounding a perishable product is different from that of normal air. Both commonly involve manipulation of CO_2, O_2 and N_2 levels; however, other gases such as CO, C_2H_4, C_2H_2 and C_3H_6 are sometimes included. MA is usually developed in a package, either by passive diffusion or purging the package with a premixed gas, after which there is no precise control over the gases surrounding the product. Surface coatings and plastic films are also used to generate MA within the tissue. Controlled atmosphere conditions involve constant control and monitoring of atmospheric gases.

The effects of CA and MA on respiration are dependent on the plant material itself and on the concentration gradient that develops between the centers of metabolic action and the outer integument of the plant material (Burton, 1978). Isenberg (1979) suggested that the effects of CA on the respiratory process depended more on the anatomy and morphology of the plant organ than on its biochemical system. According to Lougheed (1987),

identifying CA disorders in vegetables is complicated by the constant changes in cultivars and variations among vegetables in anatomy, morphology and physiology. In fruit, porosity may be important in the successful outcome of CA storage. Values for O_2 diffusivity in apples have been found to vary with cultivar, being broadly consistent with intercellular space volume (Rajapakse et al., 1990). The commercial success of CA storage of pome fruits, and more recently with MA packaging of cut vegetables, provides continued incentive to continue the investigation of gas diffusion characteristics of diverse plant species to develop strategies to increase effectiveness of CA and MA treatments, and to determine the changes required in a storage atmosphere to ameliorate CA-induced disorders (Ladeinde and Hicks, 1988; Banks and Kays, 1988; Andrich et al., 1989; Bertola et al., 1990; Lee et al., 1991; Solomos, 1989). These types of investigations are conducted with the knowledge that small changes in the storage environment such as lowering O_2 concentrations to 1.5% from 2-5% (Ultra Low O_2 Storage) or rapid establishment of CA conditions (Rapid CA) have resulted in superior quality and reduced disorder levels of stored apples (Little and Peggie, 1987).

C. BULK GAS FLOW IN BULKY PLANT TISSUES

Occurrence of Bulk Gas Flow

In addition to diffusive transfer of gases, bulk flow of gases, in which gases move collectively along a gradient of total pressure, may also occur in large plant organs. At the present time, evidence of bulk gas exchange in plant tissues comes almost exclusively from studies with wet-land plants and leaves of trees. In one such study, small pressure increases within the intercellular air spaces of water lily (Dacey, 1981) directed bulk flow of gases down petioles to

the rhizomes and out the older leaves. Bulk gas flow has also been reported in lotus leaves (Dacey, 1987), although the direction of gas flow in the petioles was not determined. Day and Parkinson (1979) calculated that the contribution of mass flow to gas exchange in leaves exposed to wind should increase rapidly with leaf oscillation frequency and a concomitant reduction in the boundary layer thickness. Although Dacey (1981) considered bulk gas flow in wet-land plants to result from gradients in temperature and water vapour concentration, factors such as flow-limiting 'pores' in palisade parenchyma, stomatal function and photosynthetic capability may be equally important (Dacey, 1987, Armstrong and Armstrong, 1991).

Although reports of mass gas flow in large plant organs are limited, there are indications that supplementary gas flow or pressurization of internal tissues may occur during postharvest activities. Reduced gas permeabilities or additional diffusion barriers to gas exchange may permit development of total gas pressure gradients between a bulky plant organ and its external environment (Corey and Tan; 1990). Research by Corey and Tan (1990) revealed that sudden temperature changes may cause mass gas flow to occur, resulting in internal gas pressure changes. Employing an apparatus designed to produce oscillating atmospheric pressure cycles, Calbo (1985) determined that mass gas flow under ambient gas concentrations had no effect on onset of the climacteric and tissue softening in "Gravenstein" apples compared to apples stored at constant pressure. Oscillating pressure treatments combined with an atmospheric concentration of 8% CO_2 had a beneficial effect on colour retention and tissue firmness compared to apples stored at ambient pressure and 8% CO_2. This effect was thought to be due to inhibition of ethylene synthesis and action by elevated internal concentrations of CO_2 although oscillating pressures in a flow-through system reduced internal and external concentrations of

ethylene (Calbo, 1985). On the other hand, experimental enhancement of mass gas flow in cabbage stored in air, by means of variable low-pressure storage, improved shelf-life and reduced trim loss (Onoda et al. 1989).

The effect of variable atmospheric pressure treatments on postharvest metabolism is largely unknown, although there is evidence that a pronounced reduction in ethylene production rate may occur in apples during storage in atmospheres with elevated CO_2/O_2 ratios (Calbo, 1985).

Application of Mass Gas Flow

It has been known for some time that by reducing the normal atmospheric pressure in the environment around plant tissue, the effective partial pressures of individual ambient gases are also lowered. A $1/5^{th}$ reduction in the total pressure of normal air would result in an effective oxygen partial pressure equivalent to 4% oxygen. Therefore, unlike CA or MA, no gas other than air need be supplied in a hypobaric system (Brecht, 1980; Jamieson, 1980). In addition to lowering the partial pressures of gases in air, including water vapour, low-pressure systems allow gases to escape more rapidly (Burg, 1975). According to Burg (1975), this is due to the fact that the diffusion coefficients of various gases, including ethylene and other volatiles are inversely proportional to atmospheric pressures. Although there are reports of the successful use of hypobaric storage for extending the storage life of tomatoes (Burg and Burg, 1966; Wu et al., 1972; Mermelstein, 1979), avocados, mangos, sweet cherries , limes, guava (Burg and Burg, 1966; Mermelstein, 1979), apricots (Wu and Salunkhe, 1972), sweet peppers, lettuce, mushrooms, floral products (Mermelstein, 1979), and tropical and subtropical fruits (Lougheed et al., 1978), this form of storage never became popular. Grumman Allied Industries developed the Dormavac System for hypobaric transportation of perishables

(Mermelstein, 1979; Jamieson, 1980), but this met with only limited commercial success and was discontinued (Sherman, 1985). Interest in hypobaric storage may have diminished due, perhaps, to the cost of constructing a storage facility capable of withstanding enormous negative pressures and evidence that indicates that flavour volatiles could be lost during storage at low pressures (Wu and Salunkhe, 1972, Lougheed et al., 1978). Geeson et al. (1986) observed that ethylene concentrations of less than 0.1 ppm in the hypobaric storage atmosphere resulted in poor and uneven ripening of tomatoes following storage, hampering flavour development.

Most of the information describing gas exchange in plant tissues has been amassed through studies with fruit rather than vegetables. The majority of those investigations indicate that the superficial tissues represent the main significant barrier to gas diffusion and are thus the primary factor regulating the internal concentrations of gases within a commodity (Burg and Burg, 1965; Ben-Yehoshua et al., 1985; Andrich et al, 1990; Rajapakse et al., 1989, 1990; Bertola et al., 1990). There is an obvious need for quantitative determination of the resistances through the various pathways for exchange of O_2, CO_2, ethylene and water vapour in vegetables in order to model and develop storage systems which are capable of creating and maintaining internal atmospheres beneficial to plant tissues.

D. FLAVOUR ANALYSIS IN FOOD RESEARCH

The ability to monitor volatile components of flavour adds a new dimension to expressions of quality often used by horticulturists such as yield, size, texture, appearance or percentage of waste and sensory assessments. However, the task of isolating and identifying volatile flavour components is formidable. Biologically generated aromas are present in low concentrations in a complex matrix and comprise a large number of compounds representing numerous chemical classes. Ideally, once the flavour compounds have been extracted, concentrated, separated and detected, the contribution of each chemical to the perception of flavour should be established. This latter task is receiving increasing attention by flavour chemists and sensory analysts in efforts to identify important components in various foods (Maarse, 1991; Grosch, 1993).

Wide availability of a technology with the capability to isolate and identify numerous flavour compounds has resulted in the publication of copious lists of constituents from many common and exotic food species. While it is true that much of the information on flavour analysis has been reported in the form of relative peak areas or height and cannot be related to the actual concentrations in the products, such data are still important when the intent of the research is considered.

Mazza and Pietrzak (1990) used GC/MS analysis and sensory analysis of headspace volatiles to identify a commercial postharvest treatment for sprout suppression as the origin of an undesirable musty, earthy aroma in potatoes. Statistical comparisons of the peak areas and peak area/total area ratios revealed that six major components were more concentrated in off-flavour potatoes than in the good quality potatoes. Three of these compounds were also isolated from an adjuvant in a commercial formulation of sprouting inhibitor used on the potatoes (Mazza and Pietrzak, 1990). This research led to the

successful resolution of a problem that threatened the continuing operation of the largest frozen French fry production plant in Canada.

Qualitative or semi-quantitative methods for determining volatile constituents in foods have been applied in many creative ways including: evaluation of exotic fruits as potential sources of novel flavours and flavour constituents (Potter and Fagerson, 1990; Wyllie and Leach, 1990; Peppard, 1992; Nisperos-Carriedo et al., 1992; Farkaš et al., 1992), identification of previously unknown compounds in common crops (Tang et al., 1990; Takeoka et al., 1991; Kuo and Ho, 1992a; Kuo and Ho, 1992b), appraisal of preservation, processing and storage methods (Chung et al., 1983; Crouzet et al., 1985; El-Nemr et al., 1988; Nisperos-Carriedo et al., 1992; St. Angelo et al., 1992; Hansen et al., 1992; Yen et al., 1992; Narain and Bora, 1992; Shamaila et al., 1992; Moshonas et al., 1993; Piggott and Othman, 1993), evaluation of cultivars (Wyllie and Leach, 1990; Kallio and Salorinne, 1990; Horvat et al., 1992; Takeoka et al., 1992; Shamaila et al., 1993), monitoring the ripening process (Chyau et al., 1992; Hansen et al., 1992; Pérez, et al., 1992; Mattheis et al., 1992), the study of processes leading to the generation of off-flavours (Seitz and Sauer, 1992; Singh, 1992; Rouseff et al., 1992), and the determination of the effect of growing conditions on flavour development (Van Wassenhove et al., 1990; Fischer, 1992).

Because of the complexity of typical fruit and vegetable volatiles, no single method of analysis will provide a flavour profile truly representative of the food. Quantitative and qualitative data on volatile compounds in many edible products, obtained using various methods, were compiled by a Dutch research organization and published recently in a three volume edition (Maarse and Visscher, 1989).

E. ISOLATION OF FOOD FLAVOURS

Techniques employed in flavour analysis of diverse food products have been reviewed elsewhere (Bemelmans and Schafer, 1981; Cronin, 1982; Ioffe and Vitenberg, 1982; Heath and Reineccius, 1986; Parliment, 1986; Burgard and Kuznicki, 1990; Maarse, 1991). A brief description of methods commonly used for isolation of aroma compounds in vegetables and fruits used as vegetables, together with examples of recent applications, are presented here.

Headspace Methods

Headspace analysis is considered the preferred technique for isolation and identification of important odour compounds in foods and beverages. The headspace techniques described in the following text are limited in their capability to isolate a complete representation of flavour components due to variation in concentration, stability, volatility and solubility of the components.

a. Static Headspace Sampling

The procedure referred to as static headspace (equilibrium) sampling, can be manual or automated. The manual technique involves use of a hand-held syringe to sample volatiles in the headspace above a sample contained in a sealed system. Automated headspace analyzers are commercially available which produce better chromatographic reproducibility than the manual method. Unfortunately, except in liquid products, it is very difficult to relate the concentration of flavour volatiles in the vapour phase to that in the product (Maarse, 1991). In addition, the normally low concentrations of aroma volatiles released by most vegetables (especially when they are intact) precludes the use of this technique, since identification is difficult if not impossible.

Methanethiol, ethyl alcohol and ethyl acetate have been identified in headspace gases of broccoli florets by Hansen et al. (1992), who used the manual method to determine the effect of low-oxygen atmosphere storage on low boiling volatiles in broccoli. Kallio and Salorinne (1990) identified onion aroma components by direct injection of headspace gas of chopped onion and on-column high-resolution capillary GC and GC-MS. Volatile flavour components in tomato homogenate (2 mL of liquefied tomato) were quantified by Baldwin et al. (1991) using an automated headspace sampler to introduce the sample onto a GC column.

b. Dynamic Headspace Sampling

Dynamic headspace (non-equilibrium) sampling involves the entrainment of odourous compounds on a solid adsorbent by purging the headspace of the holding vessel with nitrogen or some other gas. This procedure can be performed manually (in which case the volatile components are eluted with solvent), semi-automatically or automatically, with any of at least four commercially available instruments. Desorption of volatiles obtained by the semi-automated or automated procedure is effected by thermal desorption, which may cause degradation of thermally labile compounds. Commonly used adsorbents are activated coconut charcoal and synthetic polymers such as: chromasorb 105, porapack Q, Tenax TA and Tenax GC, either used alone or in combinations. Resins such as XAD-4, XAD-7 and XAD-9 are less popular adsorbents. Although Tenax GC and TA have become the most frequently used porous polymers for trapping volatiles from headspace vapours, their adsorption capacity is much less than that of activated carbon (Heath and Reineccius, 1986). Excellent recoveries of a range of organic compounds trapped on 2 mg of charcoal have been reported (Clark and Cronin, 1975). Despite the

availability of high quality carbon in convenient forms for trapping volatiles, use of this material seems to be mainly directed toward the identification and quantitation of pollutants.

Van Langenhove et al. (1991) used Tenax GC to capture and concentrate volatiles from brussels sprouts and cauliflower during the blanching process both on a lab scale and an industrial scale. Volatiles were thermally desorbed in a heating block and analyzed by GC-MS.

Tokitomo and Kobayashi (1992) entrained fresh onion volatiles on Tenax TA. Following the collection, they used a thermal desorption cold trap injector (Chrompack, The Netherlands) to desorb the volatiles, which were then cryofocused in the injector and injected onto a GC capillary column.

Kohlrabi volatiles, from the liquid fraction of a homogenate of kohlrabi and phosphate buffer, were collected on Tenax TA by Fischer (1992) and eluted with pentane/diethylether (1:1 v) for subsequent analysis by GC.

Tenax GC (10 g contained in a glass trap) was used by Hansen et al. (1992) to trap volatiles from whole broccoli florets by purging a flask containing the broccoli with purified air. The isolation was carried out for 3 h at 25°C with an air flow rate of 3 L/min. Volatiles were eluted with 100 mL of diethyl ether and concentrated by careful distillation prior to analysis by GC and GC-MS.

Simultaneous Purge and Solvent Extraction

A device developed by Umano and Shibamoto (1986), and later used by Macku and Shibamoto (1991) to extract headspace volatiles of chopped celery into solvent, has not been included in review literature. The apparatus consists of a large containment vessel with an inlet and outlet for purge gas. The outlet of the vessel is connected to a gas-washing bottle and a liquid-liquid continuous extractor joined in tandem. The extraction solvent (50 mL) was condensed to 1

mL in vacuo using a Vigreux distillation column and the volatiles were analyzed by GC and GC-MS. The main advantage of this system, compared to trapping on porous polymers, is the very high capacity of the trap.

Solvent Extraction Methods

The solvent extraction method takes advantage of the solubility of flavour compounds in organic solvents such as pentane, diethyl ether, dichloromethane and carbon disulfide. Following steam distillation of homogenates or juices, aqueous distillates can be shaken together with a solvent that is immiscible in water. As the water and solvent separate to form two layers the volatiles will remain in the solvent. Once contained in the organic solvent, aroma compounds can be concentrated allowing easier detection and identification by GC analysis.

Nonfat-containing foods can be directly extracted with solvent by using a Soxhlet apparatus (and heat) or blending the tissue or juice with the solvent followed by filtration and/or centrifugation. Often solvent extraction requires a further 'cleanup' method to eliminate nonvolatile constituents.

Kuo and Ho (1992b) prepared solvent extracts of onions and scallions by blending the samples with distilled water, adding dichloromethane and stirring for 12 hours. The samples were then filtered, dried over sodium sulfate and passed through a silica gel column to remove the chlorophyll. Following concentration of the extract, methanol was added to precipitate the waxes and the samples were filtered and analyzed by GLC and GC-MS. Block et al. (1992a, 1992b) used this method to extract flavour compounds from a number of *Allium* species except that after blending the samples with water and filtering, they saturated the filtrate with sodium chloride and extracted twice with dichloromethane. The solvent extracts were dried, concentrated, filtered and analyzed by GC-MS, HPLC and NMR spectroscopy. Weinberg et al. (1993)

blended celery and carrot juices with water and dichloromethane to extract flavour compounds. The extract was condensed on Kuderna-Danish apparatus before analysis by GC-MS.

Volatile compounds from celery have been isolated by refluxing chopped celery with diethyl ether in a Soxhlet apparatus for 4 h (Van Wassenhove et al., 1990). The extracts were dried over sodium sulfate, concentrated under reduced pressure and analyzed by two-dimensional GLC and GC-MS. In another report of volatile compounds in celery (Tang et al; 1990), the juice obtained by macerating celery in a blender was introduced onto an Amberlite XAD-2 column which was then rinsed with distilled water to eliminate sugars, acids and other substances. Pentane was passed through the column to elute the adsorbed aroma compounds (free volatiles) and methanol was used to elute aroma compounds bound to glycosides. Bound volatiles were hydrolyzed with β-glucosidase, taken up in dichloromethane and the extracts were concentrated under a stream of nitrogen and analyzed by GC and GC-MS.

Ohta and Osajima (1992) devised a cold trap apparatus to overcome the problem of emulsion formation in solvent extracts. In their procedure, they immersed fresh onion (1 kg) in ethyl ether for 5 days at 5°C, dried the ether extract (4 L) over sodium sulfate, and reduced the solvent to 100 mL *in vacuo*. The volatile fraction was recovered in liquid N_2 under vacuum using the cold trap apparatus and analyzed by GC-MS.

Distillation Methods

This technique includes steam distillation, and distillation under vacuum which allows lower temperatures to be used. Distillation methods take advantage of the volatility of flavour components and non-volatility of the major

food constituents, and is one of the oldest methods for flavour isolation from foods.

A number of newly identified volatile components of onions (*Allium cepa* L.) were reported by Farkaš et al. (1992) who used a steam-distillation technique employing a water-immiscible volatile oil separator trap. After distillation, centrifugation at high speed separated the onion oil from the water condensate. The oil was analyzed by GLC and GC-MS. This method was also used by Kuo and Ho (1992a, 1992b) to determine volatile constituents of Welsh onions and scallions (both *Allium fistulosum* L.).

Block et al. (1992a, 1992b) subjected chopped *Allium* species to high vacuum at room temperature, using an oil bath to prevent the contents of the distillation flask from freezing, and collected the aqueous extract at -196°C. They found that subsequent analysis of the methylene dichloride extract of the aqueous sample by HPLC and NMR spectroscopy, gave good qualitative thiosulfinate composition profiles. The advantages of this method over solvent extraction were reduced emulsion formation and absence of interference from pigments, waxes and other non-volatile plant components.

A popular method of isolating flavours by distillation is to use simultaneous distillation/extraction (SDE). Various modifications of the distillation head designed by Likens and Nickerson (1964) have evolved but the principle of operation remains unchanged. The design permits the mingling of vapours of the product with vapours of the extracting solvent allowing very efficient flavour extraction. The apparatus was modified by Schultz et al. (1977) for use under vacuum. Van Wassenhove and his associates (1990) used the unmodified apparatus to isolate volatiles from celery plants grown with different levels of organic and/or inorganic fertilizers. A comparison of Soxhlet and Likens-Nickerson extraction methods indicated that Soxhlet extraction was less

efficent than the Likens-Nickerson apparatus for isolation of terpenes (Van Wassenhove et al., 1990).

Supercritical Fluid Extraction Method

Isolation of flavour compounds by supercritical fluid extraction (SFE) shows promise as a sample preparation technique because of minimal losses of low boiling compounds, preservation of thermolabile constituents and rapidity of extraction. In addition, SFE grade carbon dioxide is nonflammable, inexpensive, chemically inert and leaves no toxic residue (Maarse, 1991). The technique involves pumping liquid CO_2 at a controlled pressure through a sample contained in a heated extraction vessel. Extract recovery is accomplished by using either solvent recovery or direct interfacing to a capillary column through the injector of a GC. Thus far, however, the technique has been applied mainly to spices (Moyler, 1986; Hawthorne et al.,1989; Gopalakrishnan et al., 1990; Huston and Ji, 1991), but as techniques and equipment improve the method will likely be applied to a wider range of food materials.

The progress in methods of flavour analysis offers exciting opportunities to improve the quality of fruits and vegetables at every step in the marketing chain between the producer and the consumer. Monitoring changes in flavour volatiles may be useful as an early indicator of the development of a harmful storage evironment or an unsuitable cultivar for storage. Chemical analyses are also objective, fast and easier to perform than formal sensory analyses.

SECTION 1

ACCELERATION OF GAS TRANSFER IN ONION, SWEET PEPPERS, TOMATOES AND CUCUMBERS BY VARYING ATMOSPHERIC PRESSURE

INTRODUCTION

Modification of internal gas concentrations by manipulating environmental gases, applying surface treatments or using special packaging materials can prolong storage life of a number of fruit and vegetable commodities. Refinement of CA storage has produced a number of specialized storage environments, one of these being rapid CA, where the desired temperature is achieved 1 day after harvesting and the level of low O_2 is achieved in less than 6 days after harvesting (Little and Peggie, 1987). Minimizing the time to obtain CA in pome fruit storage, results in superior quality and reduced disorder levels compared to the passive development of CA over a period of 2 to 3 weeks (Little and Peggie, 1987).

The rate and direction of diffusive exchange of gases between plant tissues and their environment depends on partial pressure gradients of each gas and is limited by available pathways for gas movement. Partial pressure gradients are a function of resistances along pathways of diffusion and the rate of production or consumption of the component gases. Jolliffe and Dyck (1988) proposed that interchange of gases between tissues and the surrounding atmosphere could be improved by supplementing diffusion with bulk gas flow induced by rapid variations in external gas pressure. Presently, however, there is little information on pressure driven gas exchange in bulky tissues.

The main purpose of this portion of my research was to study the effect of cyclic atmospheric pressure variations on CO_2 efflux rates in onion, tomatoes, sweet pepper and cucumbers. A secondary objective was to characterize the influences of temperature, oxygen concentration, and interval between pressure cycles on gas transfer in bulky plant tissues subjected to variable pressure treatments.

MATERIALS and METHODS

A. PLANT MATERIALS

Jumbo yellow onions (*Allium cepa* L.) were purchased from a local supermarket, selecting only those onions (average weight ca. 460 g) which were free of apparent botrytis neck rot or soft rot. Sweet bell peppers (*Capsicum annuum* L. c.v. Doria) and tomatoes (*Lycoperison esculentum* L. c.v. Dombito) were reared in the greenhouse at Agriculture and Agri-Food Canada's Pacific Research Center, Agassiz, B.C. The peppers, each weighting ca. 200g, were harvested at the immature green stage, and tomatoes, each weighing ca. 260 g were harvested at the red ripe stage. Slicing cucumber (*Cucumis sativa*) varieties were produced in field plots (c.v. Straight Eight) and in the greenhouse (c.v. Sweet Success) on the Research Station. Fruits 15 cm in length and weighing ca. 170 g were selected for experimentation. Immediately after harvest, tomato, pepper and cucumber fruit surfaces were sanitized with a 100 ppm hypochlorite solution. The plant material was placed in microperforated polyethylene bags and held overnight in the dark at temperatures approximating those to which the material would be subjected the following day. Experiments were repeated on 3 individual plant organs over 3 periods in the growing (or storage, as in the case of onions) season. Rates of gas exchange were measured on a fresh mass basis.

B. VARIABLE PRESSURE STORAGE AND MONITORING SYSTEM

A closed gas-exchange system was constructed to control environmental conditions while generating cycles of pressure changes in the atmosphere surrounding bulky tissues. The materials in the gas circuit comprised aluminum, stainless steel, copper and glass. The circuit had a fixed internal volume of 5.6

L (Fig. 1.1). The treatment vessel and a secondary chamber, each with a volume of 2.6L, were constructed from aluminum. They had 1.3 cm thick walls and their rims were mounted with 6 protruding screw threads. The vessels were capped by 1.1 cm thick aluminum plates with 6 smaller holes to allow the screw threads to pass through the lid. The caps had 5 holes drilled to accommodate 6 mm Swagelok bulkhead unions. A rubber O-ring was installed in the rims of the vessels to ensure a gas-tight seal between vessel and lid when wing-nuts were tightened down on the lid. A temperature sensor (Model 47 Scanning Tele-thermometer, Yellow Springs Instrument Co., Inc., TX), as well as an electrical source for fans, were passed through the bulkhead unions which were sealed air-tight with epoxy sealant. Pressure was measured by a gauge (0-18 kPa) mounted on the top of the vessel using compression fittings. A constant temperature was maintained in the insulated treatment chamber by immersing it in a Haake recirculating bath (Haake, West-Germany). Muffin fans, rated at 600 L min^{-1} at zero static pressure, were placed in the treatment chamber and the secondary chamber in order to mix gases and reduce boundary layer effects. Circulating gases were bubbled through distilled water in a glass Wheaton Purge and Trap unit to saturate the gases entering the treatment chamber. Oxygen concentration in the system was monitored with an in-line Servomex 570A Portable Oxygen Analyser. Carbon dioxide was sampled in a computer programmed sequence by means of a gas-sampling bulb installed in the gas circuit.

Room air, or commercially prepared premixed gases composed of 1% or 3% O_2, balance N_2 (Linde Specialty Gases Ltd., Edmonton, AB), were circulated around the system at a flow rate of 1.5 L min^{-1} by a pump (Metal Bellows Corp., Sharon, MA). Atmospheric pressure inside the treatment chamber was

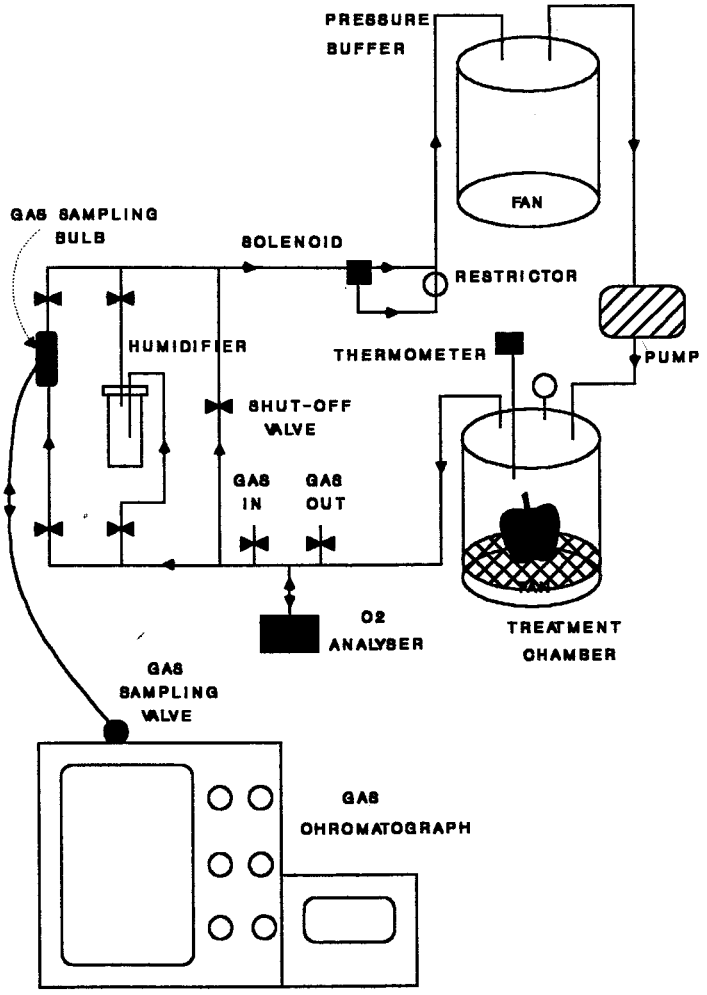

Fig. 1.1. Illustration of the variable pressure storage and monitoring system.

varied by directing the gas flow either through a bypass or against a flow restrictor. This simple design caused air pressure to increase in the downstream vessel when gas flow was directed against the restrictor, then to subside to atmospheric pressure as gas was redirected through the bypass. A programmable timer (771 Programmable Sequencer, Cole-Parmer Instrument Co.) controlled switching of a 3-way universal stainless steel solenoid valve (Ascoelectric Lt., Brantford, ON) to obtain a fluctuating pressure cycle of the desired period. This type of system allowed the redistribution of gases without varying system volume as was done in an apparatus constructed by Calbo (1985) or introducing external gases in a manner similar to the variable low pressure storage apparatus of Onoda et al. (1989). The design of this system also circumvented difficulties reportedly associated with using a paramagnetic gas measuring device in conjunction with pressure treatments, requiring that the CO_2 analyser be isolated from the gas circuit (Jolliffe and Moloney, unpublished).

Leaks were minimized in the gas exchange circuit by the use of compression fittings and valves capable of withstanding 670 kPa. To test for leaks the system was pressurized to 10 kPa above atmospheric pressure using compressed N_2, sealed and allowed to stand for 4 hours. No noticeable drop in pressure occurred over this period.

C. PRESSURE TREATMENTS

The design of the gas exchange system permitted only positive increases in total pressure to occur with a minimum value of 101 kPa (atmospheric pressure) and a maximum value of 111 kPa. Increases of 3.5 and 6.9 kPa above atmospheric pressure were chosen for the purposes of this study. Phases of the pressure cycles are illustrated in Fig. 1.2. Starting at atmospheric

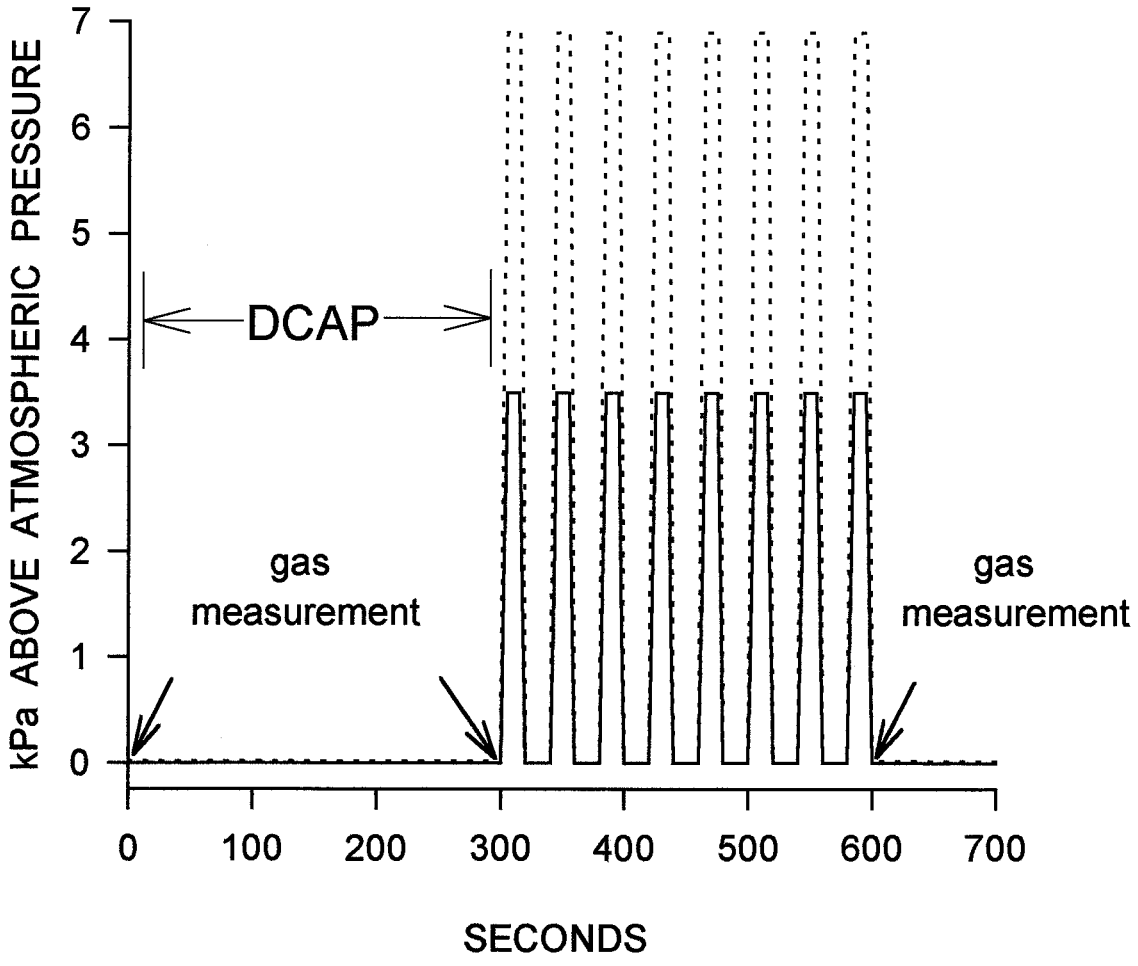

Fig. 1.2. Variable pressure cycles. A programmable timer regulating timing of administration of pressure variations at 3.5 and 6.9 kPa was co-ordinated with a computer-controlled gas sampling valve to automate gas measurements.

pressure, pressure increased rapidly to the maximum value, remained at the acrophase for about 16 s, and rapidly returned to the minimum value. Opening or closing the needle valve of the restrictor in the gas circuit controlled the level of pressure variations. The time required to complete the maximum and minimum phases of one cycle, i.e. the period of the cycle, was 40 seconds.

To begin a pressure treatment, the different plant materials were weighed and placed in the treatment chamber. The gas exchange circuit was then flushed with room air or a premixed gas using a metal bellows pump, the fans were started, the system closed and the humidifier included in the flow. Each sample of plant material was allowed to equilibrate for 2 hours before starting treatments, at which time the humidifier was excluded from the circuit. The volume of the system was sufficient to prevent the buildup of more than 1800 ppm of CO_2 before completion of the trial that day. Following treatment, the plant materials were reweighed and their volumes were determined by water displacement.

D. MEASUREMENT OF CARBON DIOXIDE

Gas measurements were performed automatically by programmed stream sampling with a 6-port air-actuated Valco valve (Chromatographic Specialties, Brockville, Ont). Sample volumes of 0.25 mL were injected onto a 6 ft, 1/8 inch ID stainless steel column packed with Porapak Q, 100/120 mesh mounted in a Shimadzu GC9A gas chromatograph. Carbon dioxide was catalytically converted to methane by means of a Shimadzu MTN-1 methanizer for detection by a Flame ionization detector. Peak integration and data calculations were performed by a Shimadzu C-R3A data processing unit. Helium (Ultra-high Purity) was used as the carrier gas at a flow rate of 40 mL/min. The flow rate of the detector gases were 340 mL/min and 50 mL/min for air (Extra Dry) and

hydrogen (Ultra-high Purity), respectively. A moisture and hydrocarbon trap was placed in the carrier gas line and each gas line was fitted with a 5 micron particle filter. GC analytical runs were isothermal with temperatures as follows: column temperature 50° C, methanizer 350° C, methanizer transfer line 80° C and detector 250° C.

Carbon dioxide efflux rates ($\mu L \cdot kg^{-1} \cdot s^{-1}$) of all tissue samples were measured several times at constant atmospheric pressure prior to initiation of pressure treatment to confirm that equilibration was complete. Data were collected as paired observations i.e. net CO_2 emission rate at constant pressure was paired with the following CO_2 emission measurement taken after a 5 min variable pressure treatment. Relative response to pressure variations was determined by the ratio (variable pressure/constant pressure) of these two rate measurements. Five consecutive paired constant and variable pressure measurements were collected for each of the different bulky tissues. Experimental treatments are outlined in Table 1.1. In addition to determining the influence of pressure level, temperature and oxygen concentration on the response to pressure variations in onions, sweet peppers, tomatoes and cucumbers, effects due to the duration of the interval of constant pressure between 5 min pressure treatments (DCAP), either 5 or 15 min, were examined.

E. STATISTICAL ANALYSES

Data for the onion, tomato, sweet pepper and cucumber trials were analyzed as a 3-way factorial design using the General Linear Model procedure of the SAS/STAT (SAS Institute Inc., 1987) procedures. A factorial model with interaction was specified. For onion data, CONTRAST statements were used in order to partition the interaction effects between temperature and pressure level,

Table 1.1. Varying pressure treatments applied to onions, peppers, tomatoes and cucumbers.

Treatments	Onion	Pepper	Tomato	Cucumber
Pressure Level				
6.9kPa	x	x	x	x
3.5kPa	x	x	x	x
DCAP[1]				
5 min	x	x	x	x
15 min	x	x	x	x
Temperature				
0°C	x			
10°C	x	x	x	x
20°C	x	x	x	x
% Oxygen[2]				
1	x			
3		x	x	x
21	x	x	x	x

[1] Duration of the interval of constant atmospheric pressure between 5 min variable pressure treatments.

[2] DCAP=15 min for trials testing the effect of different oxygen concentrations.

temperature and DCAP, and, temperature and O_2 concentration (see Appendix 1 for contrast statements). Multiple comparisons of temperature means for onion treatments were performed by the Student-Newman-Keuls multiple range test ($P \leq 0.05$).

Because of the exploratory nature of this work, and the necessity to limit the number of temperature and pressure treatment levels to those that could be managed accurately within the gas circuit system, much of the statistical analysis involved paired comparison of means using the PROC MEANS statement of the SAS procedures. A new variable was created for each of the main treatments containing the difference between the value for CO_2 efflux rate at constant pressure and the value for CO_2 efflux rate as a result of variable pressure treatments. Using statements to obtain a t statistic and probability value, the mean differences were tested to determine whether they were significantly different from zero ($P \leq 0.05$).

RESULTS and DISCUSSION

A. EFFECT OF LEVEL OF PRESSURE VARIATIONS

Both levels of pressure variation had a significant effect on CO_2 emission rate of onions ($P<0.0001$) and peppers ($P<0.001$) but no significant ($P>0.05$) effect on cucumbers and tomatoes (Fig 1.3). After each series of pressure fluctuations, carbon dioxide emission rates returned to levels close to the initial constant pressure values. The level of pressure variations, either 3.5 or 6.9 kPa, did not affect carbon dioxide emission rates for each organ type during intervals of constant pressure. Carbon dioxide efflux rates in onions increased four-fold and seven-fold, and two-fold and three-fold in peppers, during pressure variations at pressure levels of 3.5 kPa and 6.9 kPa, respectively.

Increases in bulk gas movement with increasing pressure is consistent with Darcy's law for fluid flow through porous media (Nobel, 1974). Bulk flow along a pressure gradient will be effected only if sufficient time is allowed for whole gas movement before reversal of the gradient. This response is well demonstrated in research by Jolliffe and Moloney (unpublished) which showed that increases in convective gas flow rates were proportional to the amplitude of pressure variation and that a short period of pressure oscillation prevented maximum bulk gas flow from being achieved. Lack of a response by cucumbers and tomatoes to pressure variations indicates that where there are limited avenues for gas exchange, opportunities for mass gas transfer are minimal.

In onion bulbs, it is expected that gas exchange occurred primarily through the root plate, which has been determined to be the most important avenue for gas exchange (Ladeinde and Hicks, 1988). Onions are organs

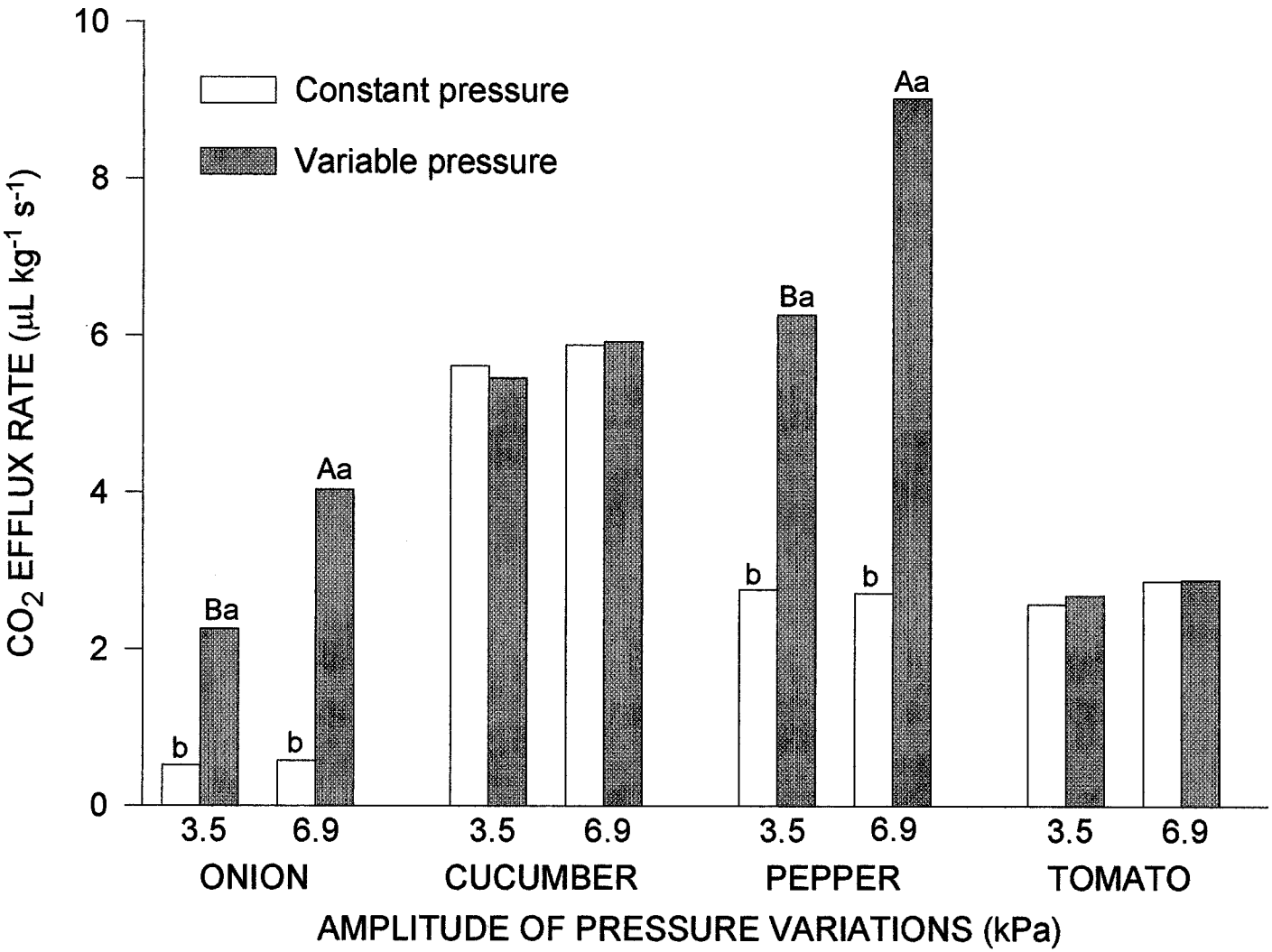

Fig. 1.3. Main effect of atmospheric pressure variations on net CO_2 efflux rate of onions, cucumbers, sweet peppers and tomatoes. [A,B] Bars associated with each plant tissue, with different letters, are significantly ($P<0.05$, F test) different. [a,b] Bars associated with each pressure level, with different letters, are significantly ($P<0.05$, paired t test) different.

composed of individual leaves, each of which is covered on both sides by an epidermis. It has been noted that air bubbles coming from onion bulbs subjected to a moderate vacuum under water came mainly from the base plate, with relatively few from the neck and none through the dry scales (Ladeinde and Hicks, 1988). Based on this observation, Ladeinde and Hicks (1988) theorized that gas exchange between adjacent leaves of mesophyll tissue may be insignificant and gas exchange takes place mainly via the root plate rather than by a lateral, shorter path of greater resistance. This observation is also consistent with the pattern of gas exchange noted in this experiment. Movement of gases between leaf scales rather than laterally from the center of the onion would result in the relatively large flux in CO_2 observed under conditions of varying atmospheric pressure.

In contrast, the major avenue for gas exchange in isolated sweet pepper fruit is at the pedicel (Burg and Burg, 1965). Burg and Burg (1965) found that CO_2 emanation was retarded by about 60% when lanolin was applied to the pedicel end. Evidence that the outer tissue layer of sweet pepper fruit is highly resistant to gas exchange was obtained by Corey and Tan (1990) who found that partial vacuums occurred in pepper fruits following their exposure to cool air and cool water. The implication of their findings is that the pressure gradient caused by exposure of warm fruit to cool water (as can occur during postharvest hydrocooling) would result in water uptake with possible bacterial contamination. On one hand, the pressure gradient caused by moving warm fruit into cool air would be beneficial in cooling internal tissues, but, moving cool fruit to warm environmental temperatures may have undesirable consequences such as significant water loss. Evidence in this study that small pressure changes result in substantial movement of internal gases could have important implications for postharvest transportation and handling practices where pressure gradients may

occur. For example, depressurization during air transportation, pressurization during CA storage, temperature gradients caused by storage room defrost cycles or movement of cold produce to a warm retail shelf, and even opening and closing cold storage room doors may contribute significantly to mass gas flow.

Diffusion of gases in tomatoes occurs through the stem scar and the epidermis. The permeability of gases through the latter depends on the solubility and diffusivity of gases in the waxy layer that normally covers the epidermis. Cameron and Yang (1982) estimated that the stem scar region is the site for 97% of the gas exchange taking place in tomato fruit. The liquid matrix of the fruit presents an obstacle in the evaluation of the stem scar as an area for mass gas transfer. Using peeled tomato fruit, Bertola et al. (1990) resolved that diffusive resistance in tomato tissue was a significant factor in the rate of internal mass gas transfer. By determining the mass transfer coefficient of intact tomato fruit and fruit with the blocked stem scar they found that the specific resistance of the peel is approximately 200 times greater than that of the stem scar, and that two-thirds of the gas diffusing in the fruit passes through the scar. This finding agrees more closely with that of Burg and Burg (1965) who determined that 60% of gas exchange takes place through the stem scar. The substantial diffusion resistance of the liquid matrix, low gas permeability of the peel and the small surface area of the stem scar, often representing no more than 1% of the tomato surface area, probably accounts for the lack of response to varying atmospheric pressure.

Part of the response to pressure variations exhibited by cucumbers and tomatoes, aside from limited opportunities for gas exchange, could be due to the period of the pressure cycle. A single period of 40 seconds was chosen for this study based on information by Jolliffe and Moloney (unpublished). They

speculated that short period cycles may be too brief to allow maximum bulk gas flow along a pressure gradient before the gradient was reversed.

Where bulk gas flow occurs, a model can be used to describe the movement of CO_2 out of internal tissues by artificial means (Fig. 1.4) where V is the tissue volume, V_i is the internal gas volume, C_{in} and C_{out} are the internal and external CO_2 concentrations (mass/L), F_d is the CO_2 efflux rate at a constant pressure (mass CO_2/fresh wt/time) and F_D is CO_2 efflux rate caused by diffusive flow. During the first cycle of pressure variations, CO_2 efflux driven by diffusive flow can be expressed as:

$$F_D t = F_d[\frac{C_{in}-C_{out}}{C_a-C_{out}}]t \qquad \text{(mass/fresh wt)}$$

where C_a equals C_{in} only under constant pressure and t is the period per cycle (time). If external gases flow back into internal gas spaces during a pressure cycle (as may happen when pressure drops back to the ambient level) then during one variable pressure cycle, CO_2 efflux driven by bulk flow will be:

$$C_{in}V_iA - C_{out}V_iA = (C_{in} - C_{out})V_iA \qquad \text{(mass/fresh wt)}$$

where A is the amplitude of the pressure cycle. When it is assumed that F_d is unaffected by atmospheric pressure cycles then CO_2 influx from internal production during one pressure cycle will equal $F_d t$.

If the initial internal concentration of CO_2 could be established, modeling of the effect of external variations in pressure on internal concentrations of CO_2 may be possible.

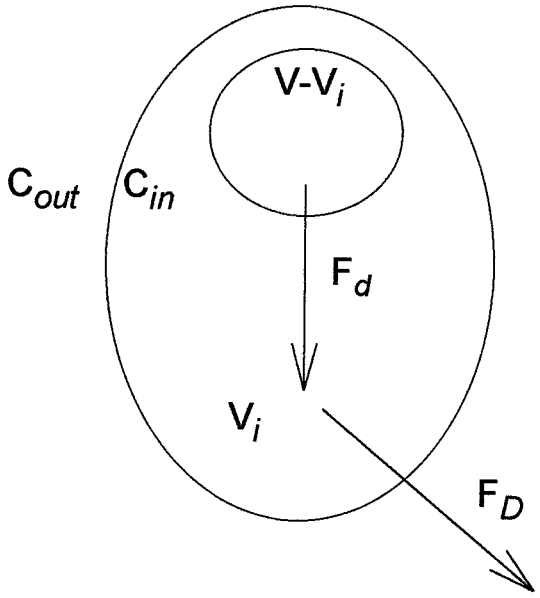

Fig. 1.4. A model of bulk gas flow.

B. EFFECT OF TEMPERATURE

Raising the storage temperature resulted in increased net rates of CO_2 emission from produce stored under both constant and varying pressures (Fig. 1.5). This increase was significant ($P<0.0001$) for all plant organs at all temperatures, except in onions where a temperature increase from 0 to 10 °C had no significant ($P>0.05$) effect at constant pressure (see Table 1.4 of Appendix 2 for more information). There was no indication that temperature affected the response to changes in atmospheric pressure nor that any significant interaction between temperature and level of pressure variations occurred for any of the plant organs. It was expected that temperature might affect the response to variable pressures particularly in bulky tissues with a more liquid matrix like tomato. The temperature coefficient of CO_2 diffusion in air is much smaller than that of its solubility in water. The differences in the diffusivity of CO_2 at different temperatures, therefore, are expected to be quite large when CO_2 is diffusing in aqueous media (Solomos, 1987). Tomatoes, however, did not show any response to pressure changes at either temperature.

The synthesis of volatile products and ethylene are among the metabolic processes not strongly suppressed by cold temperature. Therefore, even if other metabolic processes are slowed down through imposition of low temperatures, autocatalytic ethylene synthesis goes on, increasing internal concentrations of the phytohormone. When produce is removed from cold storage to the warm retail shelf, supraoptimal concentrations of internal ethylene may have a negative effect on shelf-life. Fruits and vegetables stored for long periods have a shorter shelf life upon rewarming and there are indications that a build-up of ethylene during cold storage may be an important factor in quality decline (Phan, 1987). Low ethylene storage has been found to be highly effective in reducing storage disorders and maintaining quality of many

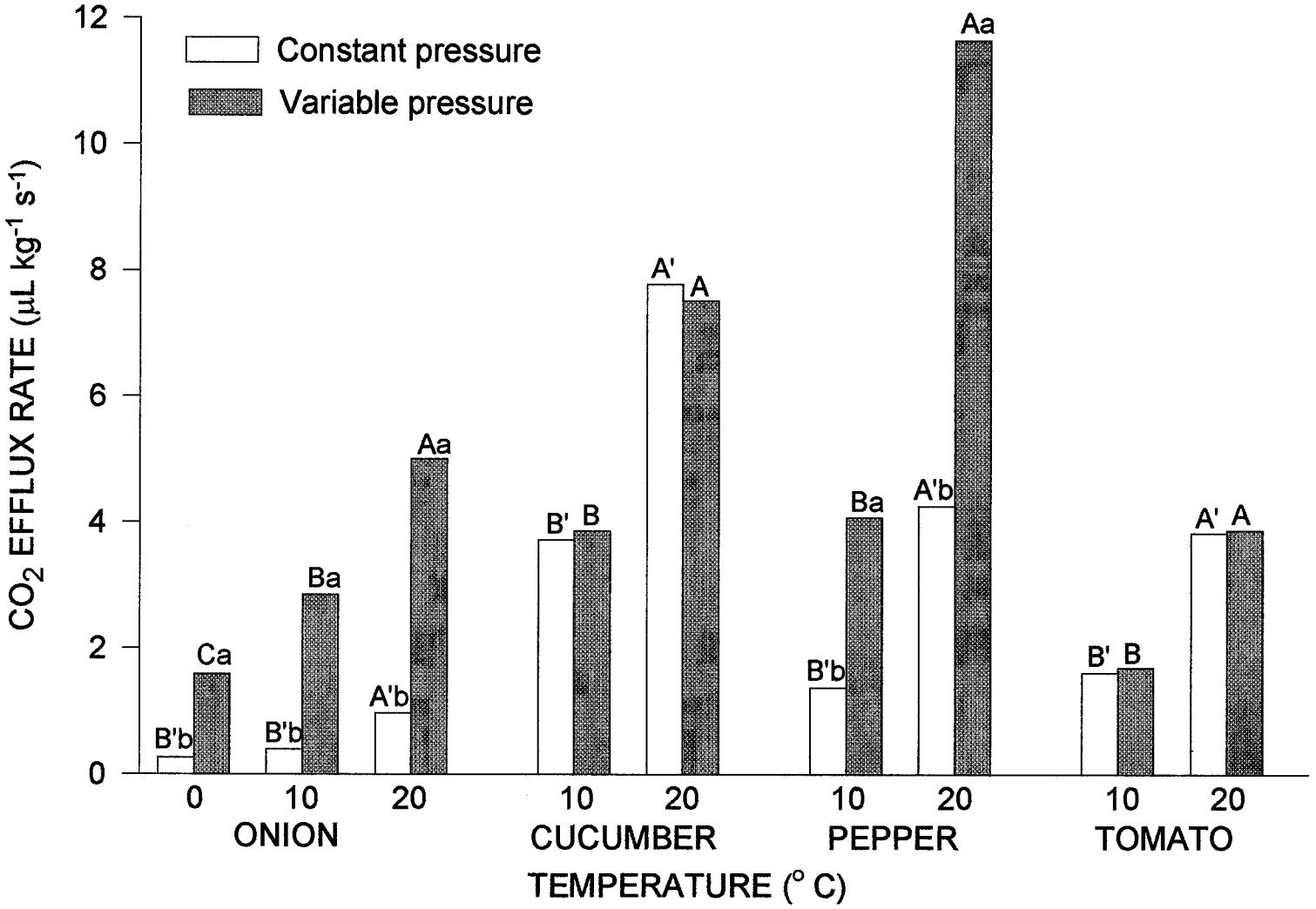

Fig. 1.5. Main effect of temperature on net CO_2 efflux rate of onions, cucumbers, sweet peppers and tomatoes. $^{A,B,C,A',B'}$ Bars associated with each plant tissue, with different letters, are significantly ($P<0.05$, F test) different. ^{a,b} Bars associated with each temperature, with different letters, are significantly ($P<0.05$, paired t test) different.

horticultural crops but, according to Sherman (1985), the technology for ethylene control during commercial storage and handling requires improvement. Research by Jolliffe and Moloney (unpublished) supplies evidence that variable pressure treatments cause bulk flow of ethylene gas in apple fruit. Since low temperatures do not appear to interfere with pathways of convective flow it is possible that external pressure variations could improve the control of internal concentrations of ethylene in fruits and vegetables. The present studies did not investigate ethylene flow in onions, cucumbers, sweet peppers and tomatoes because of the low sensitivity of the instrumentation to ethylene.

C. EFFECT OF INTERVAL OF CONSTANT PRESSURE BETWEEN PRESSURE CYCLES (DCAP)

Increasing the length of the interval of constant atmospheric pressure (DCAP) following the 5 minute variable pressure treatment from 5 to 15 minutes increased ($P<0.001$) the measured response to cycling atmospheric pressures in onions, cucumbers and tomatoes but had no effect on net CO_2 emission rate of sweet pepper (Fig. 1.6). Net CO_2 emission rate at constant pressure was significantly ($P<0.005$) reduced in onions when DCAP was increased from 5 to 15 min but significantly ($P<0.005$) increased in tomatoes and unchanged in cucumbers and sweet peppers. It is expected that the response to DCAP is similar to the response to period noted by Jolliffe and Moloney (unpublished) where short period cycles may be too brief to allow maximum bulk gas flow along the pressure gradient before the gradient is reversed. It may be useful to investigate period effects in cucumber more fully since there is some indication that lengthening DCAP results in a higher CO_2 emission rate during variable pressure treatments (Fig 1.6). The lack of a response by peppers to lengthening

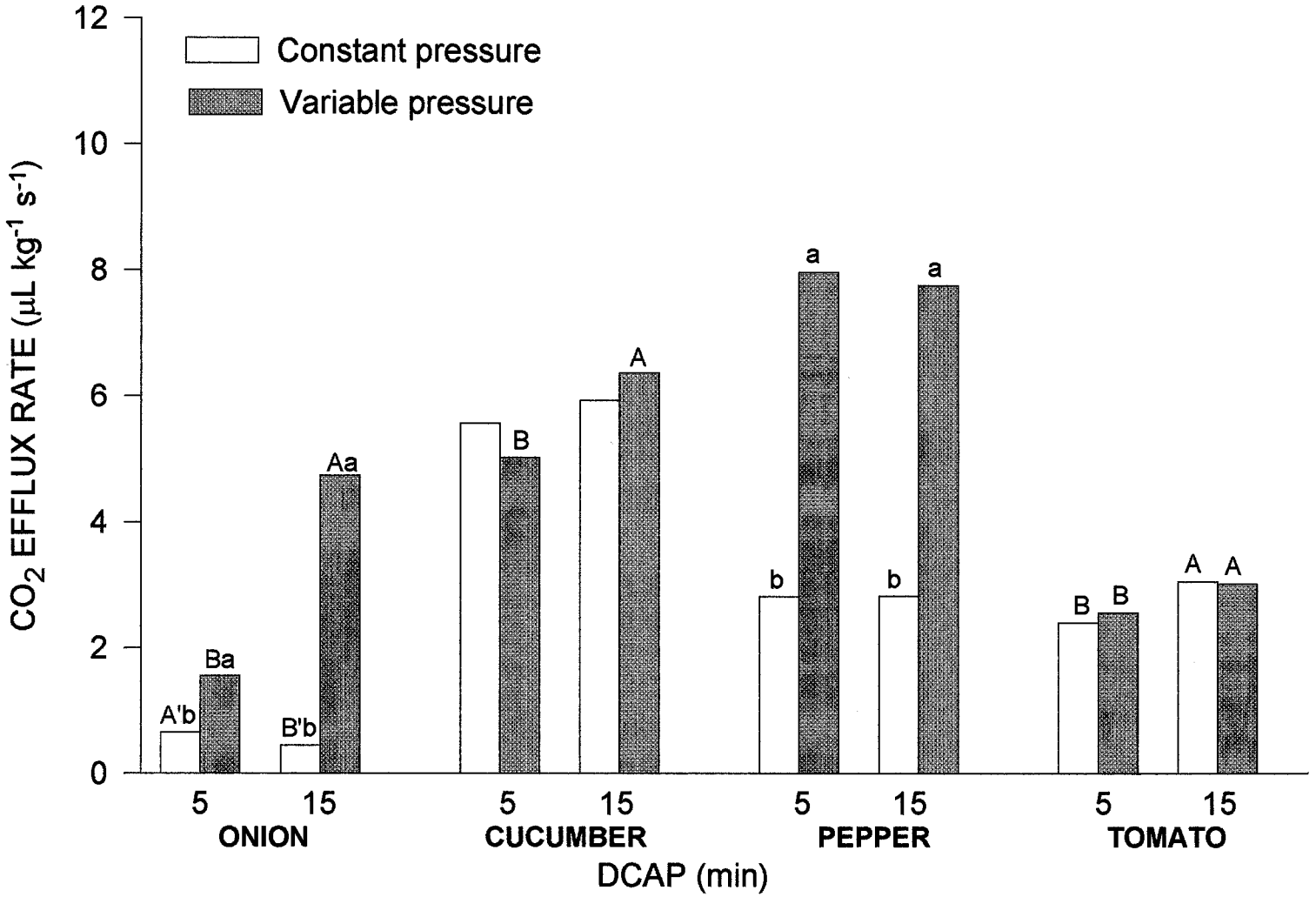

Fig 1.6. Main effect of DCAP on net CO_2 efflux rate of onions, cucumbers, sweet peppers and tomatoes. $^{A,B,A',B'}$ Bars associated with each plant tissue, with different letters, are significantly ($P<0.05$, F test) different. ^{a,b} Bars associated with each DCAP level, with different letters, are significantly ($P<0.05$, paired t test) different.

DCAP indicates that lack of internal resistance allows rapid bulk gas flow in the direction of the pressure gradient.

Interaction between temperature and DCAP, and level of pressure variations and DCAP was significant ($P<0.0001$) only for onions undergoing variable pressure treatment. The F values for temperature × DCAP (linear) and temperature × DCAP (quadratic) indicated a significant interaction for linear net CO_2 emission rates and no quadratic interaction. The effects of temperature and DCAP on net CO_2 efflux rates of onions are illustrated in Fig. 1.7 showing that the magnitude of the response to variable pressures appeared to be much greater when DCAP was 15 min rather than 5 min (see Table 1.4 in Appendix 1 for further information). In cucumber, sweet pepper and tomato fruit there were no significant interactions between DCAP and any of the other treatments as determined by measuring net CO_2 efflux rate. However, lengthening DCAP appeared to allow a fuller expression of the response to variable pressure treatment in cucumber as well as reducing the variability between cucumber samples (Fig 1.8). Although DCAP appeared to have no significant effect on response to changes in atmospheric pressure surrounding pepper fruit, variability in response among individual fruit was reduced by lengthening DCAP (Fig 1.9) suggesting that timing of the pressure variations should be examined further. In tomato fruit, lengthening DCAP resulted in higher rates of CO_2 efflux but there was no visible reduction in sample variability (Fig. 1.10). Means and standard deviations for experimental results for cucumbers, peppers and tomatoes are given in Appendix 2, Tables 1.6, 1.8 and 1.10, respectively.

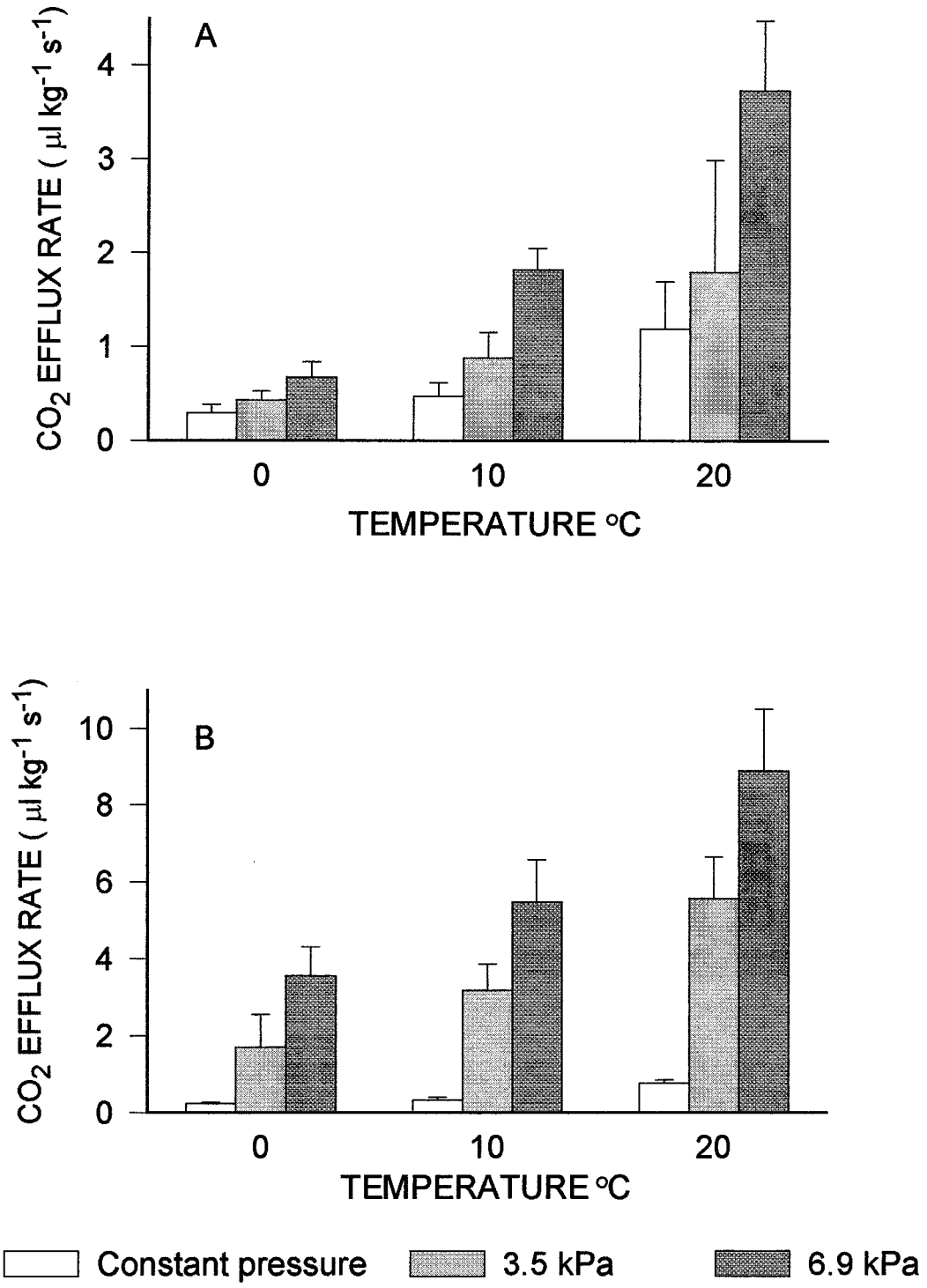

Fig. 1.7. Effect of DCAP, pressure variations and temperature on CO_2 efflux rate of onions. (A) DCAP = 5 min, (B) DCAP = 15 min. Bars represent treatment means and standard deviations for observations at constant atmospheric pressure (n=6) and variable pressure at levels of 3.5 (n=3) and 6.9 (n=3) kPa above atmospheric pressure.

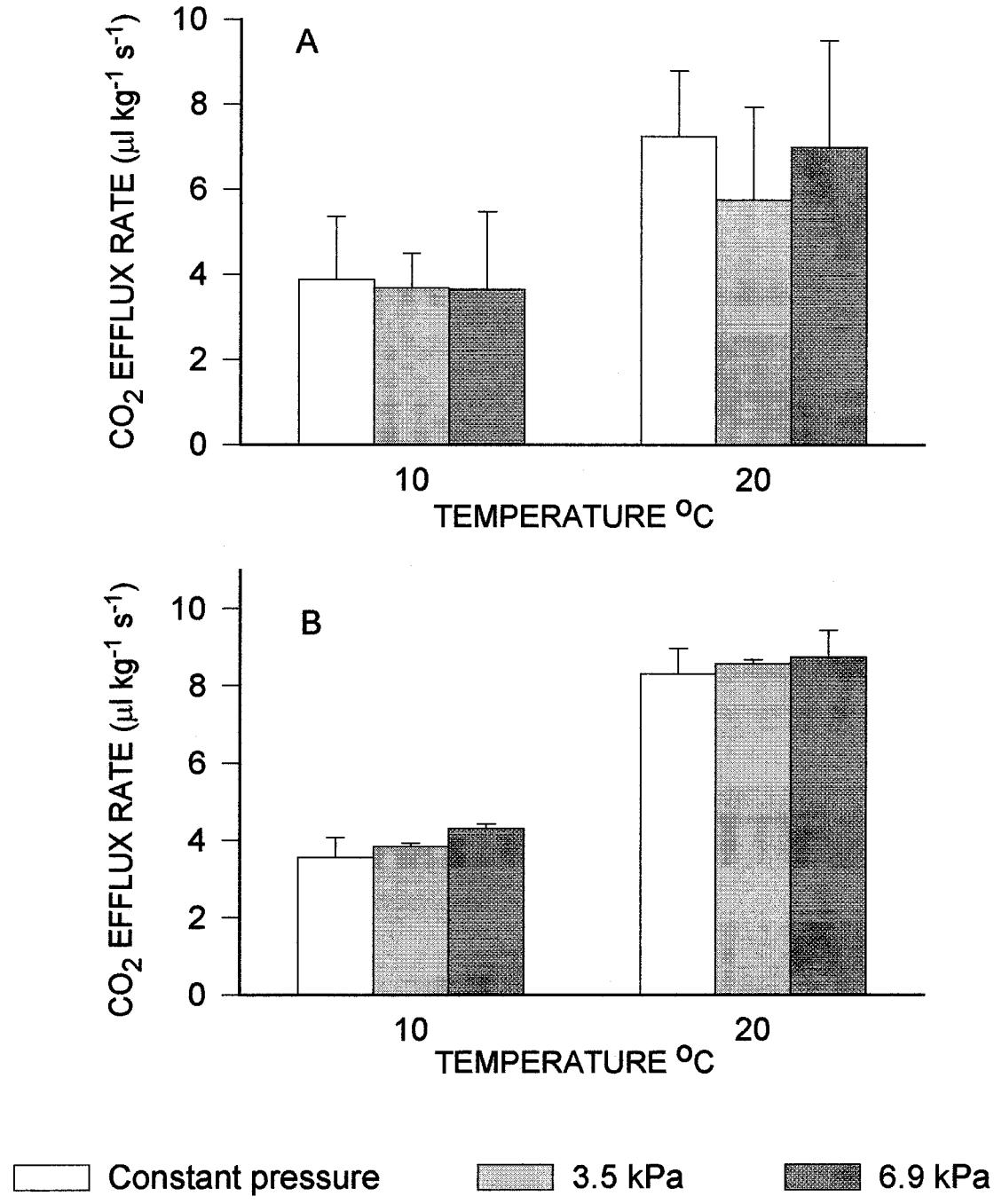

Fig. 1.8. Effect of DCAP, pressure variations and temperature on CO_2 efflux rate of cucumbers. (A) DCAP = 5 min, (B) DCAP = 15 min. Bars represent treatment means and standard deviations for observations at constant atmospheric pressure (n=6) and variable pressure at levels of 3.5 (n=3) and 6.9 (n=3) kPa above atmospheric pressure.

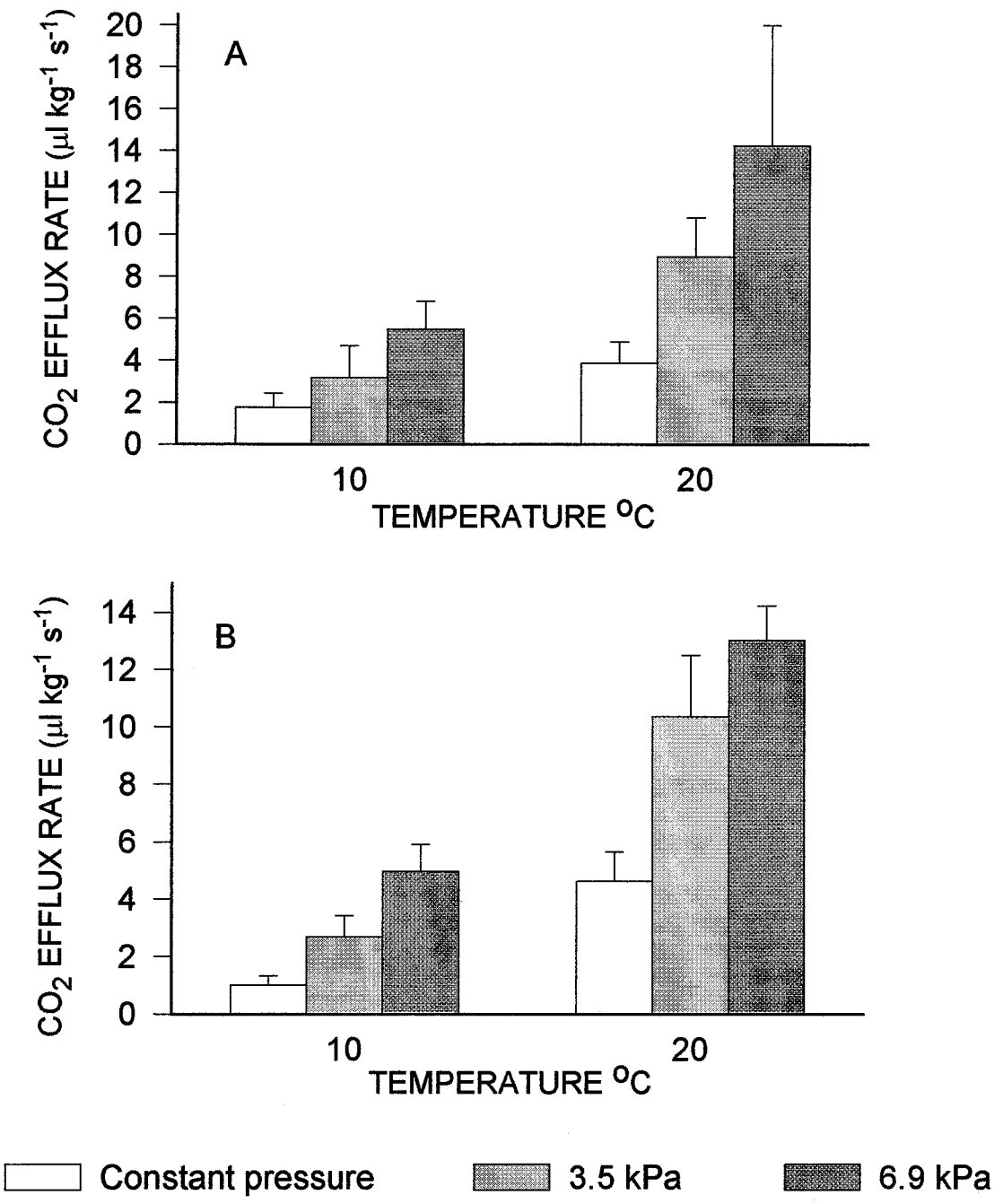

Fig. 1.9. Effect of DCAP, pressure variations and temperature on CO_2 efflux rate of sweet peppers. (A) DCAP = 5 min, (B) DCAP = 15 min. Bars represent treatment means and standard deviations for observations at constant atmospheric pressure (n=6) and variable pressure at levels of 3.5 (n=3) and 6.9 (n=3) kPa above atmospheric pressure.

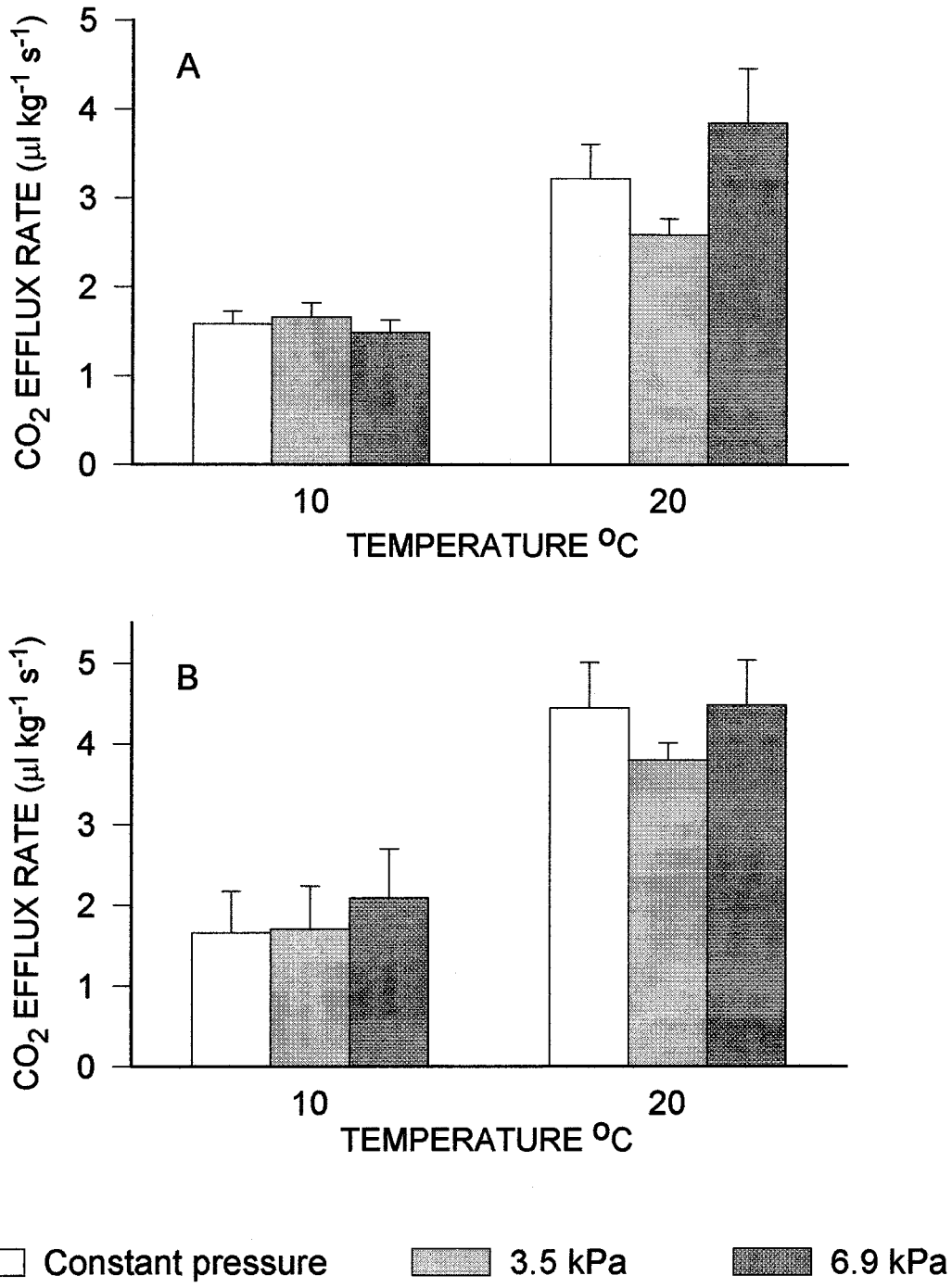

Fig. 1.10. Effect of DCAP, pressure variations and temperature on CO_2 efflux rate of tomatoes. (A) DCAP = 5 min, (B) DCAP = 15 min. Bars represent treatment means and standard deviations for observations at constant atmospheric pressure (n=6) and variable pressure at levels of 3.5 (n=3) and 6.9 (n=3) kPa above atmospheric pressure.

D. EFFECT OF PRESSURE VARIATION, TEMPERATURE AND DCAP ON RELATIVE RATE OF CO_2 EFFLUX

Analysis of ratios of net gas flow during variable pressure treatments and intervals of constant pressure (Table 1.2) indicated that temperature had no significant ($P>0.05$) effect on relative rate of CO_2 emission rate of onion, cucumbers, peppers or tomatoes. Although some of the differences in mean relative CO_2 efflux rates of onions at various temperatures were quite large, they were not significant due to sample variation. These results indicate that the response to variations in external pressures will be of the same magnitude regardless of temperature as long as the external and internal temperatures are equal.

In onions, main effects for level of pressure variation and DCAP were significant ($P<0.05$) for relative rate of CO_2 emission with the higher pressure level and longer DCAP resulting in higher relative rates. Although interaction between pressure level and DCAP was significant ($P<0.05$) this appeared to occur in the mean comparison of 15 min DCAP at 3.5 kPa and 5 min DCAP at 6.9 kPa which is not a comparison of interest. Pressure level and DCAP had little effect on relative rate of CO_2 emission in cucumbers and tomatoes while increasing the level of pressure variation from 3.5 to 6.9 kPa resulted in an increased relative CO_2 efflux rate in sweet peppers.

E. EFFECT OF OXYGEN CONCENTRATION

To determine if oxygen concentration would affect the CO_2 efflux rate of onions, cucumbers, sweet peppers and tomatoes subjected to cyclic pressure variations, pressure treatments were carried out in a low oxygen atmosphere.

Table 1.2. Effect of level of pressure variation, temperature and DCAP on relative carbon dioxide efflux rate[1] in onion, cucumber, sweet pepper and tomato.

		Pressure Level (kPa)			
		3.5		6.9	
		DCAP[2] (min)			
Tissue	°C	5	15	5	15
Onion	0	1.5 (±0.7)	7.6 (±3.5)	2.9 (±0.8)	14.9 (±4.9)
	10	1.8 (±0.2)	9.2 (±1.8)	4.6 (±1.1)	20.1 (±7.9)
	20	1.9 (±0.1)	6.9 (±2.0)	2.4 (±0.3)	12.7 (±2.6)
Cuc	10	0.9 (±0.1)	1.2 (±0.3)	0.9 (±0.1)	1.2 (±0.2)
	20	0.8 (±0.2)	1.1 (±0.1)	0.9 (±0.1)	1.0 (±0.1)
Pepper	10	2.1 (±0.7)	2.7 (±0.6)	2.9 (±0.4)	5.4 (±1.5)
	20	2.5 (±0.1)	2.3 (±0.3)	3.3 (±0.8)	2.9 (±0.6)
Tomato	10	0.8 (±0.3)	1.0 (±0.2)	1.1 (±0.2)	1.4 (±0.2)
	20	1.5 (±0.3)	1.0 (±0.2)	0.9 (±0.1)	1.0 (±0.1)

[1] Measurement of relative rate of CO_2 efflux determined by (CO_2 efflux rate during variable pressure)/(CO_2 efflux rate during constant pressure), Means ± SD.

[2] Duration of the interval of constant atmospheric pressure between 5 min variable pressure treatments.

Recommended postharvest levels of 1% O_2 for onions and 3% O_2 for cucumbers, sweet pepper and tomatoes (Saltveit, 1985) were established by flushing the gas exchange apparatus with premixed gases. Since DCAP appeared to have an effect on CO_2 emission rate, an interval of 15 min of constant pressure between pressure treatments was chosen for the low oxygen studies.

At 1% O_2, net CO_2 efflux rate of onions during variable atmospheric pressure changes was significantly ($P<0.0001$) lower than that in onions undergoing pressure treatments in air even though diffusive efflux at a constant pressure was unaffected by O_2 concentration (Fig. 1.11). However, changes in atmospheric pressure still resulted in an increase ($P<0.0001$) in net CO_2 efflux rate over constant pressure at low atmospheric O_2. In cucumbers, low oxygen concentrations reduced ($P<0.05$) net CO_2 emission rates during constant pressure and variable pressure treatments but the overall response to variable pressure treatments was the same for both O_2 levels. Low oxygen storage did not appear to affect the response of tomatoes or sweet pepper to variable pressure treatment.

It is possible that longer term storage of onions under low-oxygen may result in a damping of response to variable atmospheric pressures. Carbon dioxide measurements taken over a period of 7 hours indicate that generally the response was not diminished over this period (Fig. 1.12). Similar results were obtained with onions stored under fluctuating atmospheric pressures at 10 and 20°C (not shown). Level of pressure variation at 3 different temperatures had little effect on net CO_2 efflux rate of onions held under 1% O_2 compared to the response at ambient O_2 concentrations (Fig 1.13). As well, there were no significant ($P>0.05$) differences between efflux rates at constant or variable pressure for all pressure treatments at 1% oxygen and 0° C, and at

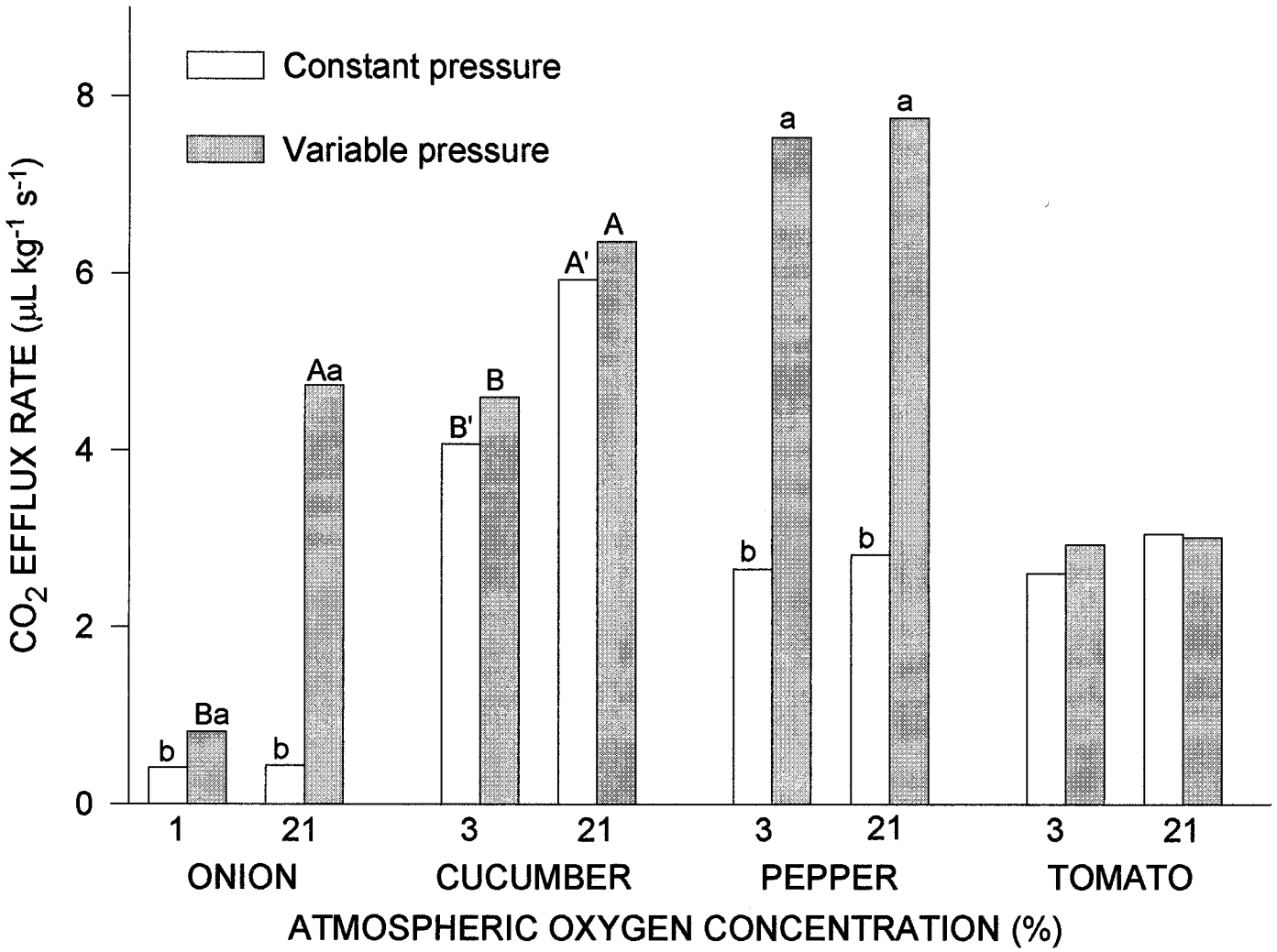

Fig. 1.11. Main effect of atmospheric oxygen concentration on net CO_2 efflux rate of onions, cucumbers, sweet peppers and tomatoes. $^{A,B,A',B'}$ Bars associated with each plant tissue, with different letters, are significantly ($P<0.05$, F test) different. ^{a,b} Bars associated with each oxygen level, with different letters, are significantly ($P<0.05$, paired t test) different.

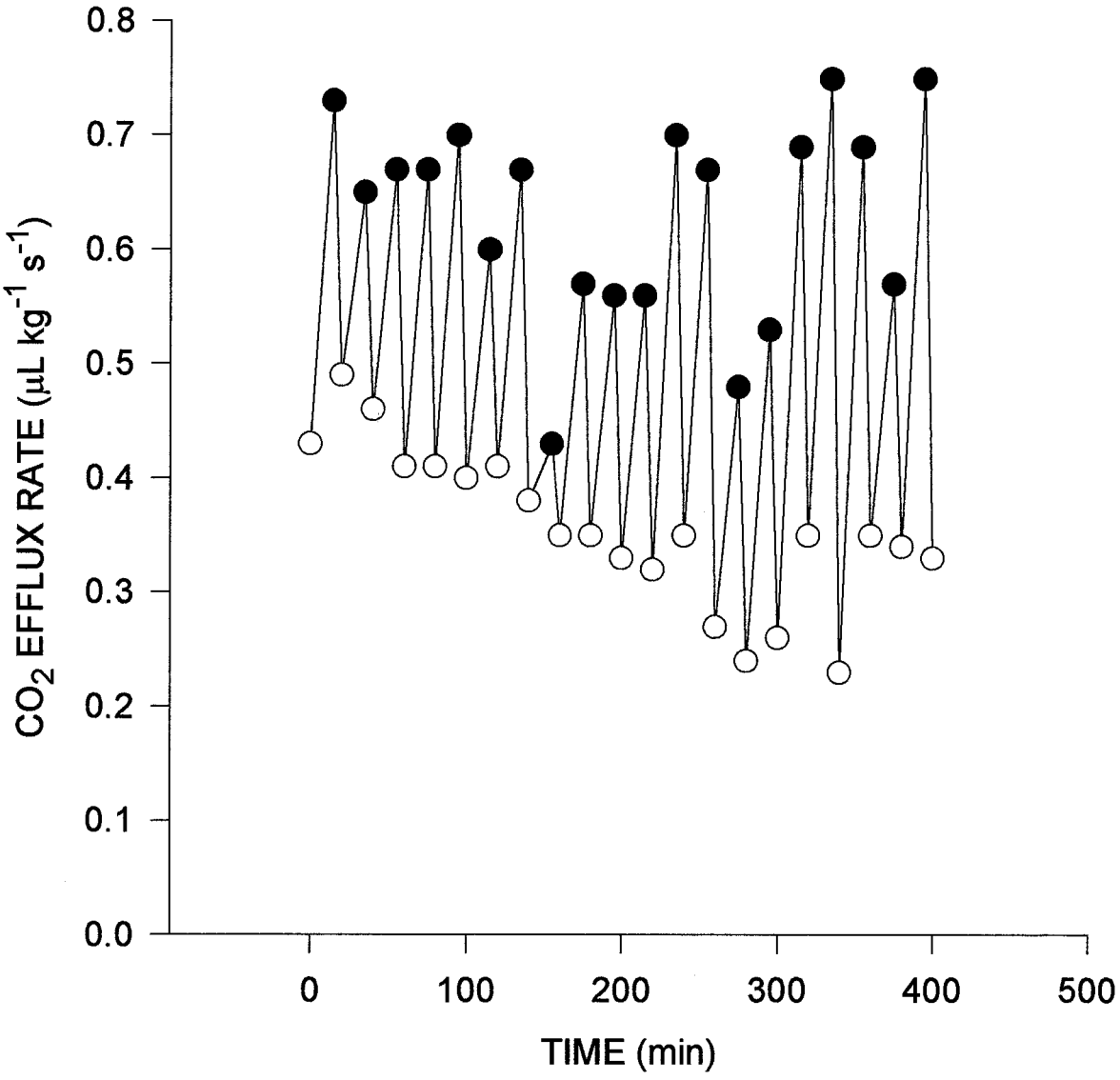

Fig. 1.12. Sustainability of mass gas flow in an onion bulb during variable pressure treatments. Net CO_2 emission rate during variable pressure treatments is represented by closed symbols and during constant pressure by open symbols. Variable pressure cycles of 6.9 kPa amplitude with a 40 s period were applied for 5 min at 0°C.

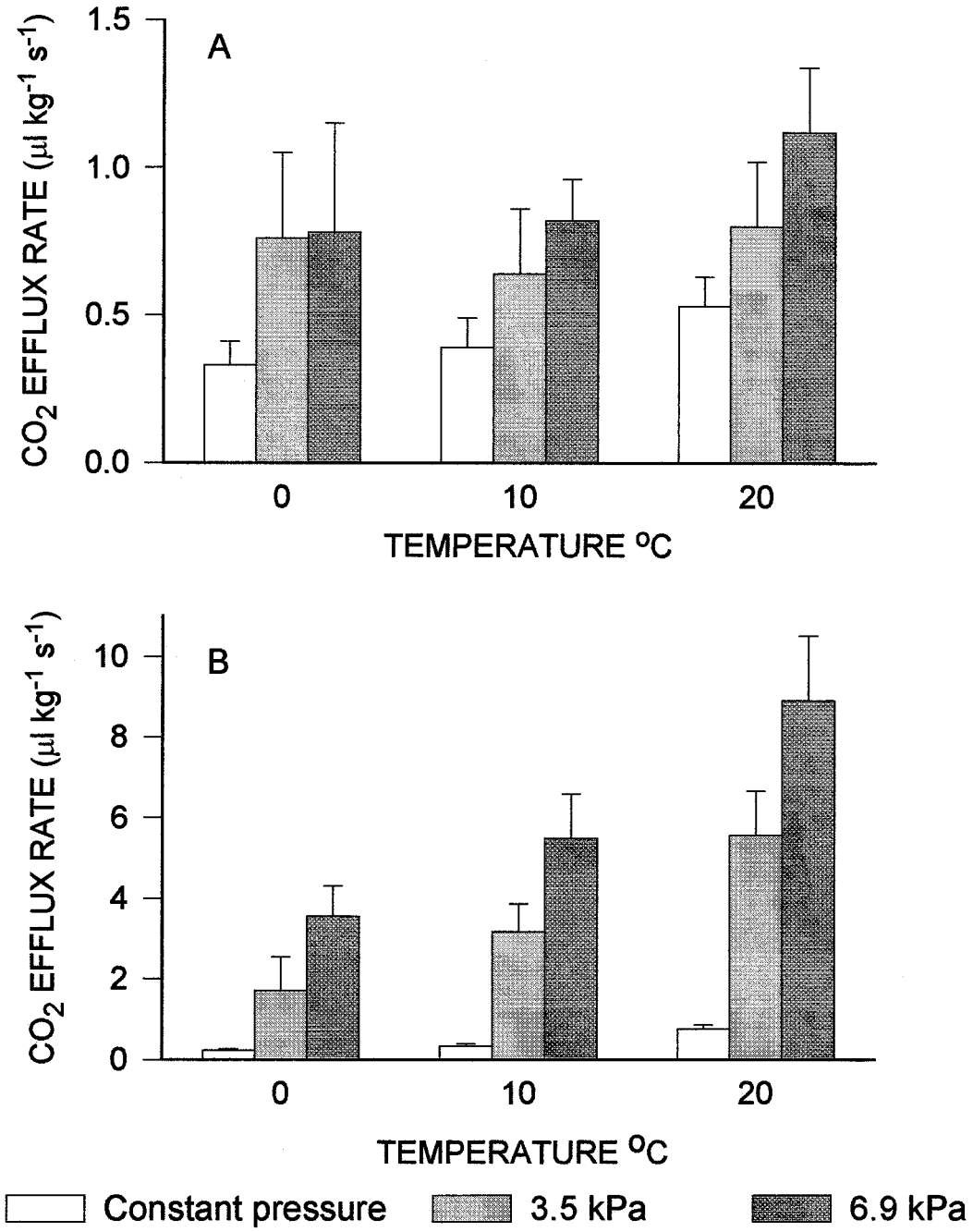

Fig. 1.13. Effect of oxygen concentration, pressure variations and temperature on net CO_2 efflux rate of jumbo onions. (A) 3% oxygen, (B) 21% oxygen. Treatment means and standard deviations for observations at constant atmospheric pressure (n=6) and variable pressure at levels of 3.5 (n=3) and 6.9 (n=3) kPa above atmospheric pressure.

constant pressure and a pressure level of 3.5 kPa at 10° C (see Table 1.5 of Appendix 2 for details). In exploring the significant interaction between O_2 concentration and temperature ($P<0.0001$), contrast statements revealed that the relationship between temperature and oxygen is linear ($P<0.0001$). The combination of low oxygen and temperature must have reduced respiration rate to such an extent that mass gas transfer was restrained. This resulted in large differences in relative rate of gas transfer between onions held at 1% oxygen compared to those held at 21% (Table 1.3).

Ladeinde and Hicks (1988) reported that 24 h was required to establish equilibrium level of internal O_2 in onions held in atmospheres containing different an O_2 concentrations. At 0°C internal O_2 concentrations equaled 75.7, 77.9 and 93.7% of external atmospheres containing 4.5, 15 and 21% O_2, respectively; at 15°C the internal O_2 concentrations equaled 55.3, 57.4 and 78.9% of external concentrations, respectively; and at 30°C, these concentrations were 6.7, 31.5 and 48.6% of external concentrations, respectively. By extrapolating these results, the level of 1% O_2 used in this study should have resulted in internal O_2 concentrations of about 0.75% at 0°C and 0.4% at 20°C. According to Kader (1985), onions can continue to respire aerobically at very low external O_2 concentrations which would explain why onion respiration rate at constant pressure under both atmospheres was unchanged. It is thought to be unlikely that changing atmospheric pressures affected respiration rate at a cellular level by increasing the internal partial pressure of O_2 during application of the pressure treatments thereby increasing CO_2 emission rate. If this were true then one might expect an increase in relative CO_2 emission rate with an increase in temperature. According to the data in Table 1.3 relative rate decreased slightly at higher temperatures as it would if O_2 became more limited in internal tissues. In other words, the fact that

Table 1.3. Effect of oxygen concentration, level of pressure variation and temperature on relative carbon dioxide efflux rate[1] in onion, cucumber, sweet pepper and tomato.

		Pressure Level (kPa)			
		3.5		6.9	
		Oxygen Concentration (%)			
Tissue	°C	1	21	1	21
Onion	0	2.1 (±0.7)	7.6 (±3.5)	2.5 (±1.2)	14.9 (±4.9)
	10	1.6 (±0.4)	9.2 (±1.8)	2.4 (±0.5)	20.1 (±7.9)
	20	1.5 (±0.1)	6.9 (±2.0)	2.3 (±0.5)	12.7 (±2.6)
		3	21	3	21
Cuc	10	1.1 (±0.1)	1.2 (±0.3)	1.2 (±0.1)	1.2 (±0.2)
	20	1.2 (±0.2)	1.1 (±0.1)	1.1 (±0.2)	1.0 (±0.1)
Pepper	10	2.9 (±0.5)	2.7 (±0.6)	4.0 (±0.5)	5.5 (±1.5)
	20	2.2 (±0.2)	2.3 (±0.3)	3.3 (±0.3)	2.9 (±0.6)
Tomato	10	1.0 (±0.1)	1.0 (±0.2)	1.2 (±0.2)	1.3 (±0.2)
	20	1.0 (±0.1)	0.9 (±0.2)	1.3 (±0.2)	1.0 (±0.1)

[1] Measurement of relative rate of CO_2 efflux determined by (CO_2 efflux rate during variable pressure)/(CO_2 efflux rate during constant pressure), Means ± SD.

at 20°C, under an atmosphere containing 1% O_2, net CO_2 efflux rate in onions was not that much greater than at 0°C may indicate that varying atmospheric pressure only affected the movement of gases in the intercellular air spaces of porous tissues. On the other hand, cycling pressures with a period longer than 40 s may allow for diffusive transfer of O_2 to centers of respiration resulting ingreater respiratory activity.

Lowering external O_2 concentration to 3% reduced CO_2 emission rate in cucumbers during constant pressure and variable pressure cycles, but the reduction in response to low O_2 was not as remarkable as that of onion (Fig. 1.14, see Table 1.7 of Appendix 2 for details). It may be possible that cucumbers must be stored for a longer period than the few hours required to carry out the treatments in this study before respiration rate stabilizes in response to a lower concentration of O_2. A study by Solomos (1982) indicates that for dense tissues like sweet potato, 48 hours is required for respiration to decline and stabilize after external O_2 concentration is lowered. The data in Table 1.3 indicates that external O_2 concentrations or levels of pressure treatments at 10 and 20°C had little effect on the relative rate of CO_2 emission.

A 20.8% reduction in respiration rate of Chili peppers held at 10°C was obtained in one published study by reducing external O_2 concentration from normal ambient levels to 5% (Kader et al, 1989). In the present study, compared to air, external concentrations of 3% O_2 resulted in a 13.5% reduction ($P>0.05$) in CO_2 emission rate in sweet pepper at 20°C (constant pressure), but there was no reduction at 10°C (Fig 1.15). Although there was a tendency for peppers subjected to pressure treatments at a low O_2 concentration and 10°C to have higher CO_2 efflux rates than the peppers pressure-treated in air, this trend was reversed at 20°C (see Table 1.9 of Appendix 2 for details).

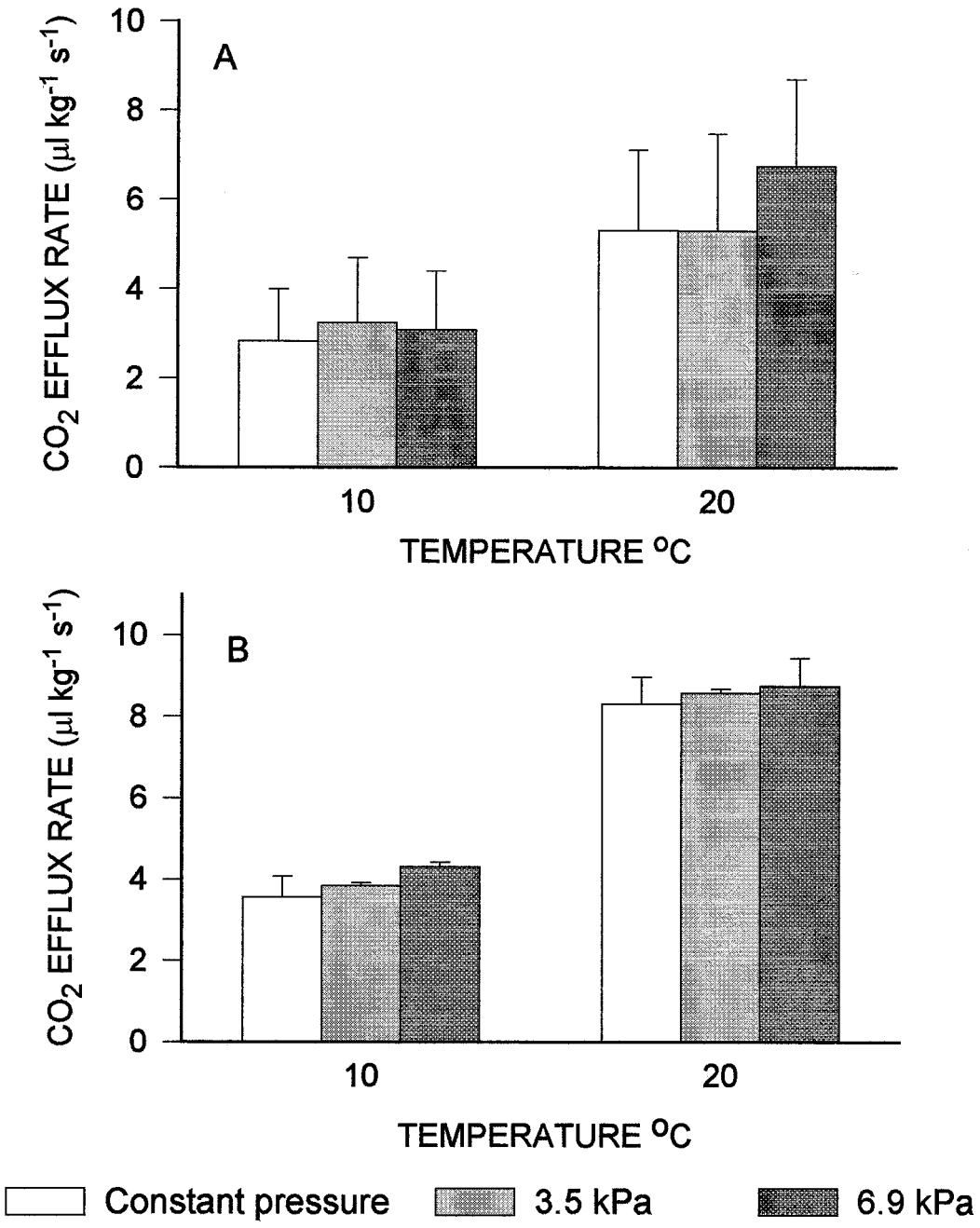

Fig. 1.14. Effect of oxygen concentration, pressure variations and temperature on net CO_2 efflux rate of cucumbers. (A) 3% oxygen, (B) 21% oxygen. Treatment means and standard deviations for observations at constant atmospheric pressure (n=6) and variable pressure at levels of 3.5 (n=3) and 6.9 (n=3) kPa above atmospheric pressure.

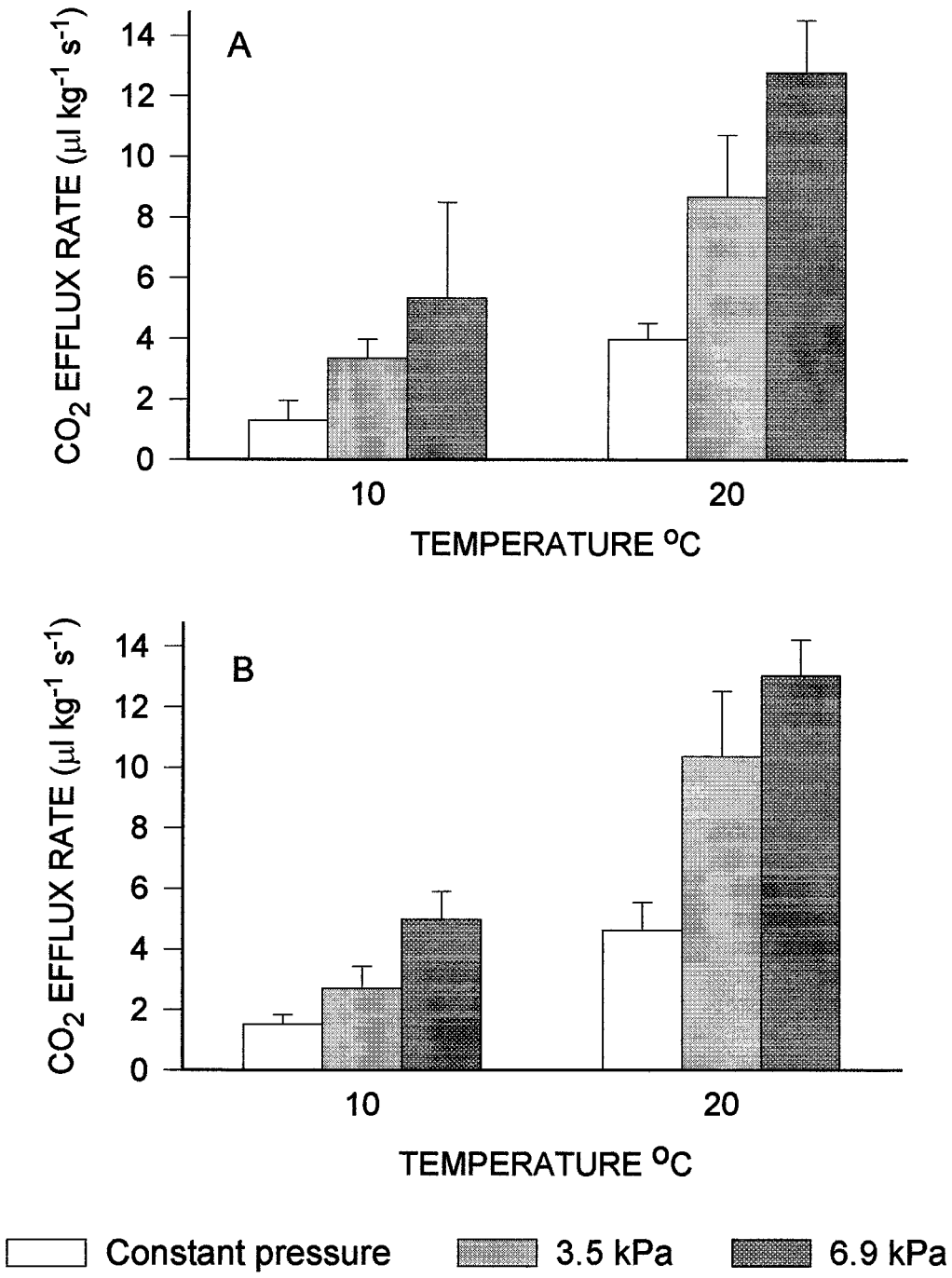

Fig. 1.15. Effect of oxygen concentration, pressure variations and temperature on net CO_2 efflux rate of sweet peppers. (A) 3% oxygen, (B) 21% oxygen. Treatment means and standard deviations for observations at constant atmospheric pressure (n=6) and variable pressure at levels of 3.5 (n=3) and 6.9 (n=3) kPa above atmospheric pressure.

There is no information, to the author's knowledge, about the effect of reduced atmospheric O_2 concentrations on red ripe tomatoes since storage research is generally done with mature-green tomatoes, which have a less fluid internal matrix and respire at a much higher rate. However, there is a great interest in bringing a ripe tomato to the retail shelf to assure the consumer of better flavour quality. There is some value therefore, in investigating techniques that could maintain high quality in tomatoes harvested at a later stage of ripeness. It is currently unknown if altering internal gas composition of ripe tomatoes has a noticeable effect on quality attributes.

External oxygen concentration and level of pressure treatment had no significant effect ($P>0.05$) on net CO_2 emission rate of tomatoes, however there was a significant temperature effect ($P<0.0001$) on tomatoes held at both oxygen concentrations. All interactions between main effects were insignificant ($P>0.05$). It was interesting to note that like peppers, the lower O_2 concentration at 10°C tended to result in a slightly higher net CO_2 efflux rate in tomatoes but this trend was reversed at 20°C (Fig 1.16, see Table 1.11 of Appendix 2 for data). Relative CO_2 emission rates for all treatments were similar (see Table 1.3).

The lack of a response to the lower O_2 concentration is likely due to the short term of the treatment. Because the internal matrix of tomato, especially ripe tomato, is very liquid and the area where most gas exchange occurs is relatively small more time was required for internal gases to equilibrate in a low oxygen atmosphere. However, it was in the interests of this study to determine if variable pressure treatments affected the rapidity of a response to low levels of atmospheric O_2.

In conclusion, this study shows that bulky fruits and vegetables with large morphological differences vary greatly in their response to changes in

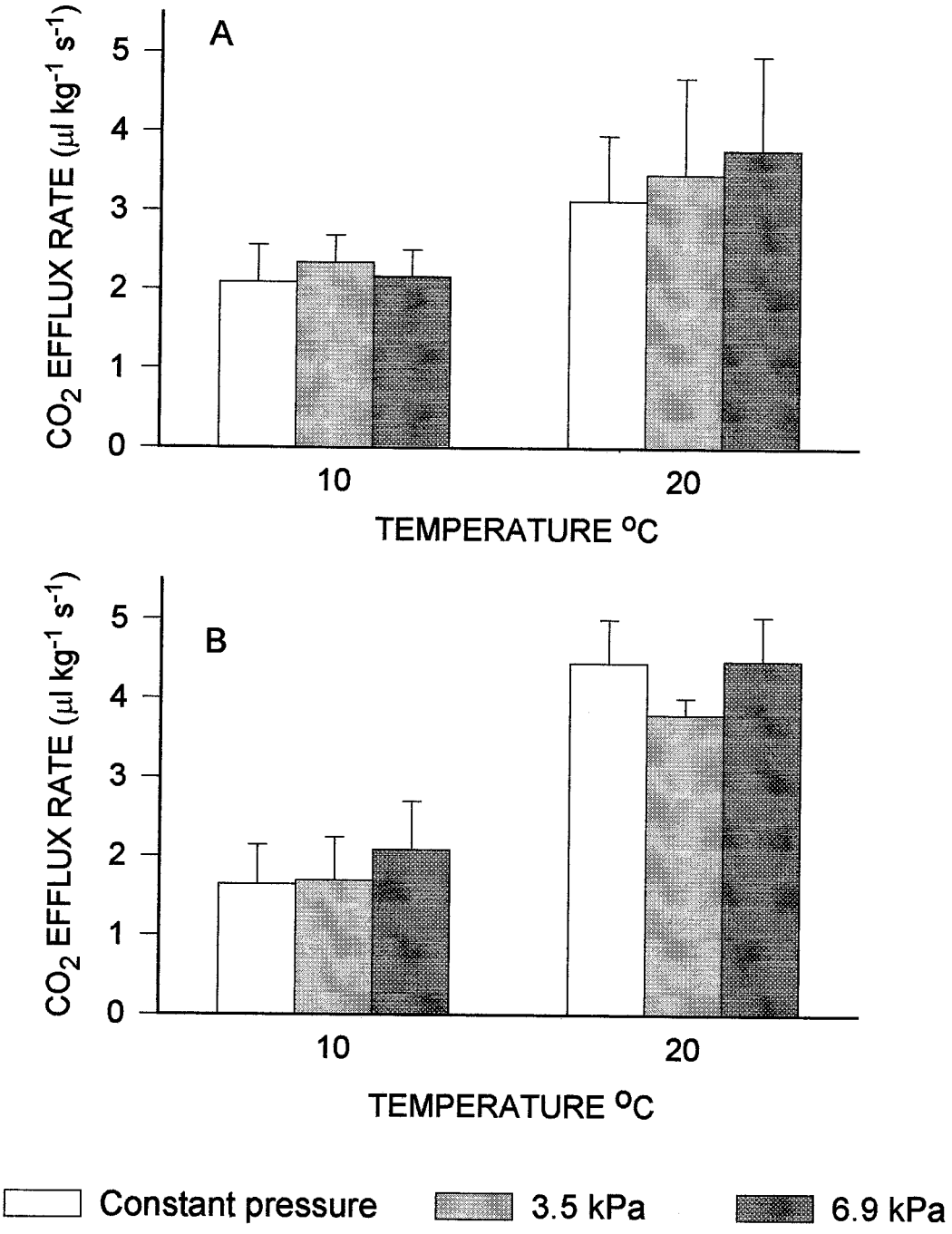

Fig. 1.16. Effect of oxygen concentration, pressure variations and temperature on net CO_2 efflux rate of tomatoes. (A) 3% oxygen, (B) 21% oxygen. Treatment means and standard deviations for observations at constant atmospheric pressure (n=6) and variable pressure at levels of 3.5 (n=3) and 6.9 (n=3) kPa above atmospheric pressure.

atmospheric pressure. Onions and sweet peppers both showed large relative responses to variable pressure treatments, but by changing temperatures, the time interval between series of pressure fluctuations and external O_2 concentration, the effect of differing pathways for gas movement became more apparent. Changing the time interval between the 5 min pressure treatments from 5 to 15 min more than doubled the relative rate of CO_2 efflux in onions while evoking little or no response in sweet peppers. This observation is probably due to the fact that there was no long time lag in movement of CO_2 from centers of production to the cavity of the pepper fruit. Although cucumbers and tomatoes were unresponsive to variable pressure treatments regardless of temperature, level of pressure treatment or O_2 concentration, there was some indication that increasing the interval between series of pressure variations had an effect on CO_2 efflux rate in cucumbers.

Researchers have focused their attention on the effects of external atmospheric concentrations of gases on fruits and vegetables while there is still much to discover about the mechanisms and pathways of gas exchange in bulky tissues. These studies suggest that pressure-driven gas exchange may accelerate movement of internal and external gases, offering the potential for better control of gas concentrations in plant tissues.

Other issues that need to be addressed are: assessment of effectiveness of positive and/or negative pressure pulses in promoting mass gas flow, whether systematically varying the atmospheric pressure surrounding bulky tissues alters total gas concentrations in core tissues or only in intercellular spaces of the superficial tissues, and determination of the effect of long term applications of variable pressures on shelf-life and rate of loss of cellular constituents.

SECTION 2

ANALYSIS OF AROMA VOLATILES OF ONIONS, PEPPERS, AND TOMATOES STORED UNDER VARYING PRESSURES

INTRODUCTION

Changes in carbohydrates, organic acids, proteins, amino acids, lipids and phenolic compounds can influence the flavour of fresh fruits and vegetables, either enhancing flavour quality or resulting in the production of undesirable off-flavours. Controlled atmosphere and MA storage have been used successfully to prolong shelf-life and maintain flavour quality in pome fruits, broccoli and root crops by suppressing senescence; to prevent enzymatic degradation of structural carbohydrates, storage starch, amino acids and lipids; and to reduce the rate of formation of harmful phenolics (Kader, 1986). Improper control of CO_2 and O_2 concentrations can result in anaerobic respiration resulting in the formation of acetaldehyde and ethanol, imparting undesirable flavour characteristics (Carlin et al., 1990). Controlled atmosphere storage for extended periods can decrease the production rate of volatiles by apples, pears and other fruits presumably by reducing the rate of alcohol synthesis (Kader, 1986). Minor disturbances in the balance of various compounds constituting flavour may render a poor sensory quality rather than a serious off-flavour, nevertheless, changes of this nature can lead to reduced consumer confidence in the product, resulting in economic loss.

Sensory panels made up of trained testers are currently utilized for determining differences in flavour characteristics among food products. Considerable effort has been expended in the development of instrumental analysis to supplement or replace sensory analysis which is considered to be expensive, difficult, labour intensive and inherently inaccurate and imprecise especially when large numbers of samples or variables are involved in an investigation (Zervos et al., 1992).

Salunke and Do (1976) asserted that the aroma of fruits and vegetables is the key factor for assessing their flavour quality. Headspace analysis is

considered to be the appropriate method for application to flavour research since it reveals the identity and the concentration in the vapour phase of those compounds that are directly responsible for the odour of the product (Maarse, 1991). Trapping headspace volatiles on a solid sorbent concentrates the trace analytes sufficiently for identification by GC/MS. The use of a solvent to desorb the volatile compounds from the solid support produces a liquid sample which can be stored or used for multiple gas chromatographic analyses.

Early in the development of experiments to test the effect of variable atmospheric pressure on mass gas flow in bulky tissues, a suggestion was made that variable pressures may enhance enzymatic degradation of vegetables due to an influx of O_2 into internal tissues (Solomos, T., private communication). Since flavour is an important factor in assessing changes in quality, this study was undertaken to determine if cyclic variations in total external atmospheric pressure would alter chromatographic profiles of aroma volatiles. The dynamic headspace sampling method of Buttery et al. (1987) was modified by the innovative use of disposable charcoal traps rather than Tenax traps which have a much lower loading capacity and are negatively affected by the presence of water vapour.

MATERIALS AND METHODS

A. PLANT MATERIALS

Tomatoes

Tomato plants (*Lycopersicon esculentum* L. cv Dombito) were grown in a greenhouse at Agriculture and Agri-Food Canada's Pacific Research Center at Agassiz. The fruit were harvested at the "turning" stage, randomly assigned to two groups and weighed. One group was placed in the treatment chamber of the gas exchange circuit described in Section 1 and the other group was placed in a forced-air, walk-in cooler.

Both containment systems were maintained at 15 °C and 95% RH. After loading, the gas-exchange circuit was closed and variable pressure treatments (see Section 1) were initiated. The 5 min treatments were applied at a level of 6.9 kPa with a 15 min interval of constant pressure between treatments. The treatments were carried out in triplicate for 3 and 7 days. At the end of each of these time periods, the tomatoes were removed from the treatment chamber and the walk-in cooler, weighed and stored in the laboratory at room temperature (average 24 °C) under normal laboratory lighting and allowed to ripen to a full red coloring. This ripening period was 13 and 11 days for the 3 and 7 day treatments, respectively. After ripening, the fruits were prepared for isolation of aroma volatiles.

Onions

Onions (*Allium cepa* L.) were purchased from a commercial source as required for the experiments, resulting in a sample selection from various origins, maintained under unknown conditions. Care was taken to select onions

free from defects and disease. The onions were divided into two groups and subjected to the same conditions as the tomatoes except the treatment periods were 7, 14, and 21 days, replicated 3 times. Following treatment the onions were immediately prepared for isolation of aroma compounds.

Sweet Peppers

Sweet pepper plants (*Capsicum annuum* L. cv Doria) were grown in a greenhouse on the Research Station at Agassiz. Immature pepper fruits weighing approximately 200 g each were harvested as required for each experimental replicate and divided into two treatment groups. The sweet peppers were subjected to the same treatments as tomato and onions over periods of 3, 7 and 14 days. In addition, a preliminary trial was performed to determine if pepper volatiles were affected by the concentration of oxygen while stored under variable atmospheric pressures. Sweet peppers were stored for 1 week, surrounded by either 21 or 3% oxygen, under a variable atmospheric pressure of 6.9 kPa. Following treatment the peppers were prepared for isolation of aroma volatiles.

B. REAGENTS and APPARATUS

Jacketed jars from Wheaton (Millville, New Jersey 08332, U.S.A.) fitted with teflon and glass connectors (Fig. 2.1) served as extraction vessels. A glass chromatography column containing a plug of silanized glass wool allowed most of the water to condense before reaching the activated charcoal tube. The temperature of the vessels was controlled by a recirculating water bath (Haake) and jars could be joined in tandem for multiple analyses. High-grade purified N_2 was delivered at a rate of 30 mL/min by four tube rotameters (Matheson, 7400

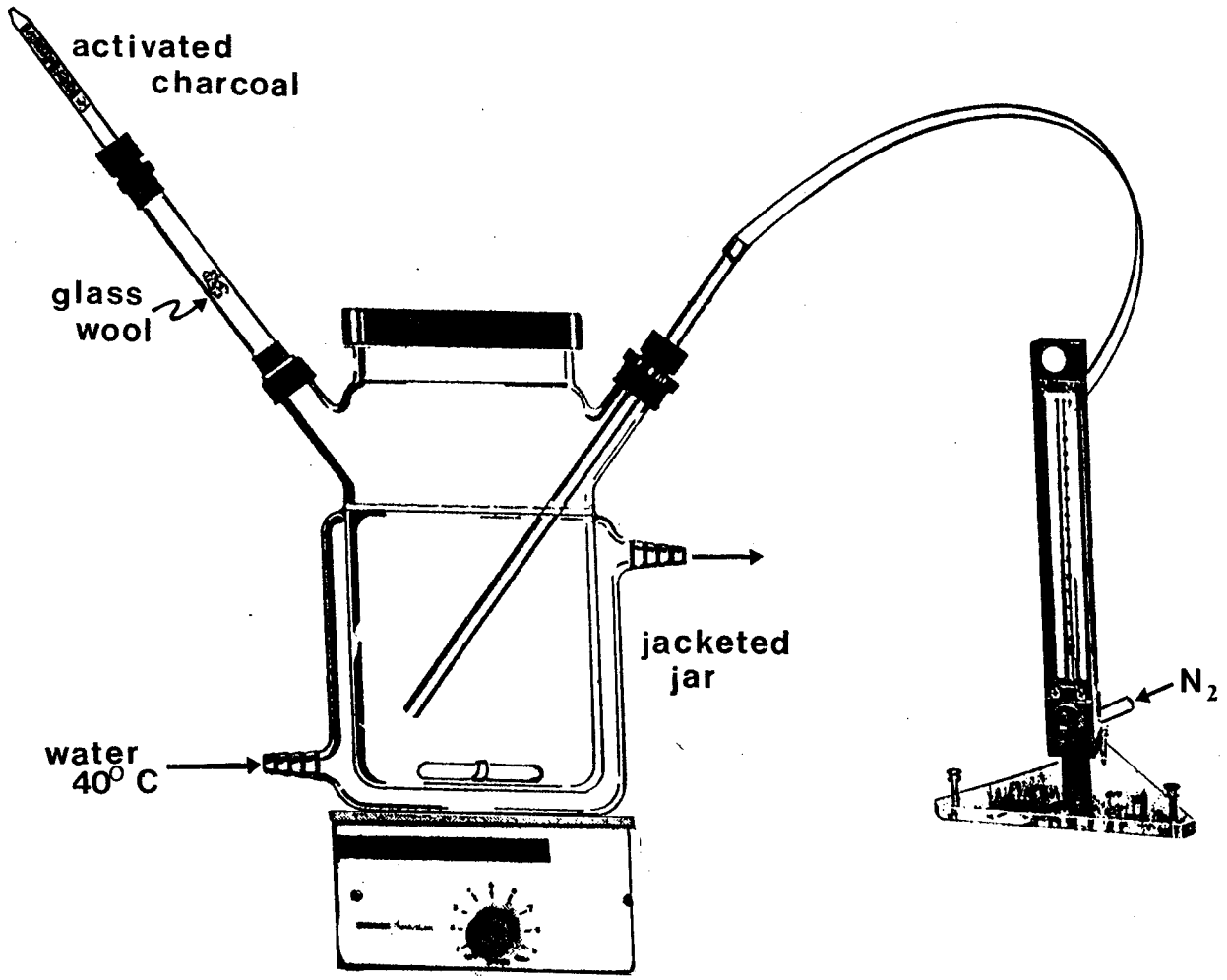

Fig. 2.1. Illustration of the dynamic purge and trap system for the collection of vegetable volatile compounds.

Series) fitted with model 610 flowmeter tubes. The activated charcoal adsorbent tubes (Orbo-32TM, 6 mm OD x 4 mm ID x 10 cm, 20/40 mesh, single bed) used as volatile traps, were obtained from Supelco (Oakville, ON). Authentic reference chemical compounds of high purity were obtained from commercial sources (Sigma Chemical Company, St. Louis, MO and Aldrich Chemical Co, Milwakee, MI). Methylene chloride, J.T. Baker "capillary analyzed", was obtained from Caledon Laboratories Ltd. (Edmonton, AB) and used as the desorbing solvent without further purification. Ethyl antioxidant 330 [1,3,5-trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl)benzene; Ethyl Corp., Baton Rouge, LA] was added to the methylene chloride (0.001%) as a preservative, just prior to desorption of volatile compounds. Internal standard solutions were prepared separately by the addition of accurately measured amounts of 2-octanone (175 µL), ethylbenzene (200 µL) and 3-pentanone (250 µL) to 5 ml methanol. A saturated $CaCl_2$ (ICN Biomedicals Canada Ltd., Mississaugha, ON) solution was prepared as previously described (Buttery et al., 1987) by adding an excess of $CaCl_2$ to grade 1 water and boiling the solution in an open Erlenmeyer flask for 1 h to remove volatile impurities.

C. ISOLATION AND CONCENTRATION OF AROMA COMPOUNDS

A tomato sample (400 g at 24 °C) of ca. equal pieces cut from four different tomatoes from each of the storage treatments, was blended for 45 sec in a Braun food processor. Saturated $CaCl_2$ solution (400 mL at 24 °C) was added all at once and the mixture blended for 15 seconds. The mixture was placed in a preheated Wheaton purge and trap jar. The jar was sealed and the homogenate was purged briefly with nitrogen gas flowing in through one of the side arms of the jar. Five microliters of the 3-pentanone solution was added through the other side arm on the vessel, the charcoal trap was attached and the

jar resealed. Tomato volatiles were captured in the trap by bubbling nitrogen through the vigorously stirred mixture for 4 h. Following the collection period, the charcoal trap was removed and volatile compounds were extracted with 3 mL of methylene chloride. The extract was concentrated to ca. 30 µL on ice under a gentle flow of purified nitrogen.

Volatile concentrates were obtained from onion and sweet pepper in essentially the same way except that 300 g of onion or sweet pepper tissue was blended with 400 mL of saturated $CaCl_2$. Onion homogenates were allowed to stand for 100 sec before the addition of $CaCl_2$ since the characteristic flavour of the onions develops only after rupture of the cells. Control over the time period before addition of $CaCl_2$ was important in replication of the sample collection. Five microliters of 2-octanone were added to sweet pepper homogenates as the internal standard and for onion, 5 µL of the 3-pentanone standard solution was used.

The area count ratio between peaks of interest and that of the internal standard was calculated to determine relative quantities of volatile compounds.

D. CAPILLARY GC-MS ANALYSIS

Analytical Conditions

The analytical column used in this study was a 30 m x 0.32 mm (i.d.) bonded phase DB-1 fused-silica capillary column (J&W Scientific, Folsom, CA) with a 1 µm phase thickness. Helium (ultra high purity) carrier gas, set at a flow velocity of 30 cm/sec, was further purified by placing a 5 µm screen, an oxygen and a hydrocarbon trap in line. The GLC oven was held at 35 °C for 10 min after injection, programmed at 5 °C/min to 180 °C, then to 240 °C at 10 °C/min and

held at the final temperature for 10 min. The injector temperature was 220 °C. A sample size of 2 µL was injected in splitless mode.

A Hewlett-Packard 5890 gas chromatograph, mounted with a HP 7673A autoinjector and interfaced to an HP 5970B quadrupole Mass Selective (MS) Detector was used for all volatile compound analyses. The MS detector was operated in the electron impact mode at 70 eV, taking scans from 30 to 350 m/z in a 1-s cycle. The temperature of the open-split interface was 245 °C.

Identification of Vegetable Volatiles

Chromatographic peaks were identified by using a software driven library search routine which automatically compared user-specified sample spectra to a user-generated or NBS (National Bureau of Standards) library. Authentic standards with a purity of at least 95% were chromatographed under the same conditions as the samples. Mass spectra from these standards were compiled on hard disk in a custom-made library and, together with the retention times, were used to confirm the identity of unknown peaks in the total ion chromatogram.

In this study, n-paraffins C_6-C_{24} were chromatographed under the same conditions as the samples to develop retention indices for unknown peaks for comparison with published indices. A comparison of linear retention indices is often useful to tentatively identify unknown compounds. The most widely used index is the Kovats retention index, I, which utilizes the linear relationship between log retention time, t'_R , and the carbon number of an n-alkane standard (Lee et al., 1984). The retention time of compound $x[t'_R]$ is interpolated between the values of t'_R for two adjacent n-alkanes with carbon numbers z and z + 1 [retention times $t'_R(z)$ and $t'_R(z+1)$]:

$$I(x)=100z+100\frac{\log t'_R(x)-\log t'_R(z)}{\log t'_R(z+1)-\log t'_R(z)}$$

In the case of temperature-programmed gas chromatography, $\log t'_R$ is replaced by the elution temperature, T_R. The temperature programmed retention index, $I_P(x)$, for a compound eluted between two n-alkanes with carbon numbers z and z + 1 is given by the following equation provided that the program begins at a low enough temperature.

$$I_p(x)=100z+100\frac{T_R(x)-T_R(z)}{T_R(z+1)-T_R(z)}$$

Recovery and Precision Tests

Two model systems are described for testing the recovery rate and precision of the methodology for capturing aroma volatiles. Two mixtures of compounds representative of vegetable aromas were prepared (see Table 2.1 in Results and Discussion on p. 81). For each mixture, 20 µL of each compound (weight determined from density) was accurately measured into a container and 0.1% of Ethyl Antioxidant 330 was added. The mixtures were stored at -4 °C.

Two microliters of the first model mixture were added to 400 mL of a $CaCl_2$ solution (near saturation) together with the 2-octanone standard solution in each of 6 Wheaton jars. The volatile compounds were collected in the same manner as vegetable volatile samples. The volatiles were desorbed with 3 mL of dichloromethane which was reduced to a volume of ca. 30 µL. Three microliters of a 50 ppm standard of ethylbenzene were added to each sample which was then taken up in a 100 µL syringe and the volume brought to 50 µL. One microliter of the extract was injected automatically and analyzed by GC-MS.

Similarly, 2 µL of the second model mixture were added to a homogenate

of 300 g of green tomato and 400 mL of saturated $CaCl_2$ solution. A large homogenate of green tomato was first prepared and divided into 6 jars for even replication. Five microliters of 3-pentanone internal standard solution were added to the homogenate instead of 2-octanone. Sample collection, elution and analysis for the second model mixture were the same as for the first mixture.

Response factors (MS) were determined relative to the internal standards 3-pentanone, 2-octanone and ethylbenzene by making known solutions in dichloromethane.

Statistical Analysis

Data for the different components were analyzed by analysis of variance using the General Linear Model (GLM) procedure, a program of the SAS Institute Inc. (Cary, NC). The experiment was analyzed as a one-way analysis of variance with 3 replications. Treatment comparisons were carried out using Fisher's (protected) least significant difference (LSD) test (Steel and Torrie, 1980) at a 5% level of significance. Principal component analysis (PCA) was applied to the GC data to determine if, by condensing the variability into a limited number of variables, the effect of variable pressure storage of tomatoes, onions and peppers could be distinguished from a constant pressure treatment (SAS, 1987; Zervos and Albert, 1992).

RESULTS and DISCUSSION

A. Method Development for Isolation of Volatiles

There were many considerations in selecting a technique to isolate volatile compounds such as: the cost of the more commonly used specialized glassware and instrumentation such as the Likens-Nickerson apparatus, Hewlett Packard headspace analyzer or Teckmar purge and trap unit, the limited availability of fume hood space, the low concentrations and thermolability of the compounds of interest and the need for easy replication. Another important consideration was the need for a liquid rather than a gaseous sample so analysis by GC-MS could be automated.

The dynamic headspace sampling technique using a solid sorbant developed by Buttery et al. (1987), was modified for use in this study. In employing this technique, Buttery and his associates used 10 g of Tenax in each collection tube and a very fast flow of purified air (3 L/min) for entrainment of tomato volatiles. Attempts were made in this lab to use smaller amounts of Tenax GC and TA (150 mg) and a lower gas flow rate (30 mL/min), in a manner similar to Shamaila et al. (1992, 1993). The amount of volatile compounds from tomatoes and sweet peppers that could be captured on this adsorbent, however, was insufficient for analysis by GC-MS. Although Tenax can be purified and reused, a less expensive sorbant which did not require time-consuming regeneration was desired.

Tubes containing activated coconut charcoal (custom ordered Orbo-32™, 6 mm O.D. x 10 cm long with a single 7.5 cm bed of charcoal) of acceptable purity, were inexpensive, simple to use and elute, and had a greater capacity to adsorb volatile compounds than the same weight of Tenax. Since this study utilizing activated charcoal was initiated, there have been two reports of the use

of the same product for isolation of strawberry (Pérez et al. 1992) and apple (Olías et al. 1992) volatiles.

In order to deactivate enzyme systems, plant tissues were homogenized with a saturated solution of $CaCl_2$ as described by Buttery et al. (1987). Enzyme deactivation was necessary due to the long collection period (4 h) and the presence of an anaerobic atmosphere in the sample jar, both of which would alter the volatile composition. The use of $CaCl_2$ rather than heavy metal salts was more environmentally sound and also increased the air/water partition coefficient of many volatile compounds, causing a "salting out" effect noted by Buttery and his associates (1987). In other words, $CaCl_2$ in the homogenate decreased the solubility of aroma compounds in the aqueous mixture.

Unlike Buttery et al. (1987), surrogate internal standards (2-octanone, ethylbenzene and 3-pentanone) were prepared in methanol rather than water to improve reproducibility of the standard addition. Due to its low molecular weight methanol is not retained well on charcoal. Nevertheless, amounts of methanol were kept to a minimum to avoid binding some of the active sites on the charcoal.

Air cannot be used as the purge gas with activated charcoal because of its reactivity, but the charcoal sorbant can be eluted with a wide range of solvents and its activity is unaffected by water in headspace vapours. Dichloromethane was chosen as the eluting solvent because fresh batches could be quickly obtained from the manufacturer allowing maximum shelf-life of the solvent. The use of carbon disulfide was attempted but a supply of acceptable purity could not be found and redistillation on an efficient fractionating column proved unsatisfactory. It was observed during the course of this work that Tenax can only be eluted with non-polar solvents. At low initial

temperatures in the GC oven, pentane or diethyl ether both cause excessive peak tailing on a non-polar capillary column, obscuring early eluting peaks.

B. Precision and Accuracy

Precision is a measure of the reproducibility of an analytical procedure accounting for individual variabilities from the sampling, extraction and instrumental analysis procedures. Overall precision is estimated by calculation of the standard deviation of a sample population which is normalized to the mean value to yield the relative standard deviation (RSD) or coefficient of variation (CV) as in the following equation:

$$RSD_i = s_i / \overline{X_i} \times 100$$

To reduce variability due to instrumental precision, a calibration mixture was run daily and the Mass Selective Detector was calibrated by a software controlled program.

Accuracy refers to how close the reported value is to the true value. The accuracy of the calculated result depends on the precision of the procedure, interferences in the determination, effects of the matrix, instrumental calibration and losses that occur during sample preparation. Attempts were not made in this study to discover the true bias in the found vs. known results since the headspace capture methodology will not extract total volatiles. By reporting the amounts of identified compounds in relation to an internal standard some of the sources of error involved in extraction and analysis of minute amounts of volatiles are defined.

The results of the accuracy and precision tests are given in Table 2.1. Measurement of extraction recoveries was not performed since, in applying the purge and trap method to fresh vegetables, it would have been impossible to achieve complete recovery of aroma compounds. Complete validation of the

Table 2.1. Accuracy and precision determinations with a water (Model 1) and a green tomato (Model 2) matrix.

Model 1	Accuracy (%)[a]	CV
α-terpineol	5.8	4.6
(S)-(-)-limonene	21.5	6.9
1-heptanol	11.3	4.9
2-ethylfuran	15.0	3.2
2-furaldehyde	3.2	9.8
2-methyl-1-butanol	13.6	5.8
2-octanone	49.6	3.2
eugenol	trace	-
geranyl tiglate	trace	-
hexenal	31.1	8.3
terpinen-4-ol	11.9	10.7
trans-2-heptenal	24.0	11.0
trans-2-hexenal	34.2	5.2

Model 2	Accuracy (%)[a]	CV
α-terpineol	1.5	6.5
β-ionone	trace	-
1-penten-3-one	5.6	8.9
2-isobutylthiazole	41.2	6.3
3-methyl-1-butanol	10.4	4.8
3-pentenone	17.7	5.4
6-methyl-5-hepten-2-one	11.3	9.5
geraniol	25.4	8.6
geranyl acetone	trace	-
hexenal	24.4	8.2
linalool	32.5	10.5
terpinen-4-ol	8.7	7.4
trans-2-heptenal	18.1	4.2

[a] Accuracy % = observed amount/known amount × 100.
n = 6

method would have required the availability of all of the chemical compounds identified in this work.

Buttery et al. (1987, 1988) observed that percent recovery of 3-pentanone, 2-octanone, 1-penten-3-one, geraniol, 6-methyl-5-hepten-2-one, hexenal, trans-2-hexenal, 3-methyl-1-butanol, 2-isobutylthiazole and linalool on Tenax was approximately twice as great as the amounts trapped on activated charcoal in the present study. Since the volume of the sweep gas used by Buttery et al. (1987, 1988) was 2.5 times greater than in the present study this result was not totally unexpected. The poor rate of capture of geranylacetone was unexpected, however, since Buttery et al. (1988) observed an absolute recovery of 36% of this compound from water solutions. It appears therefore that geranylacetone must have been tightly adsorbed to the activated charcoal. Eugenol, β-ionone and geranyl tiglate could have been strongly bound to the adsorbent as well since only traces were found in the extracts (Table 2.1). Recovery of some of the volatiles could be related to a their affinity for water and hence their volatility [air to water partition coefficient] (Buttery et al., 1988).

The green tomato matrix appeared to affect the amount of α-terpineol, hexenal, trans-2-heptenal and terpene-4-ol captured on activated charcoal. Green tomatoes were chosen as an organic matrix for the precision tests because, other than trans-2-hexenal, the amounts of volatiles are very low. Petro-Turza (1987) noted that the acidity of the medium or the conditions of sample preparation (enzyme activity, the amount of oxygen in the sample or the extent of comminution) affect the quality and quantity of aldehydes and alcohols recovered from tomato.

C. Effect of Atmospheric Pressure Treatments on Onion Volatiles

The characteristic aroma of onions is attributed to the numerous sulfur-containing volatiles in these plants. One hundred and forty compounds, most of which contain sulfur, have been isolated from fresh and cooked onions by various extraction methods. The composition and formation of volatiles in onion have been recently reviewed (Carson, 1987; Whitfield and Last, 1991).

The twenty-three organic compounds listed in Table 2.2 were recovered from the headspace of onion homogenates and are numbered according to their order of elution from the gas chromatographic column. The aroma compounds were identified by comparing GC peak retention indices with those in the literature (RI) or matching sample spectra with spectra from authentic compounds (CS) as well as spectra contained in the NBS library (MS). Total ion chromatograms of volatile components from onions subjected to variable pressure treatment for 1 to 3 weeks were compared to those from onions stored for the same length of time under constant pressure (Fig. 2.2, see Fig. 2.20 to 2.24 in Appendix 3). Peak numbers in the Table 2.2 correspond to numbered peaks in Fig. 2.2.

Pure, authentic chromatographic standards for comparison with onion volatiles are not easily obtained from commercial sources, due the extreme toxicity of such compounds. As a result, much of the identification of onion aroma compounds by a number of researchers (Brodnitz and Pascale, 1971; Boelens et al., 1971; Galetto and Hoffman, 1976a, 1976b; Kallio and Salorinne, 1990; Farkaš et al., 1992; Block et al., 1992a, 1992b; Kuo and Ho, 1992a, 1992b; Tokitomo and Kobyagashi, 1992; Ohta and Osajima, 1992) has been accomplished through in-lab or custom synthesis of the desired compounds, interpretation of MS fragmentation patterns, comparison of spectra and retention indices with previous reports, or the use of other instrumentation such as Nuclear Magnetic Resonance (NMR) to assist in confirming the identity of a

Table 2.2. Flavour constituents of onion.

Peak Number[a]	Component	I_p (DB-1)[b]	ID
1	1,3,5-cycloheptatriene	749	MS
2	5-hexyn-3-ol	769	MS
3	2-methyl-2-pentenal[1,2,3]	816	MS RI CS
4	S-propyl thioacetate	851	MS RT
5	1,4-dimethyl benzene	853	MS CS
6	2,4-dimethyl thiophene[1,2,3]	855	MS RI
7	3,4-dimethyl thiophene[1,2,3]	882	MS RI
8	unknown	896	
9	unknown	903	
10	methylpropyl disulfide[1,2,3]	905	MS RI
11	methyl 1-propenyl disulfide[1,2,3]	915	MS RI
12	propanthioic acid	940	MS
13	unknown	960	
14	2-pentylfuran[2,3] & unknown	978	MS CS RI
15	decane	1000	MS CS
16	dipropyl disulfide[1,2,3]	1095	MS RI
17	propyl 1-propenyl disulfide[1,2,3]	1097	MS RI
18	unknown	1146	
19	unknown	1224	
20	unknown	1226	
21	dipropyl trisulfide[1,2,3]	1270	MS RI
22	3,5-diethyl-1,2,4-trithio-lane[1,2,3]	1280	MS RI
23	3,5-diethyl-1,2,4-trithiolane (isomer)[1,2,3]	1283	MS RI

MS, mass spectra of unknown tentatively matched with spectra in the mass spectral library of the National Bureau of Standards (Probability Based Matching); RT, retention time of unknown peak matched with retention time of peak chromatographed under similar conditions in the published literature; RI, retention index of unknown peak matched against retention index in published literature; CS, mass spectra of unknown matched with spectra and retention times of authentic compounds.

[a] Peak number matches peaks numbered in Fig. 2.2

[b] Kovat's linear retention indices with temperature programming (Lee et al., 1984).

[1] Farkaš et al. (1992), [2] Kuo and Ho (1992a), [3] Kuo and Ho (1992b)

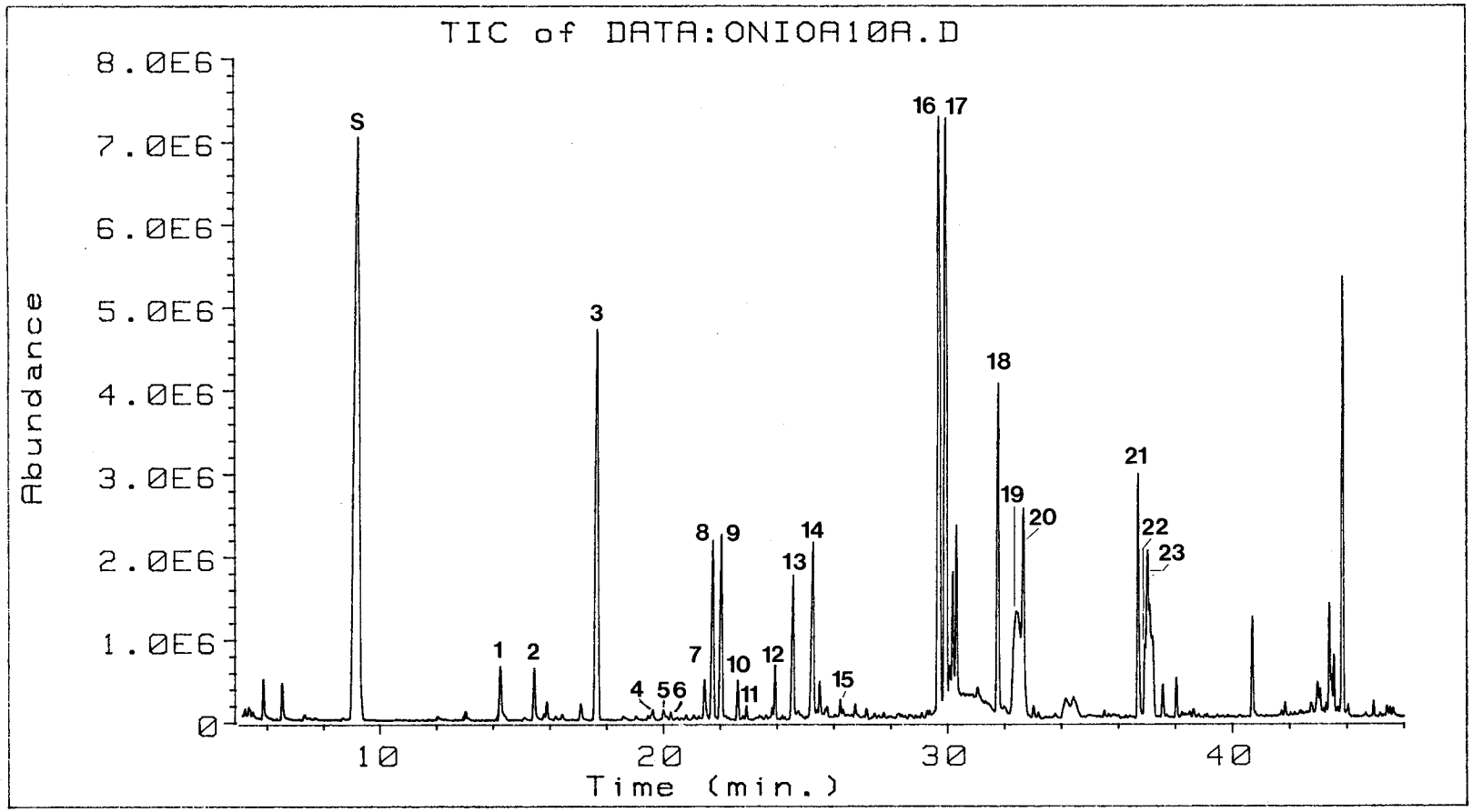

Fig. 2.2. Total ion chromatogram of volatiles extracted from onion stored under constant pressure at 15C for 7 days. Numbers refer to compounds listed in Table 2.2.

compound.

Some relatively large peaks in the onion samples were unidentifiable either by comparing spectra or relative retention index (Table 2.2). These peaks could be overlapping compounds (which makes spectral identification very difficult), degradation products of thermolabile sulfur-containing constituents and not identifiable from the literature or true unknowns. The thiosulfinates found in onions are unstable and break down nonenzymatically to yield alkyl and alkenyl mono-, di- and trisulfides or may be oxidized to the corresponding sulfonates (Whitfield and Last, 1991).

The main purpose of this study was not to study onion volatile components, but to determine if storage under variable atmospheric pressure had an effect on the chemical composition of aroma extracts. A visual comparison of the total ion chromatograms reveals very little about changes in volatile composition due to storage treatment (Fig. 2.2 and Fig. 2.20 to 2.24 in Appendix 3). Moreover, the complexity of the data obtained from GC/MS analysis (Table 2.3) does not allow the observer to readily distinguish major treatment effects although individual peak differences are apparent.

Variable pressure treatments increased relative amounts of 2-methyl-2-pentenal recovered from onions compared to onions held under constant pressure (Table 2.3). This increase, however, was significant ($P<0.05$) only for the 7 and 14 day treatment periods. Total relative concentration of volatiles was higher in the pressure-treated onions than in the onions stored at constant pressure although this effect was significant ($P<0.05$) only for the comparison between onions stored 1 week under variable pressure and onions stored 1-3 weeks under constant pressure. By week 3, volatile composition of variable pressure-treated onions was very much like that of the constant pressure treatments.

Table 2.3. Means[1] of volatile compounds identified in onion.

Peak Number	Component	Relative amounts of compounds[3]					
		CON(7)[2]	CON(14)	CON(21)	VAR(7)	VAR(14)	VAR(21)
1	1,3,5-cycloheptatriene	0.38	0.54	0.39	0.41	0.42	0.40
2	5-hexyn-3-ol	0.19	0.25	0.40	1.27	1.42	0.25
3	2-methyl-2-pentenal	5.55^{cd}	3.11^{d}	8.28^{cd}	20.91^{a}	17.09^{ab}	11.99^{bc}
4	S-propyl thioacetate	0.08	0.11	0.08	0.10	0.09	0.11
5	1,4-dimethyl benzene	0.02^{c}	0.04^{bc}	0.07^{abc}	0.07^{abc}	0.11^{a}	0.08^{ab}
6	2,4-dimethyl thiophene	0.05	0.05	0.16	0.18	0.14	0.19
7	3,4-dimethyl thiophene	0.38^{ab}	0.31^{b}	0.32^{b}	0.75^{a}	0.74^{a}	0.42^{ab}
8	unidentified	2.47	1.23	7.19	13.99	10.65	7.44
9	unidentified	2.94^{ab}	1.17^{b}	5.71^{ab}	9.48^{a}	7.97^{ab}	5.66^{ab}
10	methylpropyl disulfide	0.14	0.22	0.17	0.14	0.12	0.18
11	methyl 1-propenyl disulfide	0.07	0.11	0.05	0.18	0.17	0.09
12	propanthioic acid	0.14^{b}	0.15^{b}	0.18^{b}	0.49^{a}	0.22^{b}	0.17^{b}
13	unidentified	2.18^{b}	1.18^{b}	2.95^{ab}	6.41^{a}	3.13^{ab}	4.57^{ab}
14	2-pentylfuran & unidentified	2.83^{ab}	1.49^{b}	3.73^{ab}	7.40^{a}	4.35^{ab}	5.57^{ab}
15	decane	0.08	0.10	0.06	0.08	0.08	0.06
16	dipropyl disulfide	3.09^{ab}	5.42^{a}	2.64^{b}	3.64^{ab}	4.25^{ab}	3.93^{ab}
17	propyl 1-propenyl disulfide	4.86^{ab}	7.80^{a}	3.95^{b}	7.03^{ab}	6.38^{ab}	4.91^{ab}
18	unidentified	2.24^{bc}	2.84^{abc}	1.52^{c}	4.10^{a}	3.31^{ab}	1.78^{bc}
19	unidentified	0.72	0.38	0.84	2.06	1.33	1.60
20	unidentified	1.19^{b}	0.43^{b}	1.05^{b}	3.85^{a}	1.27^{b}	1.70^{b}
21	dipropyl trisulfide	0.89^{bc}	1.40^{abc}	0.57^{c}	1.93^{a}	1.76^{ab}	1.02^{abc}
22	3,5-diethyl-1,2,4-trithio-lane	0.80	1.62	1.31	2.09	1.93	1.65
23	3,5-diethyl-1,2,4-trithiolane (isomer)	1.37^{bc}	1.88^{abc}	0.67^{c}	4.35^{a}	3.79^{ab}	1.97^{abc}

Table 2.3 continued

	Relative amounts of compounds[3]					
	CON(7)[2]	CON(14)	CON(21)	VAR(7)	VAR(14)	VAR(21)
Total amounts relative to internal standard	32.67[b]	31.81[b]	42.27[b]	90.91[a]	70.78[ab]	55.89[ab]

[1] means of three observations.
[2] CON - constant pressure, VAR - variable pressure, number of days of treatment in parenthesis.
[3] Amounts are relative to 3-pentanone (internal standard) peak.
[abc] Means in the same row with different letters are significantly ($P<0.05$) different.

Because of the complexity of flavour analysis, statistical treatment of one variable (i.e. one compound) at a time is often not sufficient to determine relationships among treatments. Principal component analysis (PCA) condenses the variability into a limited number of variables and extracts the eigenvectors and eigenvalues used to perform the calculation of sets of coordinates. Two-dimensional plots of the points indicated by these coordinates may suggest some natural groupings of sample materials. According to Zervos and Albert (1992), one of the simplest analyses of flavour data is a point cloud on a plane formed by a pair of principal components. Zervos and Albert (1992), using data from the flavour analyses of orange juice, illustrate how samples associated with a treatment sometimes cluster in different regions of a system of normal orthogonal axes, presenting an identifiable pattern.

Principal component analysis was used to extract the eigenvectors and eigenvalues from the onion volatile data (Table 2.4). According to Afifi and Clark (1984), a rule of thumb adopted by many investigators is to select only the principal components explaining 5% or more of the total variance. In this case, the first 5 principal components (eigenvector numbers 1 to 5) account for 85.2% of the variability in the onion data (Table 2.4). Examination of the coefficients defining each of the principal components reveals the correlation between a given variable and the principal component (Table 2.5). For each principal component, the variables with a correlation greater than 0.5 with that component, are underlined. The value of 0.5 was chosen for illustration because it was used by Afifi and Clark (1984) in their discussion on interpretation of multivariate analysis. The correlation between the i^{th} principal component C_i and the j^{th} variable x_j is $r_{ij} = a_{ij}\sqrt{VarC_i}$ therefore, a coefficient a_{ij} is underlined if it exceeds $0.5/\sqrt{VarC_i}$.

Table 2.4. Principal component analysis (PCA) of volatile compounds from onions stored under constant and variable pressures.

Eigenvector number	Eigenvalue	Variance preserved	
		Each	Total
1	10.741	46.7	46.7
2	3.883	16.9	63.6
3	2.058	8.9	72.5
4	1.650	7.2	79.7
5	1.277	5.5	85.2
6	1.067	4.6	89.9
7	0.626	2.7	92.6
8	0.503	2.2	94.8
9	0.310	1.3	96.1
10	0.259	1.1	97.2
11	0.224	1.0	98.2
12	0.164	0.7	98.9
13	0.104	0.5	99.4
14	0.069	0.3	99.7
15	0.043	0.2	99.9
16	0.017	0.1	100.0
17	0.004	0.0	100.0

Table 2.5. Coefficients of the first five principal components of the onion volatiles data set.

Peak Number	Principal Component 1	2	3	4	5
PK1	0.058632	0.409061	-.108862	0.360228	0.054402
PK2	0.175463	0.007802	0.275465	0.044524	-.510911
PK3	0.280391	-.162314	0.034219	-.058251	0.027632
PK4	0.096838	0.242732	-.338864	0.153860	0.142915
PK5	0.213376	-.080227	0.176382	0.363961	-.008061
PK6	0.147642	0.029712	0.412833	0.362022	0.269231
PK7	0.290305	-.004466	0.035958	-.021501	-.188106
PK8	0.261106	-.123413	-.188010	0.098871	0.159776
PK9	0.263306	-.186953	-.132618	0.049956	0.034413
PK10	-.081276	0.237002	0.438254	0.125573	0.086326
PK11	0.222694	0.193197	-.028676	0.238520	-.260267
PK12	0.159617	0.091409	0.325464	0.035036	0.137405
PK13	0.252381	-.224238	-.041702	-.036783	0.163831
PK14	0.253247	-.200429	0.009109	-.014892	0.143866
PK15	0.081509	0.323757	-.416617	0.200462	-.062269
PK16	0.020687	0.346202	0.222071	-.331318	0.242101
PK17	0.143371	0.331550	-.029688	-.389036	0.142632
PK18	0.219496	0.207498	0.105198	-.300986	-.211122
PK19	0.223856	-.202076	-.014757	-.022174	-.153123
PK20	0.222727	-.110101	-.032914	-.159171	0.444817
PK21	0.260163	0.133881	-.007829	-.244089	-.214168
PK22	0.232647	0.209034	-.034991	0.064651	0.182379
PK23	0.286055	0.072699	-.049405	-.089328	-.103541
Var C_i[a]	10.741	3.883	2.058	1.650	1.277
CPE[b]	46.7	63.6	72.5	79.7	85.2
$0.5/\sqrt{VarC_i}$	0.153	0.254	0.348	0.389	0.442

[a] Eigenvalues

[b] Cumulative proportion explained

For each principal component, the variables with a correlation greater than 0.5 with that component, are underlined.

Table 2.5 shows that many peaks were highly correlated (greater than 0.5) with the first component, with all of the correlations being positive. Peaks 1, 15, 16, and 17 were highly correlated with the second component and peaks 6, 10 and 15 (a strong negative correlation) with the third component. The third component is a contrast of peaks 6 and 10 against 15.

The factor pattern calculated by the statistical procedure can be plotted for each onion treatment. The arrangment of points (each representing a treatment rep) in a cloud plane (Fig. 2.3 and Fig. 2.4) indicate that variables associated with the first principal component were important in separating treatment effects. The first three eigenvectors extracted from the onion volatile data accounted for 72.5% of the variability and were, therefore, important in identifying differences in aroma compounds between variable and constant pressure treatments. It appears from the plots in Fig. 2.3 and 2.4 that variable pressure treatments had the greatest effect on onion volatile constituents in week 1 and week 2 of storage. Due to extensive variation in onion samples, there was no clear separation of the treatments according to duration of storage period in the principal component plots.

Since early eigenvectors may not always best show the existing class separation in the sample space it is worthwhile plotting later values against the first component (Zervos and Albert, 1992). The plots in Fig. 2.5 and Fig. 2.6 show a tighter grouping of onion volatiles from onions stored under constant pressure, although onion volatile constituents from the variable pressure treatments were very similar.

Onion aroma contains of a number of strong chemical irritants. Sensory properties of a fresh vegetable such as this are very difficult to assess by sensory evaluation. Although the onions in this study were not evaluated formally by a trained panel, it was noted that the presence of the

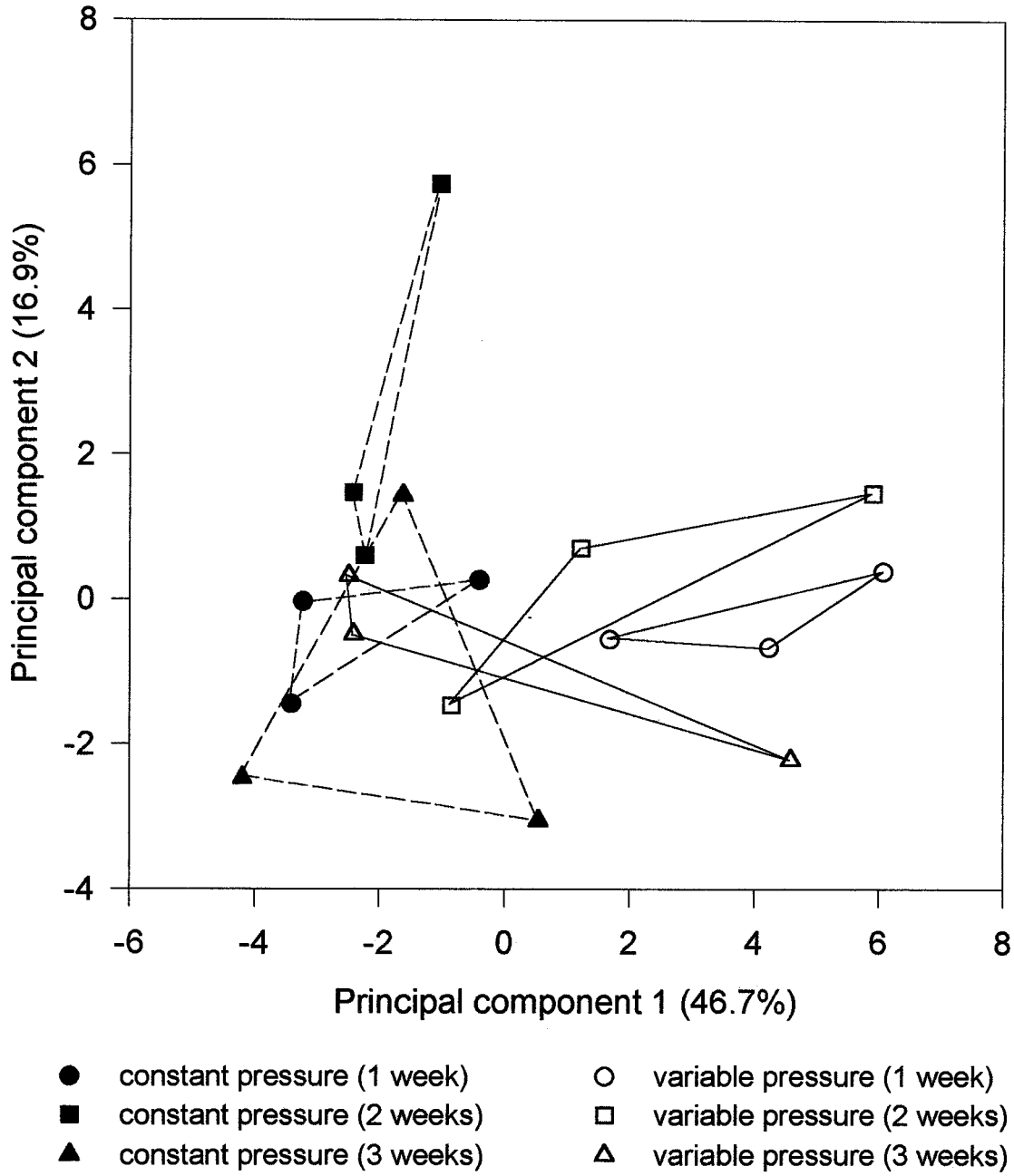

Fig. 2.3. Principal component analysis of aroma compounds from onions stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and second eigenvectors.

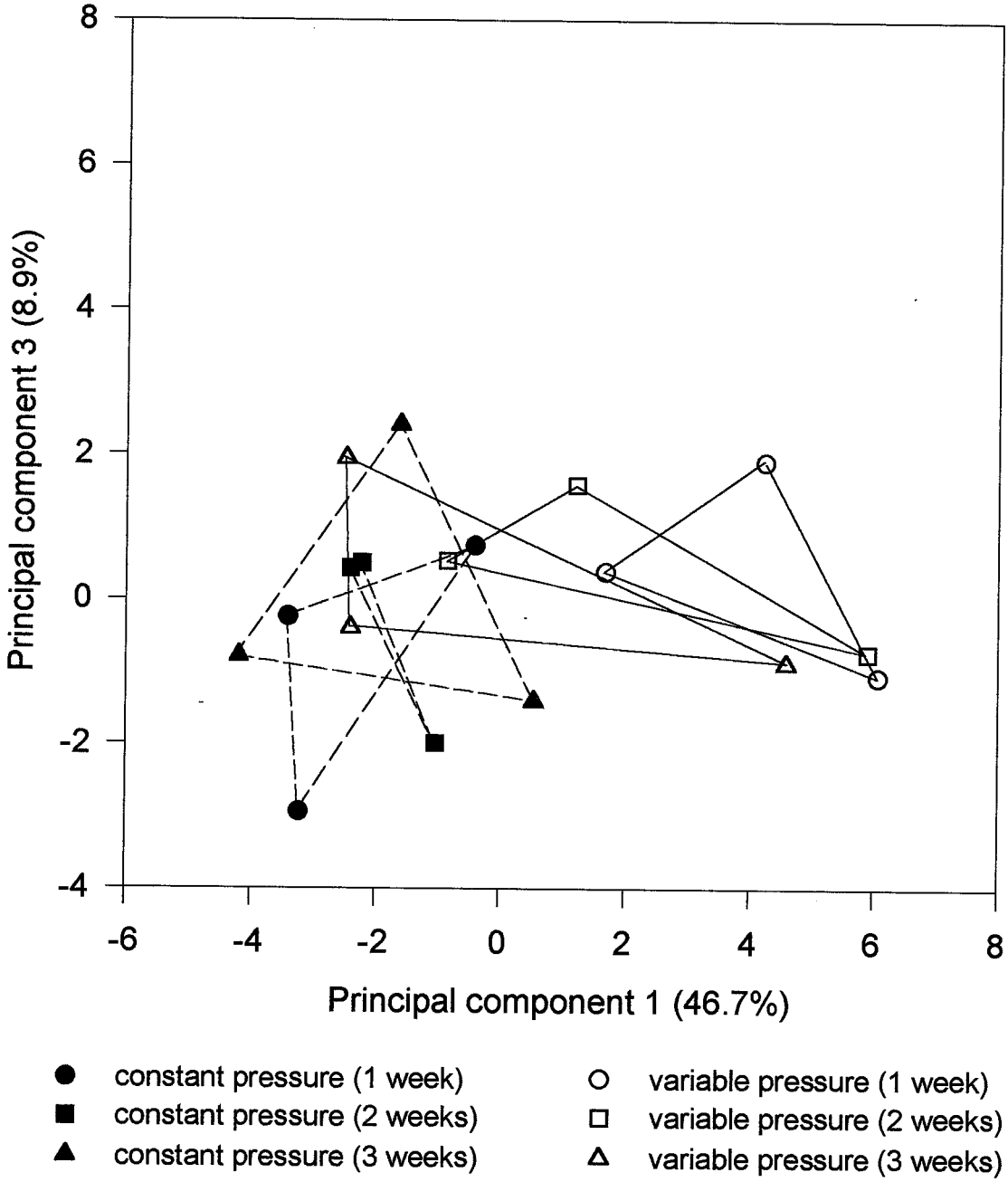

Fig. 2.4. Principal component analysis of aroma compounds from onions stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and third eigenvectors.

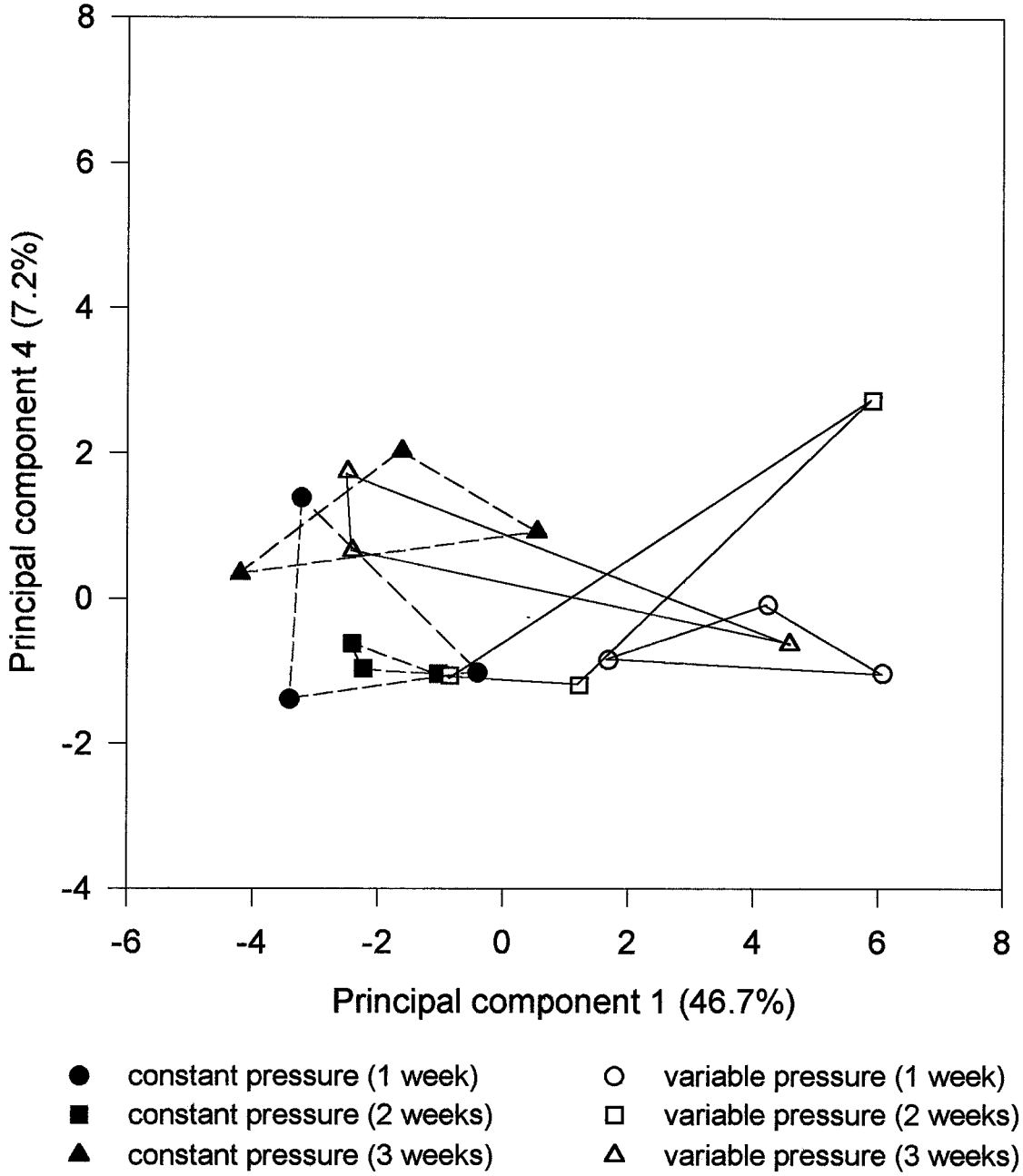

Fig. 2.5. Principal component analysis of aroma compounds from onions stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and fourth eigenvectors.

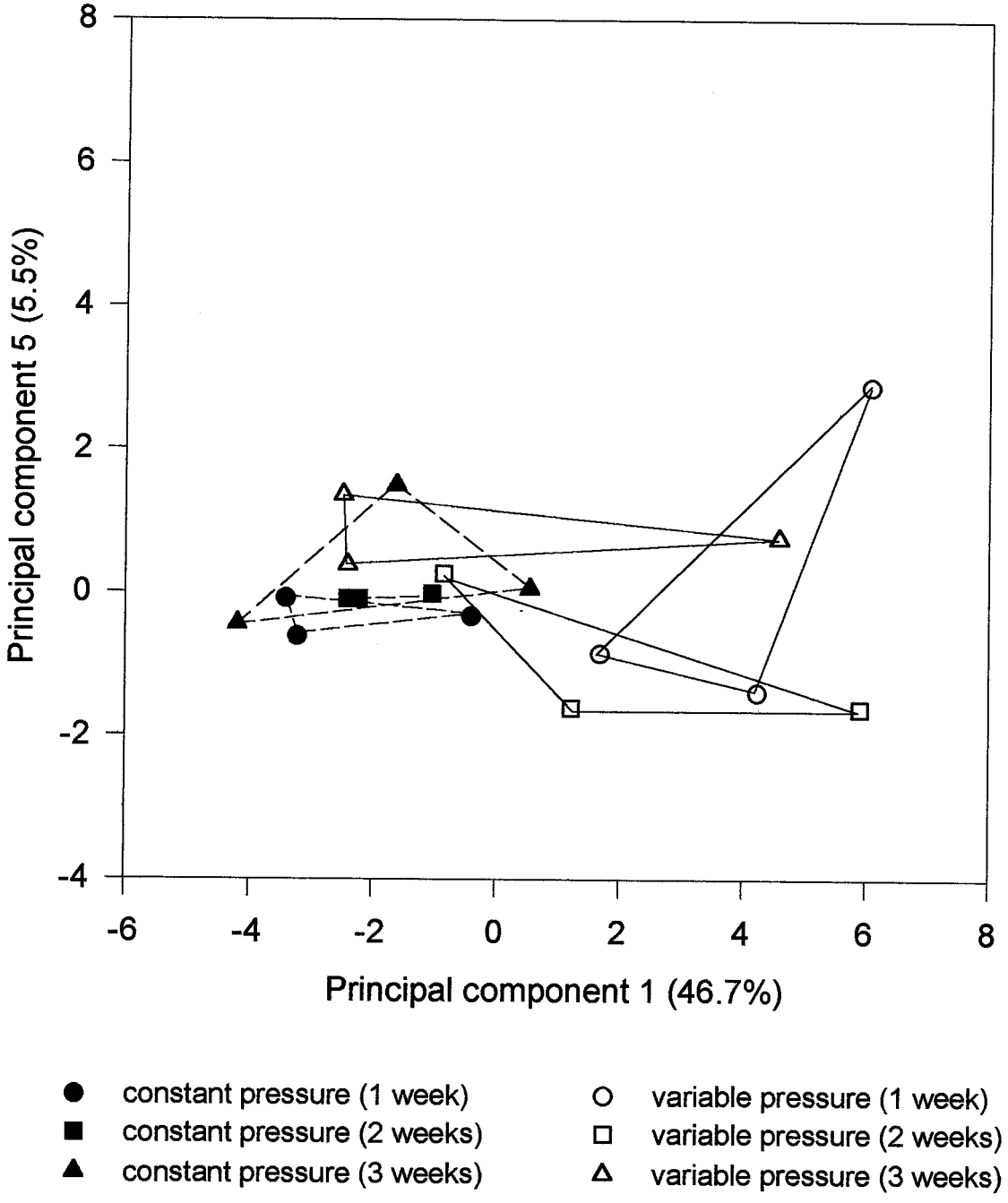

Fig. 2.6. Principal component analysis of aroma compounds from onions stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and fifth eigenvectors.

lachrymatory factor, thiopropanal S-oxide (not found in GC-MS analysis due to degradation in the hot GC oven), did not seem as intense in onions stored under variable pressure compared to onions stored under constant pressure.

D. Effect of Atmospheric Pressure Treatments on Tomato Volatiles

Volatile substances, imparting the characteristic flavour and aroma of tomatoes, develop during ripening and during cellular disruption as a result of enzyme action. Flavour constituents are derived mainly from fatty acids and amino acids (Petró-Turza, 1987). Of the approximately 400 volatile compounds so far identified, compounds considered to be most important in the aroma of raw tomato are Z-3-hexenal, 3-methylbutanal, β-ionone, 1-penten-3-one, hexanal, Z-3-hexen-1-ol, E-2-hexenal, 2- and 3-methylbutanol, 2-isobutyl-thiazole, eugenol, 6-methyl-5-hepten-2-one and dimethyl trisulfide (Petró-Turza, 1987; Whitfield and Last, 1991). Aroma volatiles identified in tomatoes stored under constant and variable pressure treatments for 3 and 7 days are listed in Table 2.6 and total ion chromatograms showing typical volatile profiles for each treatment are shown in Fig. 2.7 and Fig. 2.25 to 2.27 in Appendix 3. Eugenol and β-ionone were not captured well by the head-space methodology used in this study so the concentration of these compounds was insufficient for identification by GC-MS.

Volatiles are principally derived from two different classes of precursors, the straight-chain compounds from unsaturated fatty acids and the branched-chain compounds from free amino acids (Petró-Turza, 1987). Alanine, leucine and valine are considered to be the most important free amino acids in tomato for formation of volatile compounds such as 3-methylbutanal and 3-methylbutanol (Yu and Spencer, 1970). Linoleic and linolenic acids are oxidized to hydroperoxides which yield mainly hexanal and Z-3-hexenal (Whitfield and

Table 2.6. Flavour constituents of tomato.

Peak Number[a]	Component	Ip (DB-1)[b]	ID
1	2-methylfuran	601	MS CS
2	2-methyl-1-propanol	613	MS CS
3	3-methylbutanal	633	MS CS
4	2-methylbutanal	641	MS CS
5	1-penten-3-one	660	MS CS
6	1-penten-3-ol	664	MS CS
7	unidentified	698	
8	E-2-methyl-2-butenal	715	MS CS
9	Dimethyl disulfide and 3-methyl-1-butanol	721	MS CS
10	2-methyl-1-butanol and E-2-pentenal	724	MS CS
11	3-methylpentanal[3]	743	MS RT
12	Z-3-hexenal[3]	769	MS RT
13	hexanal	780	MS CS
14	2-methylpropanoic acid[2]	786	MS
15	octane	800	MS CS
16	E-2-hexenal	818	MS CS
17	3-methyl-1-pentanol[1]	822	MS RT
18	Z-3-hexen-1-ol	840	MS CS
19	ethylbenzene	845	MS CS
20	p- or m-xylene	851	MS CS
21	2,4-hexadienal	871	MS CS
22	5-ethyl-2(5H)-furanone	903	MS
23	E-2-heptenal	920	MS CS
24	dimethyl trisulfide[1]	940	MS
25	6-methyl-5-hepten-2-one	960	MS CS
26	2-octanone	965	MS CS
27	2-pentylfuran	978	MS CS
28	2-isobutylthiazole and unknown	1005	MS CS
29	linalool	1073	MS CS
30	undecane	1100	MS CS
31	Dimethyl tetrasulfide	1200	MS

MS, mass spectra of unknown tentatively matched with spectra in the mass spectral library of the National Bureau of Standards (Probability Based Matching); RT, retention time of unknown peak matched with retention time of peak chromatographed under similar conditions in the published literature; CS, mass spectra of unknown matched with spectra and retention times of authentic compounds.

[a] Peak numbers match peaks in Fig. 2.7

Table 2.6 continued

[b]Kovat's linear retention indices with temperature programming (Lee et al., 1984).
[1]McGlasson et al., 1987 [2]Petró-Turza, 1987 [3]Maarse and Visscher, 1989

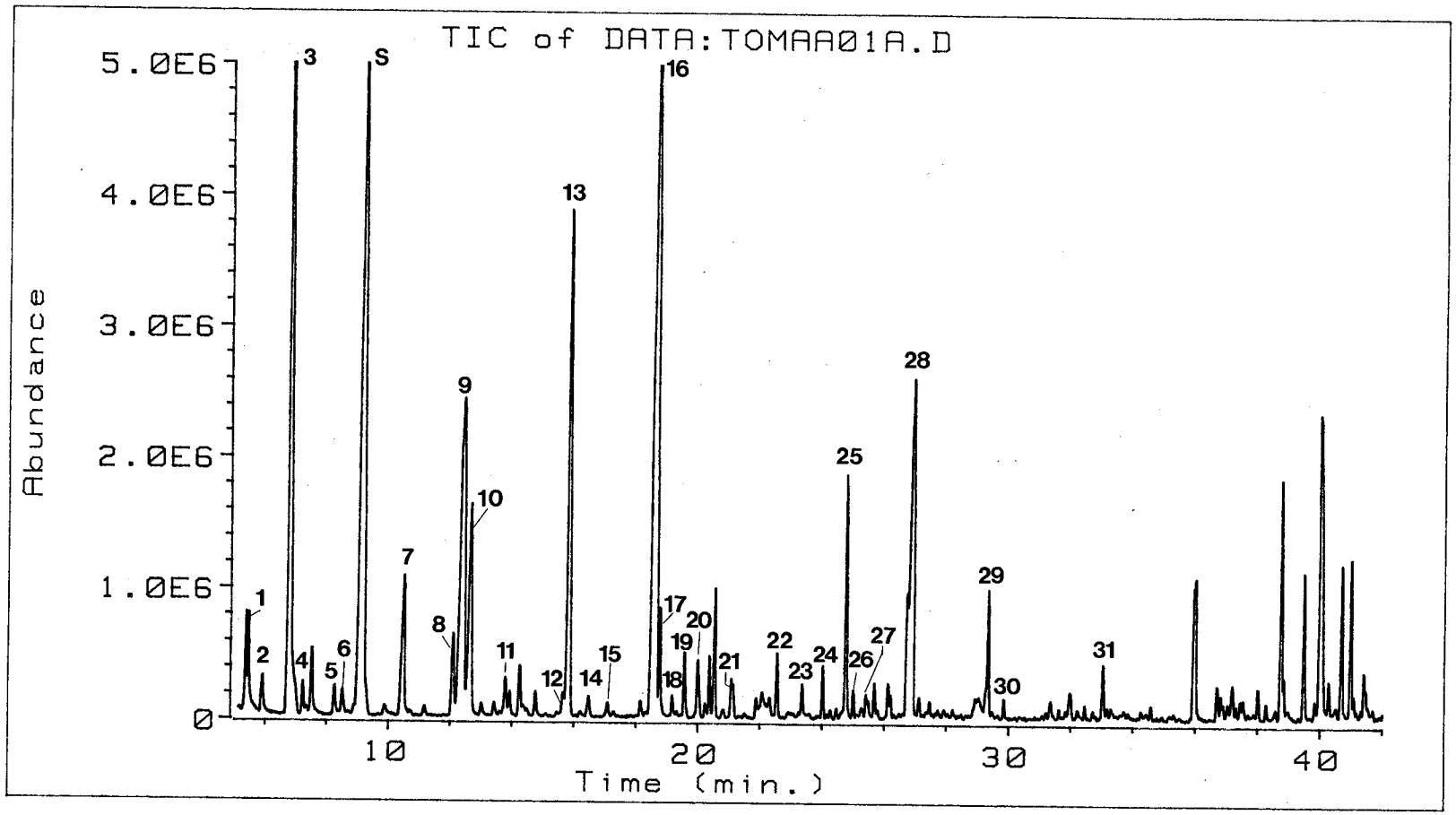

Fig. 2.7. Total ion chromatogram of volatiles extracted from tomatoes stored under constant pressure at 15C for 3 days. Peak numbers correspond to peak names in Table 2.6.

Last, 1991). In an acidic environment, Z-3-hexenal is quickly converted to E-2-hexenal (Petró-Turza, 1987). E-2-hexenal is considered to be an important aroma component and is associated with 'green tones' of tomato flavour. However, too high a concentration of this component is associated with an unpleasant, rancid aroma (Petró-Turza, 1987).

The abundances of dimethyl disulfide + 3-methyl-1-butanol, E-2-hexenal and Z-3-hexen-1-ol, and were greater in the tomatoes stored 7 days compared to tomatoes stored 3 days regardless of the atmospheric pressure treatment although these compounds tended to be significantly ($P<0.05$) more concentrated in extracts from tomatoes subjected to varying pressure treatment for 7 days (Table 2.7). Total relative amounts of volatiles were not significantly ($P>0.05$) affected by treatment although there was a tendency for the tomatoes stored for 7 days to have greater amounts of volatiles.

Principal Component Analysis of the chromatographic data revealed that 11 components are required to account for the total variability with the first three components explaining 61.2% of the variance (Table 2.8). Plots of principal components 1 and 2 (Fig. 2.8) and components 1 and 3 (Fig. 2.9) were closely overlapped for tomatoes stored 3 days regardless of atmospheric pressure treatment. Nevertheless, variable pressure treatment appeared to have some effect on tomato volatile profiles after 7 days of storage although variability in concentration of aroma compounds associated with the second and third components indicated by the spread of points on the Y axis may have obscured the differences. The data seem to indicate that a delay in ripening caused by cold storage affected the subsequent development of volatile compounds. The construction of a table of coefficients defining each of the principal components (Table 2.9) is helpful in determining how variable atmospheric pressures may have affected the development of flavour compounds, based on chemical

Table 2.7. Means[1] of volatile compounds identified in tomato.

Peak Number	Component	Relative amounts of compounds[3] CON(3)[2]	CON(7)	VAR(3)	VAR(7)
1	2-methylfuran	0.54	0.70	0.47	0.91
2	2-methyl-1-propanol	0.28[b]	0.42[ab]	0.39[ab]	0.58[a]
3	3-methylbutanal	4.04	5.49	5.17	6.90
4	2-methylbutanal	0.18	0.18	0.20	0.29
5	1-penten-3-one	0.14	0.11	0.06	0.04
6	1-penten-3-ol	0.18	0.14	0.13	0.08
7	unidentified	0.97[b]	1.35[b]	1.32[b]	1.99[a]
8	E-2-methyl-2-butenal	0.56	0.60	0.83	0.92
9	Dimethyl disulfide and 3-methyl-1-butanol	3.73[c]	6.38[b]	4.96[bc]	11.21[a]
10	2-methyl-1-butanol and E-2-pentenal	2.10	2.24	2.73	3.02
11	3-methylpentanal	0.46	0.29	0.84	0.39
12	Z-3-hexenal	0.28	0.21	0.25	0.22
13	hexanal	4.12	3.16	3.44	3.00
14	2-methylpropanoic acid	0.45	0.51	0.81	1.20
15	octane	0.04	0.06	0.07	0.05
16	E-2-hexenal	8.66[b]	12.46[a]	8.47[b]	13.85[a]
17	3-methyl-1-pentanol	0.62[a]	0.16[b]	0.61[a]	0.27[ab]
18	Z-3-hexen-1-ol	0.16[b]	0.46[a]	0.05[b]	0.36[a]
19	ethylbenzene	0.47	0.84	0.44	0.58
20	p- or m-xylene	0.34	0.42	0.39	0.30
21	2,4-hexadienal	0.37	0.49	0.30	0.51
22	5-ethyl-2(5H)-furanone	0.46	0.31	0.48	0.42
23	E-2-heptenal	0.18	0.19	0.25	0.20

Table 2.7 continued

Peak Number	Component	Relative amounts of compounds[3] CON(3)[2]	CON(7)	VAR(3)	VAR(7)
24	dimethyl trisulfide	0.23	0.22	0.18	0.23
25	6-methyl-5-hepten-2-one	1.52[ab]	1.64[ab]	1.16[b]	2.21[a]
26	2-octanone	0.15	0.12	0.66	0.07
27	2-pentylfuran	0.12	0.08	0.10	0.09
28	2-isobutylthiazole and unknown	6.33	5.47	6.11	3.05
29	linalool	0.53[b]	0.88[ab]	0.57[b]	1.41[a]
30	undecane	0.07	0.12	0.06	0.11
31	Dimethyl tetrasulfide	0.25	0.28	0.34	0.39
	Total amounts relative to internal standard	38.50	45.97	41.92	54.79

[1] means of three observations.
[2] CON - constant pressure, VAR - variable pressure, number of days of treatment in parenthesis.
[3] Amounts are relative to 3-pentanone (internal standard) peak.
[abc] Means in the same row with different letters are significantly ($P<0.05$) different.

Table 2.8. Principal component analysis (PCA) of volatile compounds from tomato stored under constant and variable pressure.

Eigenvector number	Eigenvalue	Variance preserved	
		Each	Total
PRIN1	7.193	23.2	23.2
PRIN2	6.451	20.8	44.0
PRIN3	5.329	17.2	61.2
PRIN4	4.294	13.9	75.1
PRIN5	2.705	8.7	83.8
PRIN6	1.984	6.4	90.2
PRIN7	1.231	3.9	94.1
PRIN8	0.836	2.7	96.8
PRIN9	0.701	2.3	99.1
PRIN10	0.151	0.5	99.6
PRIN11	0.125	0.4	100.0

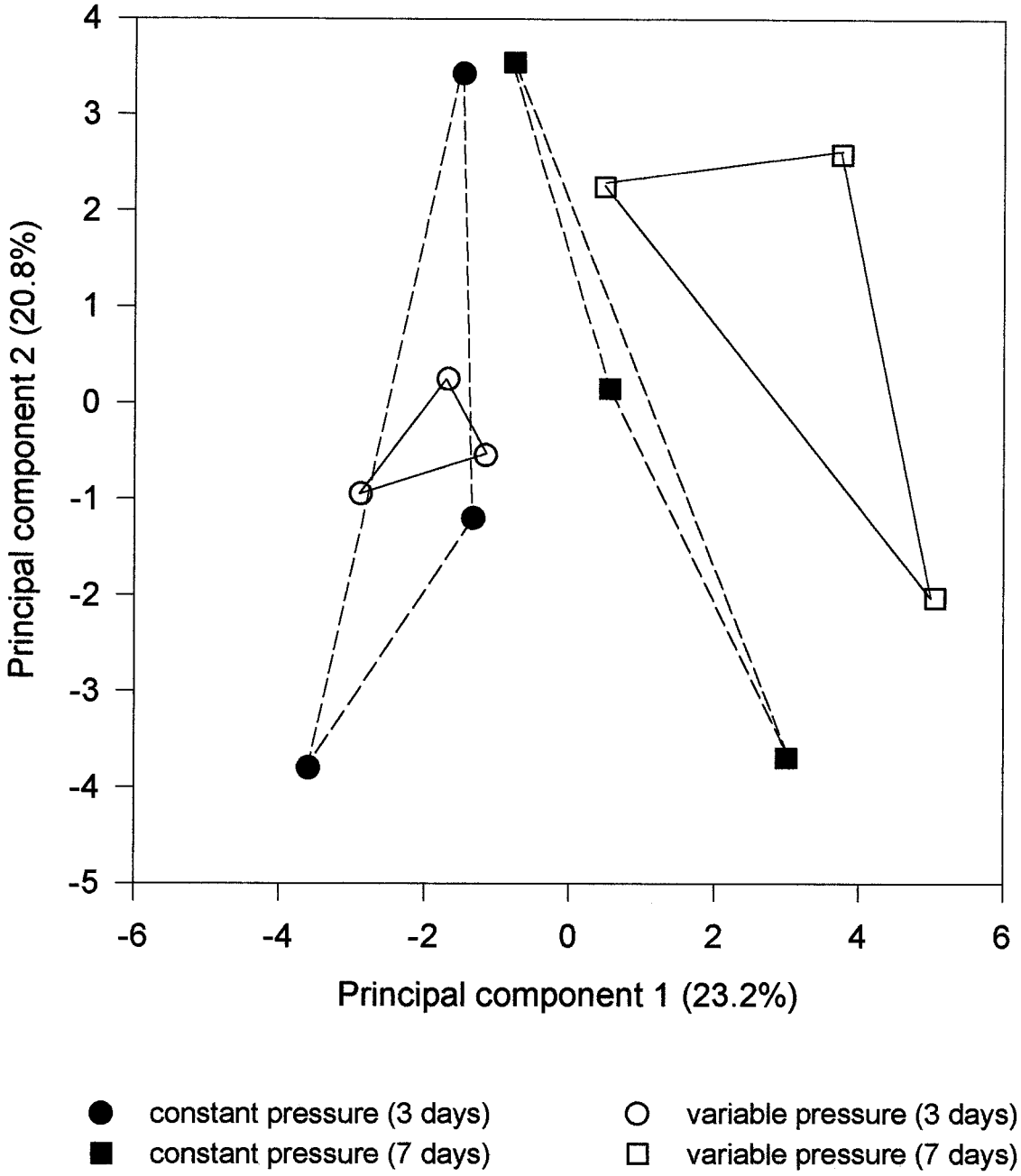

Fig. 2.8. Principal component analysis of aroma compounds from tomatoes stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and second eigenvectors.

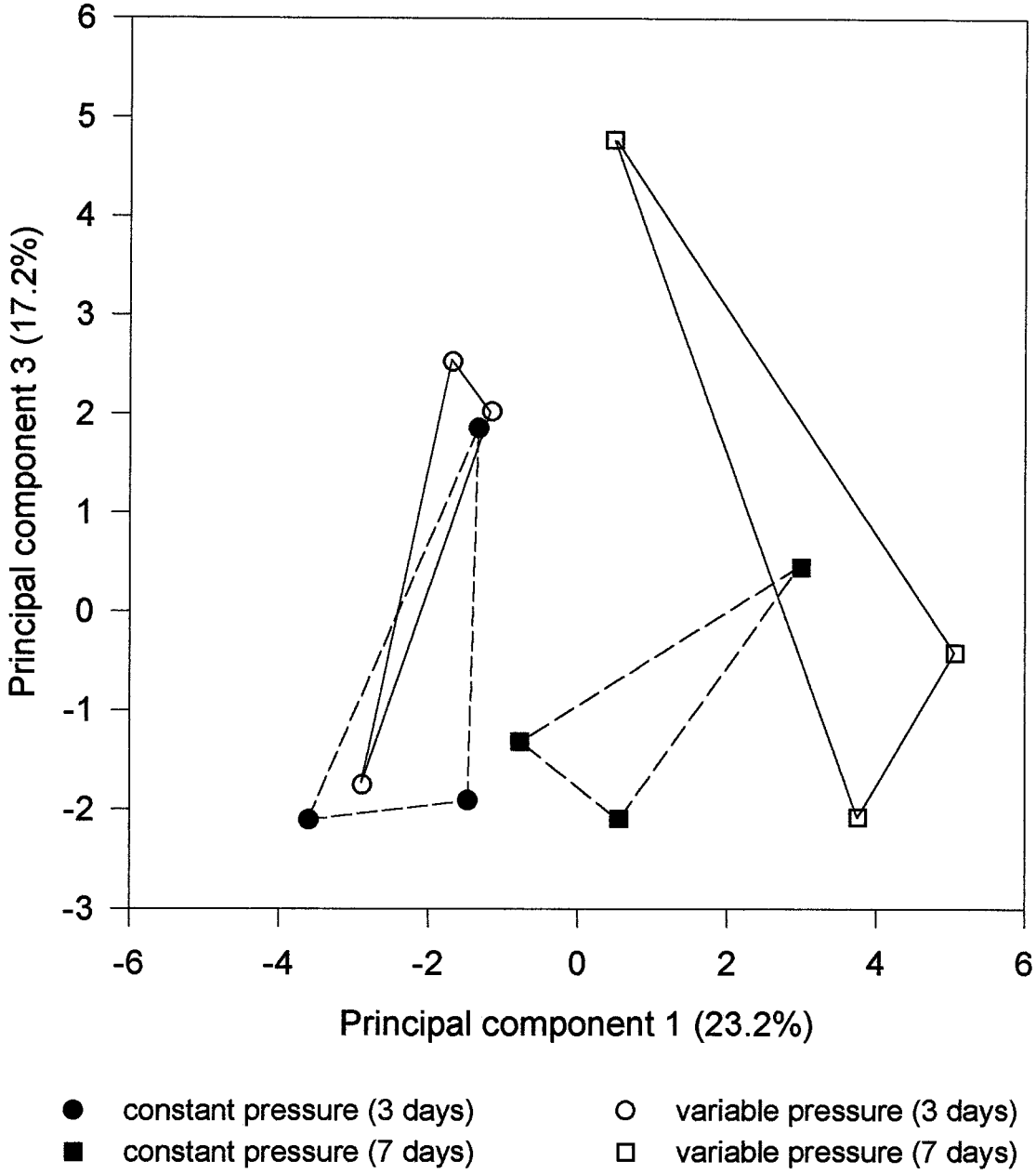

Fig. 2.9. Principal component analysis of aroma compounds from tomatoes stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and third eigenvectors.

Table 2.9. Coefficients of the first six principal components of the tomato volatiles data set.

Peak Number	Principal Component 1	2	3	4	5	6
PK1	0.252032	0.157853	-.098856	-.009496	-.262972	0.094547
PK2	0.349089	0.026923	-.008700	0.066610	0.065681	-.187940
PK3	-.037968	0.124601	0.376815	-.157032	-.060335	0.035525
PK4	-.157412	0.237867	0.180744	-.155755	0.199438	0.017477
PK5	-.088230	-.263780	0.166253	0.103648	-.044905	0.053191
PK6	0.068281	0.023008	0.015759	0.408863	0.247513	0.154121
PK7	0.251459	0.156650	0.157450	-.134568	-.142740	-.168451
PK8	0.059803	0.286327	0.199294	-.103065	0.149525	-.157223
PK9	0.328465	0.050693	0.148247	-.086978	-.035705	-.124941
PK10	0.097800	0.327133	0.127607	-.065856	0.073625	-.086879
PK11	-.062941	0.287549	-.164758	0.116210	0.241082	-.137224
PK12	-.083164	0.327261	-.068987	0.135887	-.091068	0.223969
PK13	-.051688	0.255068	0.142042	0.244018	0.063609	0.250140
PK14	0.218469	0.161268	-.260067	-.034317	-.031964	-.223456
PK15	0.057688	-.051890	0.039888	0.029413	0.492967	-.088616
PK16	0.246465	-.197375	0.163415	0.169055	0.054763	-.043240
PK17	-.287818	0.038309	0.180820	0.061190	0.008431	-.176823
PK18	0.189719	0.026330	-.028933	-.170745	-.298354	0.326941
PK19	0.123062	-.243536	0.143572	0.133327	-.080313	0.170681
PK20	0.028776	-.133787	0.155281	0.181516	-.058526	0.063506
PK21	0.236399	0.165580	-.196637	0.128836	-.082803	0.242290
PK22	-.075907	0.321134	0.002560	0.184894	-.158270	-.062825
PK23	0.082163	0.020385	0.166854	0.386052	-.183155	-.177331
PK24	0.037510	-.074906	0.278474	-.176020	0.178984	0.300664
PK25	0.252065	0.154381	-.063406	-.041746	0.240462	0.263189
PK26	-.074397	0.029598	0.129079	0.275780	-.318249	-.343365
PK27	0.111087	-.059665	-.036927	0.369364	0.277530	-.025560
PK28	-.209085	0.169795	0.062553	0.250435	-.121010	0.244396
PK29	0.330287	-.085813	0.119169	0.049650	0.045549	-.114948
PK30	0.159990	0.017667	0.347329	0.055881	-.058634	0.193321
PK31	-.043743	0.113218	0.392060	-.094411	0.013422	-.078511
Var C_i[a]	7.193	6.451	5.329	4.294	2.705	1.984
CPE[b]	0.232	0.440	0.612	0.751	0.838	0.902
$0.5/\sqrt{VarC_i}$	0.186	0.197	0.217	0.241	0.304	0.355

[a] Eigenvalues

[b] Cumulative proportion explained

Table 2.9 continued

For each principal component, the variables with a correlation greater than 0.5 with that component, are underlined.

analysis. In tomato, variables highly correlated (more than 0.5 absolute correlation) with the first principal component were 2-methylfuran, 2-methy-1-propanol, an unidentified peak, dimethyl disulfide + 3-methyl-1-butanol, 2-methylpropanoic acid, E-2-hexenal, Z-3-hexen-1-ol, 2,4-hexadienal, 6-methyl-5-hepten-2-one and linalool. Of these compounds, E-2-hexenal, Z-3-hexen-1-ol and 6-methyl-5-hepten-2-one are important in determining tomato flavour and the concentrations of two of these compounds were found to be significantly higher in tomatoes stored for seven days at low temperatures (Table 2.7).

Coefficients for the second component indicate that 2-methylbutanal, 1-penten-3-one, Z-3-hexenal, hexanal, ethylbenzene and 5-ethyl-2(5H)-furanone were the variables which correlate well with this component (Table 2.9) and two of these compounds, namely 2-methylbutanal and hexanal, have been identified in the literature as important flavour constituents of tomatoes. Although loadings for this component appeared to be quite variable among tomatoes used in this study, there is some indication that the second component captured enough of the variance structure of the data matrix to group the treatments into 3 main groups (Fig. 2.8). An explanation of a negative correlation between 1-penten-2-one and ethylbenzene and the second component may be found in the data contained in Table 2.7. Concentrations of these chemical compounds were higher for tomatoes stored for 7 days under constant pressure compared to tomatoes stored 7 days under variable pressure and may in part, have led to the separation between the two treatments in the plot. Variables having more than 0.5 absolute correlation with the third component were 3-methylbutanal, 2-methylpropanoic acid, dimethyl trisulfide, undecane and dimethyl tetrasulfide (Table 2.9). Of these compounds 3-methylbutanal and dimethyl trisulfide have been previously identified as important odor compounds of tomato. It appears from the plot of the first and third principal components (Fig. 2.9) that these

variables not only confirm the class difference between number of days in storage but also determine the commonality of the treatment for 7 days at constant pressure and the treatment for 7 days at variable pressure since the plots for these treatments overlap.

Again it is worthwhile plotting later values against the first component to identify variables responsible for differentiation between treatments. Referring to the plot of the first and fourth eigenvectors in Fig. 2.10 and the table of coefficients in Table 2.9, it appears that 1-penten-3-ol, E-2-heptenal, 2-octanone, 2-pentylfuran and 2-isobutylthiazole + unknown were important variables in the separation of treatment classes, namely, variable pressure treatment for 7 days. Plots of the first and fifth eigenvectors (Fig. 2.11) and first and sixth eigenvectors (Fig. 2.12) also show that distinct differences in volatile production occur in tomatoes stored for 3 days compared to tomatoes stored for 7 days. There is some indication in Fig. 2.12 that differences in volatile composition exist between tomatoes stored for 3 days at constant and variable pressures. However, although the sixth principal component accounted for 6.4% of the variablility, none of the coefficients listed in Table 2.9 had a significant association with that component.

Informal tasting of the various treatments found that all tomatoes possessed a good flavour, although it was noted upon removal from 3 or 7 days storage, that tomatoes subjected to atmospheric pressure variations were more evenly colored than tomatoes stored at constant pressure. The deleterious effect of refrigerated storage on certain tomato volatiles is well supported in the literature although this is thought to occur only at chilling temperatures, i.e. below 12 °C (Whitfield and Last, 1991).

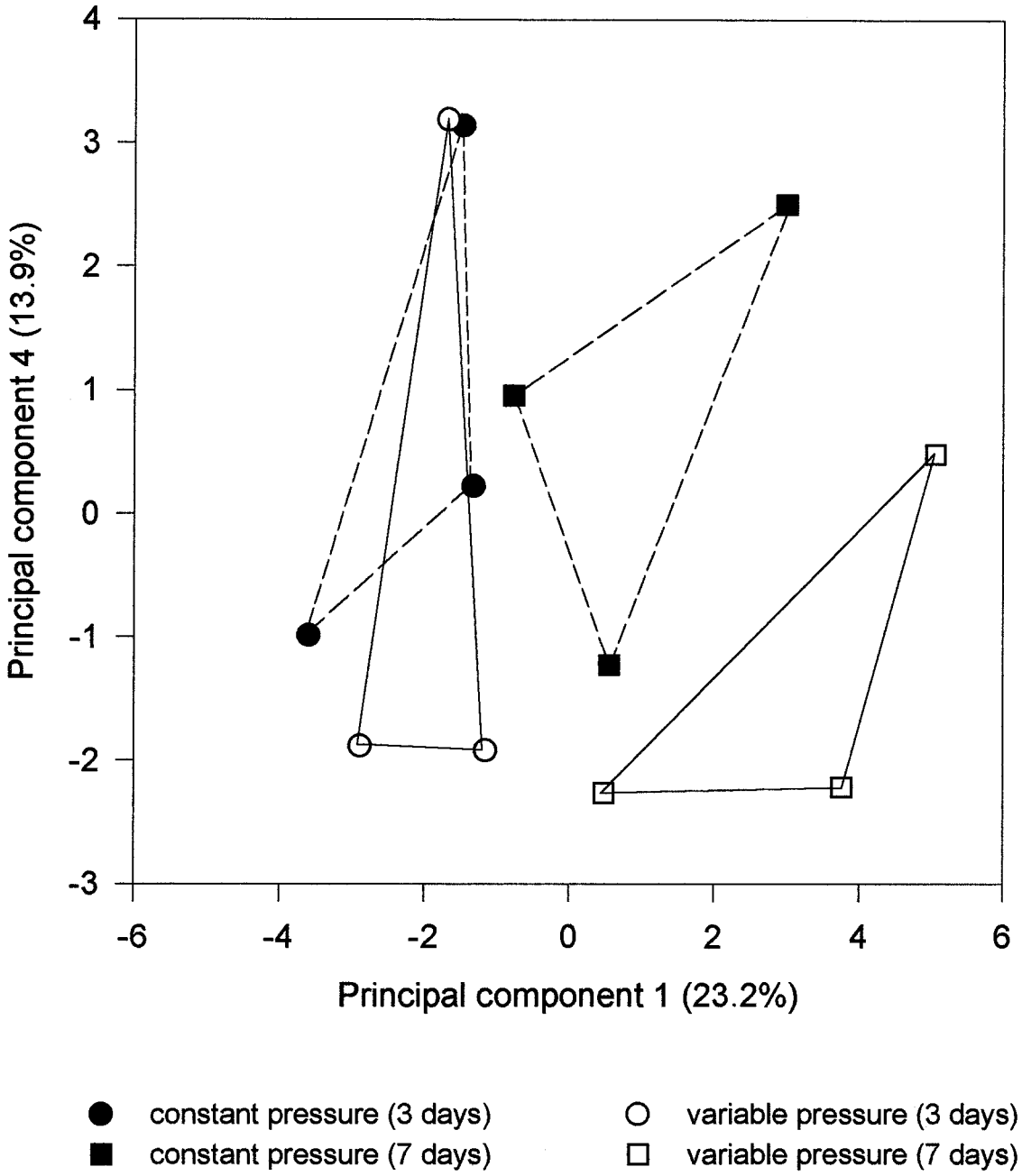

Fig. 2.10. Principal component analysis of aroma compounds from tomatoes stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and fourth eigenvectors.

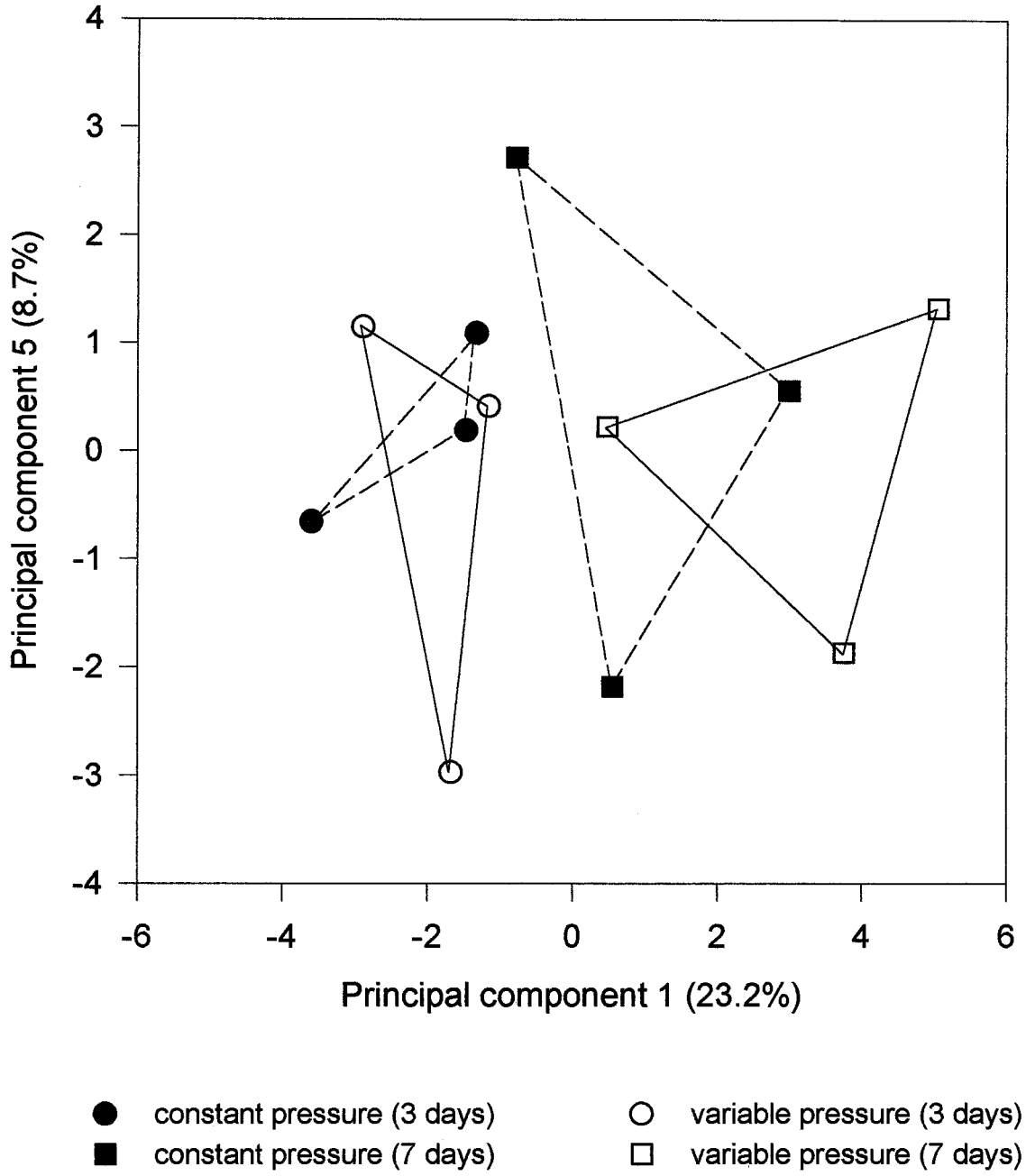

Fig. 2.11. Principal component analysis of aroma compounds from tomatoes stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and fifth eigenvectors.

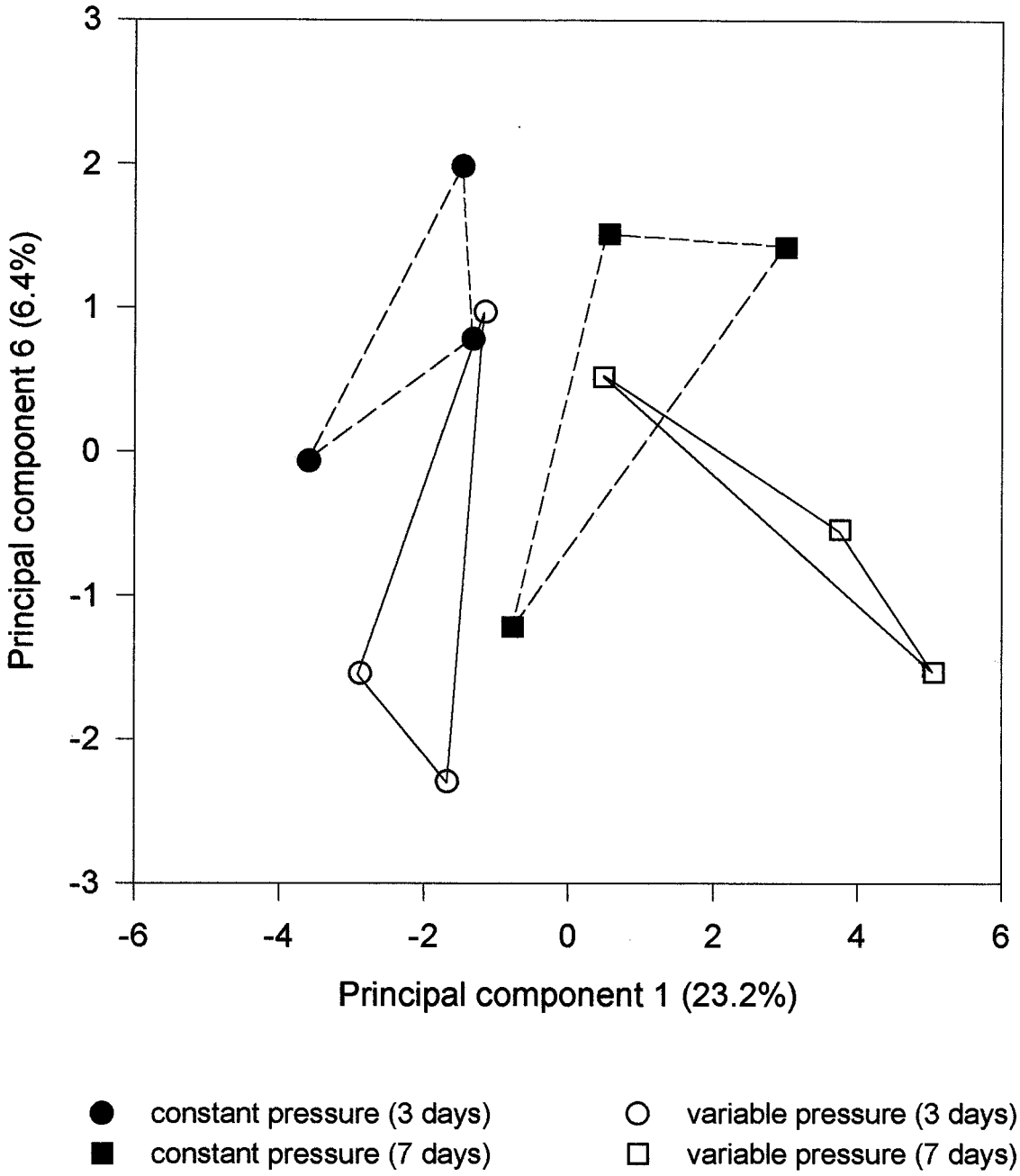

Fig. 2.12. Principal component analysis of aroma compounds from tomatoes stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and sixth eigenvectors.

E. Effect of Atmospheric Pressure Treatments on Sweet Pepper Volatiles

According to Whitfield and Last (1991) a total of 23 compounds have been identified in the volatile oil of uncooked peppers with the most important compounds being; (E)-β-ocimene, methyl salicylate, limonene and 2-(2-methylpropyl)-3-methoxypyrazine. Other pyrazines such as 2-isopropyl-3-methoxypyrazine and 2-sec-butyl-3-methoxypyrazine have been captured in appreciable quantities by a headspace concentration technique (Murray and Whitfield, 1975).

Total ion chromatograms of sweet peppers stored for 3, 7 and 14 days at constant atmospheric pressure or varying atmospheric pressure are shown in Fig. 2.13 and Fig. 2.28 to 2.32 in Appendix 3. The compounds identified in the chromatograms are listed in Table 2.10. Much of the published literature on sweet pepper volatiles appeared before 1975, and contains little in the way of capillary 'aromagrams' [other than that of Buttery et al. (1969)] and no retention indices. Consequently, it was not possible to confirm the identity of some of the later eluting compounds in the absence of authentic standards, and as a result many of the unknown peaks were identified by matching spectra with those contained in the computer mass spectral library. Means as shown in Table 2.11, indicate that variable pressure treatment may have had an effect on C_6 compounds. Means for relative amounts of hexanal, E-2-hexenal and Z-3-hexen-1-ol were higher for peppers stored under variable pressure treatments compared to peppers stored at constant pressure, but this increase was not significant ($P>0.05$) for all storage periods. Although 2-isobutyl-3-methoxy-pyrazine and total volatiles were more abundant in the variable pressure-treated peppers compared to peppers stored under constant pressure, this difference was not significant ($P>0.05$).

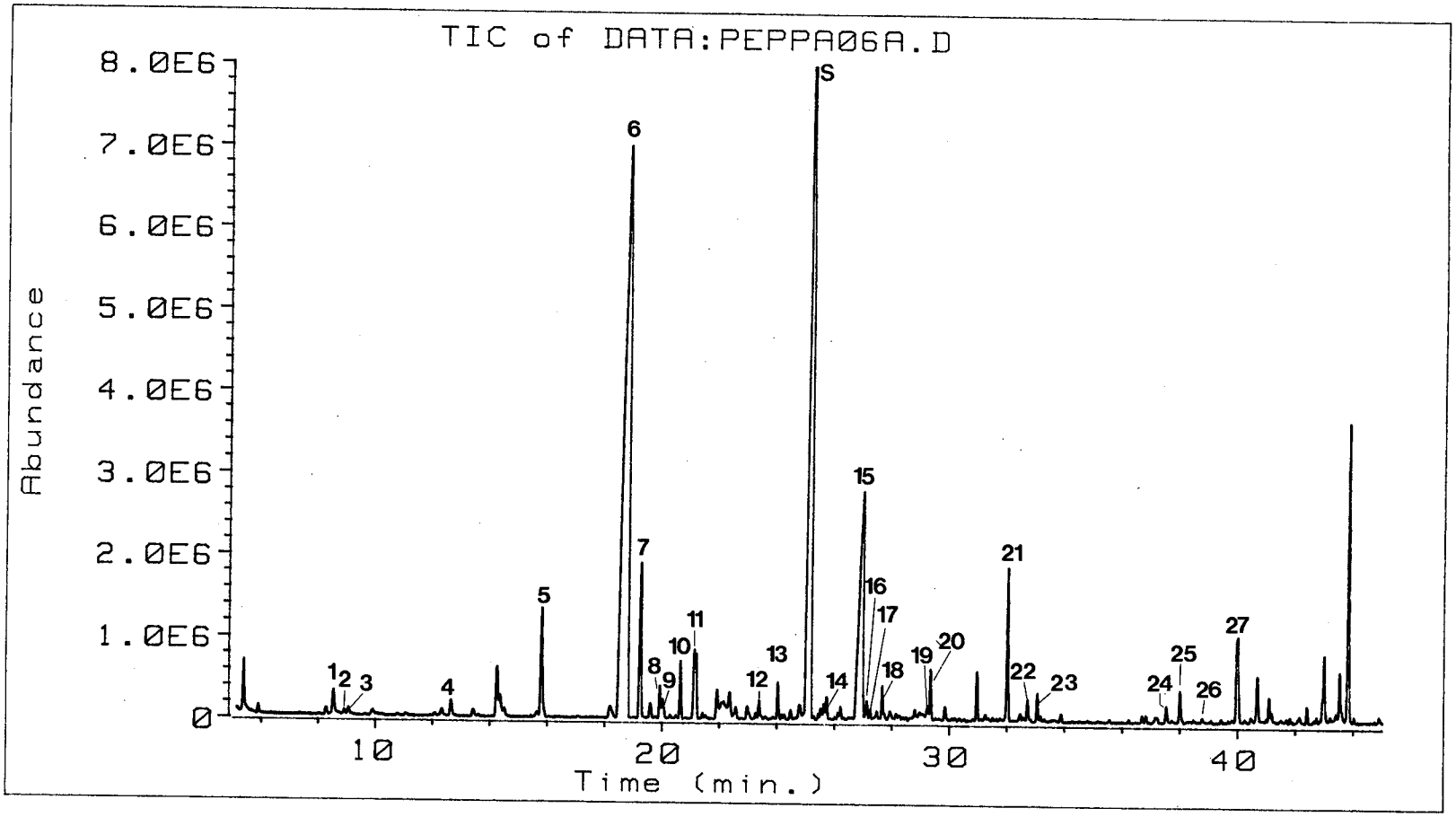

Fig.2.13. Total ion chromatogram of volatiles extracted from sweet pepper stored under constant pressure at 15C for 3 days. Peak numbers correspond to peak names in Table 2.10.

Table 2.10. Flavour constituents of sweet pepper.

Peak Number[a]	Component	I_p (DB-1)[b]	ID
1	1-penten-3-one	660	MS CS
2	unidentified	671	
3	3-pentanone	673	MS CS
4	E-2-pentenal	723	MS CS
5	hexanal[1,2]	780	MS CS
6	E-2-hexenal[3]	820	MS CS
7	Z-3-hexen-1-ol[3]	841	MS CS
8	1-hexanol[3]	851	MS CS
9	1,3-dimethylbenzene[1,2]	853	MS CS
10	2-heptanone[2,3]	861	MS CS
11	2,4-hexadienal	871	MS CS
12	E-2-heptenal[1,3]	920	MS CS
13	dimethyl trisulfide	940	MS
14	5-ethyl-2-(5H)-furanone	980	MS
15	cyclobutanone oxime	1016	MS
16	D-limonene[1,2,3]	1017	MS CS
17	E-3,7-dimethyl-1,3,6-octatriene[1](Ocimene)	1021	MS
18	E-3,7-dimethyl-1,3,6-octatriene[1,2](β-ocimene)	1029	MS
19	3,7,7-trimethyl-bicyclo[4.1.0]hept-2-ene	1074	MS
20	3,7-dimethyl-1,6-octadien-3-ol	1076	MS
21	2-isobutyl-3-methoxypyrazine[1,2]	1164	MS
22	methylsalicylate[2]	1168	MS CS
23	dimethyl tetrasulfide	1200	MS
24	2-methyltridecane	1290	MS
25	unidentified	1400	MS
26	copaene[1]	1405	MS
27	unidentified	1430	

MS, mass spectra of unknown tentatively matched with spectra in the mass spectral library of the National Bureau of Standards (Probability Based Matching); CS, mass spectra of unknown matched with spectra and retention times of authentic compounds.

[a] Peak numbers match peaks in Fig. 2.13

[b] Kovat's linear retention indices with temperature programming (Lee et al., 1984).

[1] Maarse and Visscher (1989) [2] Buttery et al. (1969) [3] Whitfield and Last (1991)

Table 2.11. Means[1] of volatile compounds identified in sweet pepper

Peak Number	Component	Relative amounts of compounds[3] CON(3)[2]	CON(7)	CON(14)	VAR(3)	VAR(7)	VAR(14)
1	1-penten-3-one	0.31^{a}	0.31^{ab}	0.09^{b}	0.31^{a}	0.40^{a}	0.29^{ab}
2	unidentified	0.10	0.12	0.21	0.20	0.15	0.18
3	3-pentanone	0.10^{ab}	0.11^{ab}	0.01^{b}	0.14^{a}	0.16^{a}	0.19^{a}
4	E-2-pentenal	0.19^{ab}	0.22^{ab}	0.08^{b}	0.28^{a}	0.37^{a}	0.31^{a}
5	hexanal	2.88	3.67	3.38	4.90	4.06	5.27
6	E-2-hexenal	17.11^{b}	18.45^{ab}	25.70^{ab}	22.39^{ab}	24.51^{ab}	26.77^{a}
7	Z-3-hexen-1-ol	1.20^{abc}	1.21^{abc}	0.78^{c}	1.38^{abc}	1.85^{a}	1.62^{ab}
8	1-hexanol	0.20	0.25	0.20	0.21	0.28	0.25
9	1,3-dimethylbenzene	0.25^{ab}	0.10^{b}	0.39^{a}	0.25^{ab}	0.16^{ab}	0.18^{ab}
10	2-heptanone	0.23^{c}	0.22^{c}	0.34^{ab}	0.25^{bc}	0.32^{abc}	0.37^{a}
11	2,4-hexadienal	0.30^{b}	0.20^{b}	0.08^{b}	1.19^{a}	0.38^{ab}	0.32^{b}
12	Z-2-heptenal	0.18	0.16	0.14	0.23	0.19	0.19
13	dimethyl trisulfide	0.18	0.15	0.25	0.18	0.22	0.17
14	5-ethyl-2-(5H)-furanone	0.14^{ab}	0.12^{ab}	0.08^{b}	0.18^{a}	0.18^{a}	0.17^{a}
15	cyclobutanone oxime	3.27	2.83	2.18	3.84	3.56	3.58
16	D-limonene	0.13^{a}	0.08^{ab}	0.10^{ab}	0.04^{b}	0.09^{ab}	0.05^{ab}
17	E-3,7-dimethyl-1,3,6-octatriene	0.39	0.26	0.34	0.27	0.28	0.25
18	E-3,7-dimethyl-1,3,6-octatriene	1.88	1.51	0.76	1.48	1.54	1.62
19	3,7,7-trimethyl-bicyclo[4.1.0]hept-2-ene	0.12	0.16	0.15	0.81	0.30	0.27
20	3,7-dimethyl-1,6-octadien-3-ol	0.37^{b}	0.36^{b}	0.61^{ab}	0.78^{a}	0.44^{ab}	0.45^{ab}
21	2-isobutyl-3-methoxypyrazine	1.00	1.04	1.03	1.08	1.25	1.27
22	methylsalicylate	0.43	0.22	0.19	0.34	0.30	0.19
23	dimethyl tetrasulfide	0.19^{b}	0.17^{b}	0.40^{a}	0.20^{b}	0.23^{ab}	0.20^{b}

Table 2.11 continued

Peak Number	Component	Relative amounts of compounds[3] CON(3)[2]	CON(7)	CON(14)	VAR(3)	VAR(7)	VAR(14)
24	2-methyltridecane	0.17	0.12	0.14	0.17	0.18	0.16
25	unidentified	0.28	0.19	0.16	0.28	0.25	0.17
26	copaene	0.15	0.03	0.06	0.24	0.07	0.07
27	unidentified	1.81^{bc}	1.74^{bc}	0.57^{c}	3.32^{a}	2.87^{ab}	3.22^{ab}
Total amounts relative to internal standard		33.55	33.98	38.43	44.95	44.52	47.84

[1] means of three observations.
[2] CON - constant pressure, VAR - variable pressure, number of days of treatment in parenthesis.
[3] Amounts are relative to 2-octanone (internal standard) peak.
[abc] Means in the same row with different letters are significantly ($P<0.05$) different.

Principal component analysis indicated that 84.7% of the sample variability could be accounted for by the first six principal components (Table 2.12). To ascertain if a recognizable treatment pattern could be discerned, the latter 5 components were plotted against the first component. The first 2 plots (Fig. 2.14 and Fig. 2.15) illustrate the close relationship of all the variable pressure treatments. Calculation of the coefficients of the first six principal components indicates that 16 of the 27 compounds were important variables in the first component (Table 2.13). An unidentified peak, hexanal, E-2-hexenal, 1,3-dimethylbenzene, dimethyl trisulfide and dimethyl tetrasulfide were the variables associated with the second component. Smaller differences between concentrations of these compounds among the variable pressure treatments would account for close overlapping of these treatment classes in Fig. 2.14. Clear separation of the treatment class representing constant atmospheric pressure treatment for 14 days from other pressure treatment classes may be evidence that over time, metabolic events taking place in peppers stored at constant pressure were different than peppers stored under variable atmospheric pressure (Fig. 2.15). Plotting later eigenvalues against the first component did not assist in further treatment class separations (Fig. 2.16 to Fig. 2.18).

The gas chromatographic data indicate that extensive oxidation of cellular constituents of sweet peppers did not occur as a result of variable atmospheric pressure storage. If oxidation occurred, significant lipid changes would have been detected by the observation of much higher concentrations of E-2-hexenal and Z-3-hexen-1-ol in peppers stored under variable pressures compared to those stored under constant pressure.

Data from preliminary experiments to determine if pepper volatiles were affected by O_2 concentration during storage under variable atmospheric

Table 2.12. Principal component analysis (PCA) of volatile compounds from sweet pepper stored under constant and variable pressures.

Eigenvector number	Variance preserved		
	Eigenvalue	Each	Total
1	8.889	32.9	32.9
2	4.388	16.3	49.2
3	3.550	13.1	62.3
4	2.750	10.2	72.5
5	1.909	7.1	79.6
6	1.389	5.1	84.7
7	1.189	4.4	89.1
8	1.130	4.2	93.3
9	0.626	2.3	95.6
10	0.418	1.6	97.2
11	0.279	1.0	98.2
12	0.173	0.6	98.9
13	0.111	0.4	99.3
14	0.081	0.3	99.6
15	0.046	0.2	99.8
16	0.043	0.2	99.9
17	0.021	0.1	100.0

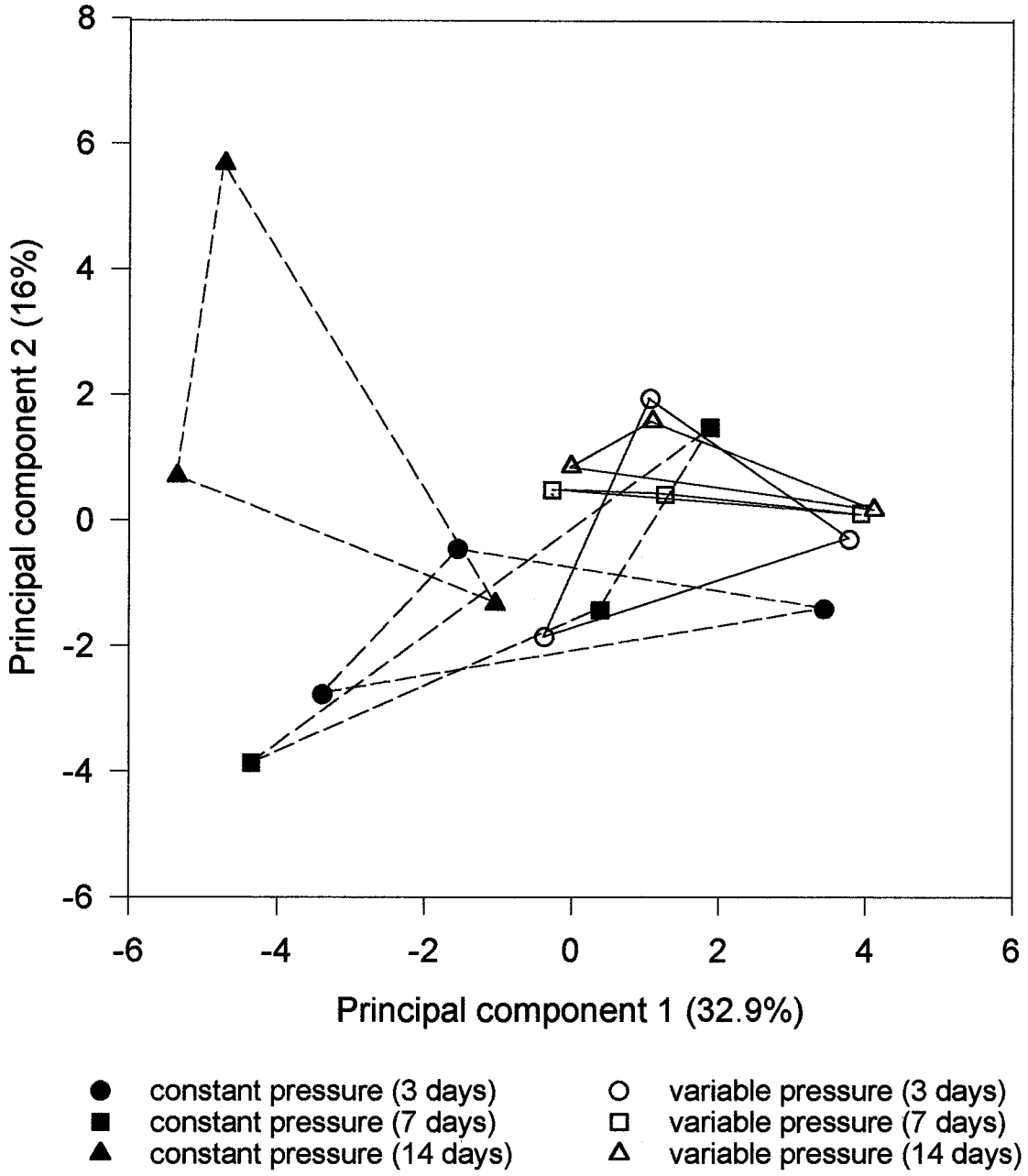

Fig. 2.14. Principal component analysis of aroma compounds from peppers stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and second eigenvectors.

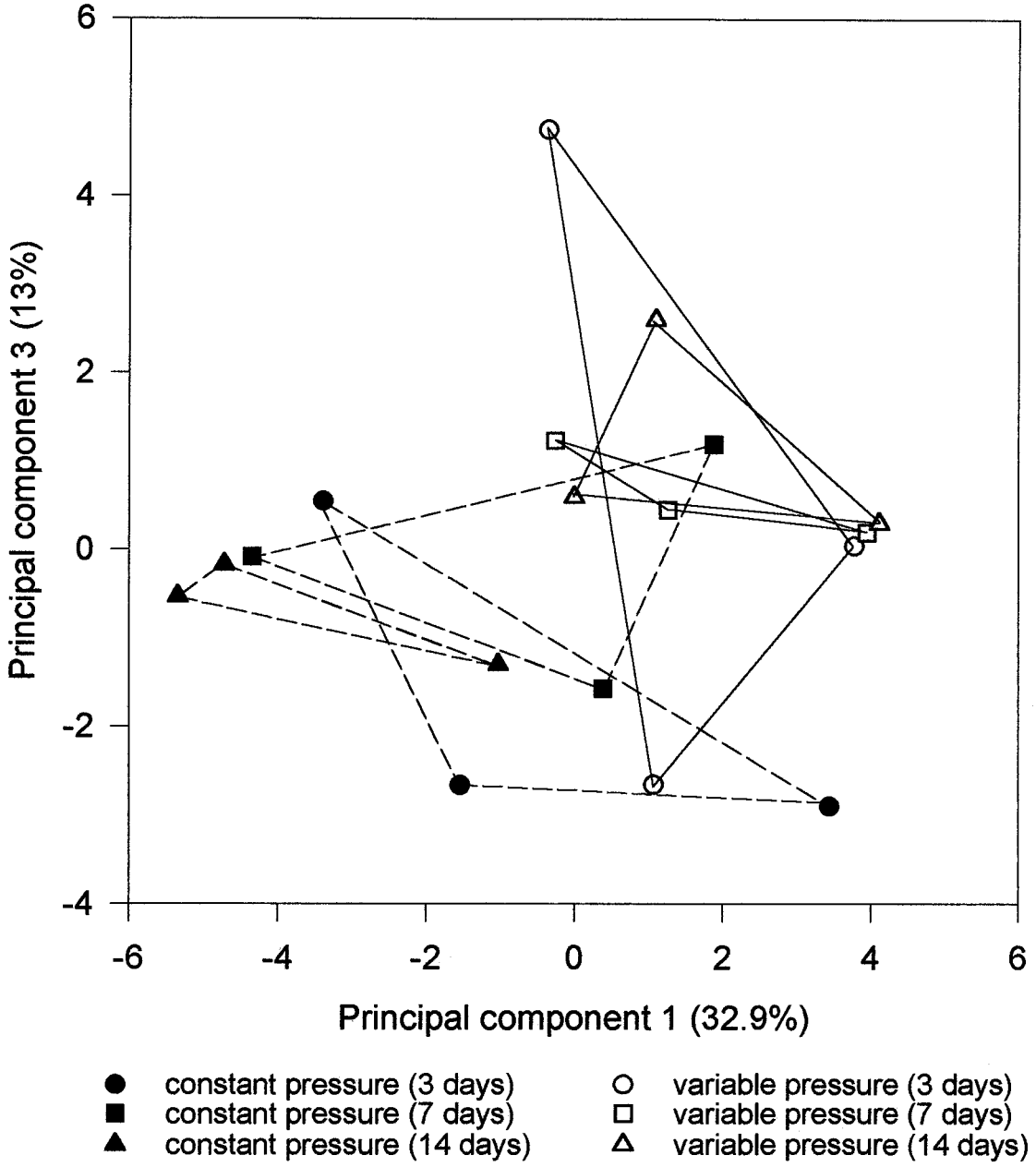

Fig. 2.15. Principal component analysis of aroma compounds from peppers stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and third eigenvectors.

Table 2.13. Coefficients of the first six principal components of the pepper volatiles data set.

Peak Number	Principal Components 1	2	3	4	5	6
PK1	0.286491	-.051463	-.030297	-.057584	0.062031	0.275943
PK2	0.044831	0.391753	-.048385	0.121284	-.192524	-.149661
PK3	0.295805	0.102107	0.070661	-.069663	-.092605	0.225534
PK4	0.297260	0.049597	0.129180	-.120951	-.053125	-.066998
PK5	0.218747	0.252239	-.015331	0.042901	-.286418	-.193381
PK6	0.128078	0.353740	0.109818	-.123300	0.162832	-.237491
PK7	0.231918	0.021079	0.226030	-.126472	0.115434	0.379242
PK8	0.123794	0.213074	0.164193	-.249695	-.181762	0.250550
PK9	-.182691	0.309925	-.130620	0.213528	-.013021	0.001360
PK10	-.037210	0.237325	0.255213	-.168507	0.230813	-.316406
PK11	0.050870	-.126713	0.337249	0.418142	-.007870	-.016764
PK12	0.250579	0.031291	0.007334	0.008172	-.107308	0.019841
PK13	-.107026	0.277162	-.099847	0.081339	0.406171	0.262334
PK14	0.273671	-.152741	0.134559	0.072374	0.216445	-.034324
PK15	0.220148	-.196614	-.050694	0.064611	0.233225	-.209830
PK16	-.210767	-.093251	0.040428	0.003087	0.186615	0.423850
PK17	0.168635	-.005485	-.356483	0.027254	0.234116	-.095602
PK18	0.235876	0.041436	-.251467	-.003370	0.044716	0.036915
PK19	0.029491	-.113212	0.351315	0.396945	0.049591	-.066591
PK20	-.025922	0.139842	0.255089	0.418095	0.168514	0.058172
PK21	0.218292	0.225512	0.055490	-.047581	0.276006	0.138810
PK22	0.198119	-.116106	-.351135	0.139805	0.122347	0.014449
PK23	-.138218	0.374425	-.074671	0.084030	0.255930	0.014536
PK24	0.227363	-.108670	-.192253	0.137787	0.226308	-.212262
PK25	0.079881	0.080971	-.175292	0.334763	-.136691	0.167931
PK26	0.011705	0.131575	-.256993	0.283360	-.267427	0.199368
PK27	0.278338	0.071777	0.109348	0.169802	-.202438	0.015097
Var C_i[a]	8.889	4.388	3.550	2.750	1.909	1.389
CPE[b]	0.329	0.492	0.623	0.725	0.796	0.847
$0.5/\sqrt{VarC_i}$	0.168	0.239	0.265	0.302	0.362	0.424

[a] Eigenvalues

[b] Cumulative proportion explained

For each principal component, the variables with a correlation greater than 0.5 with that component, are underlined.

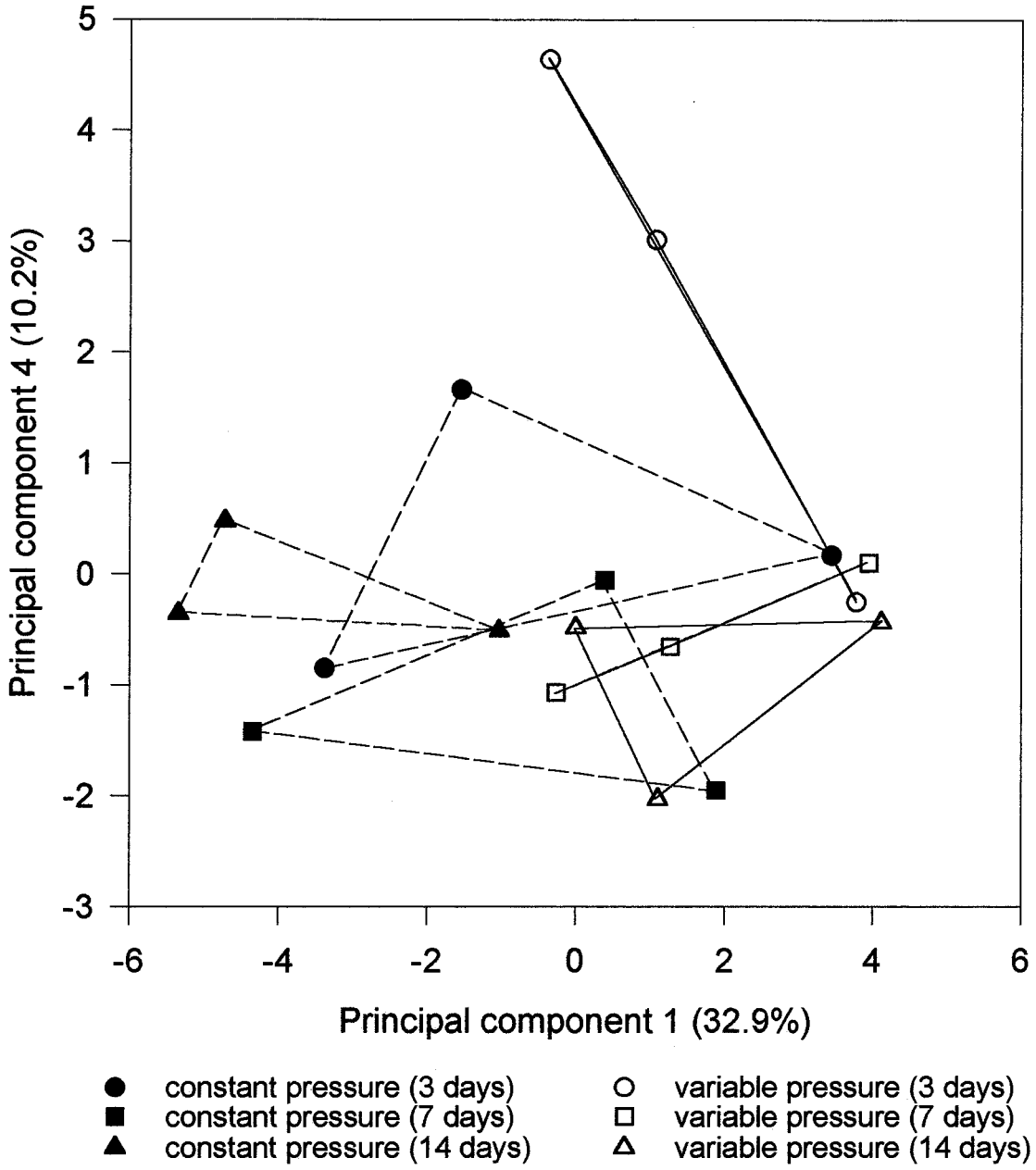

Fig. 2.16. Principal component analysis of aroma compounds from peppers stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and fourth eigenvectors.

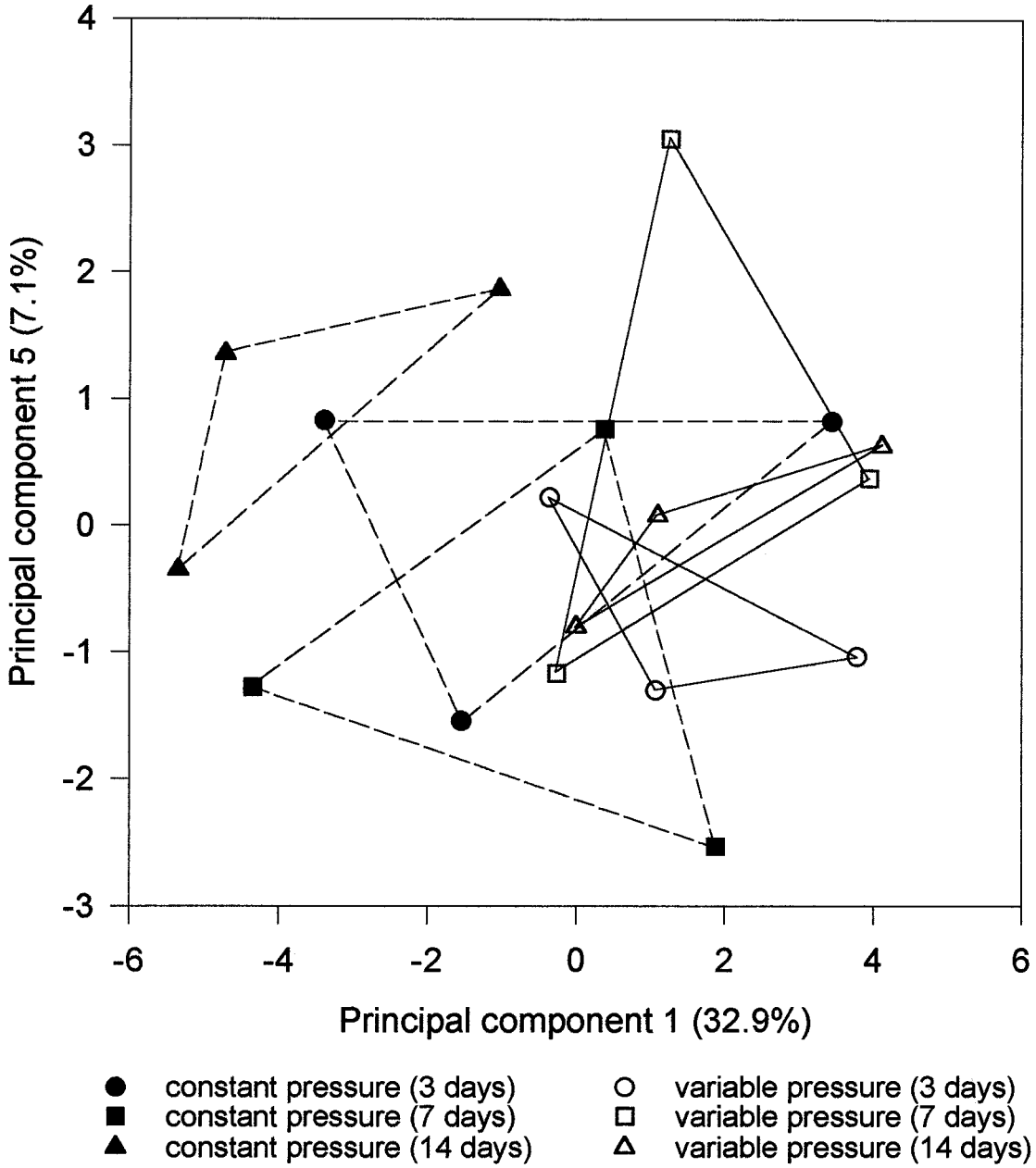

Fig. 2.17. Principal component analysis of aroma compounds from peppers stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and fifth eigenvectors.

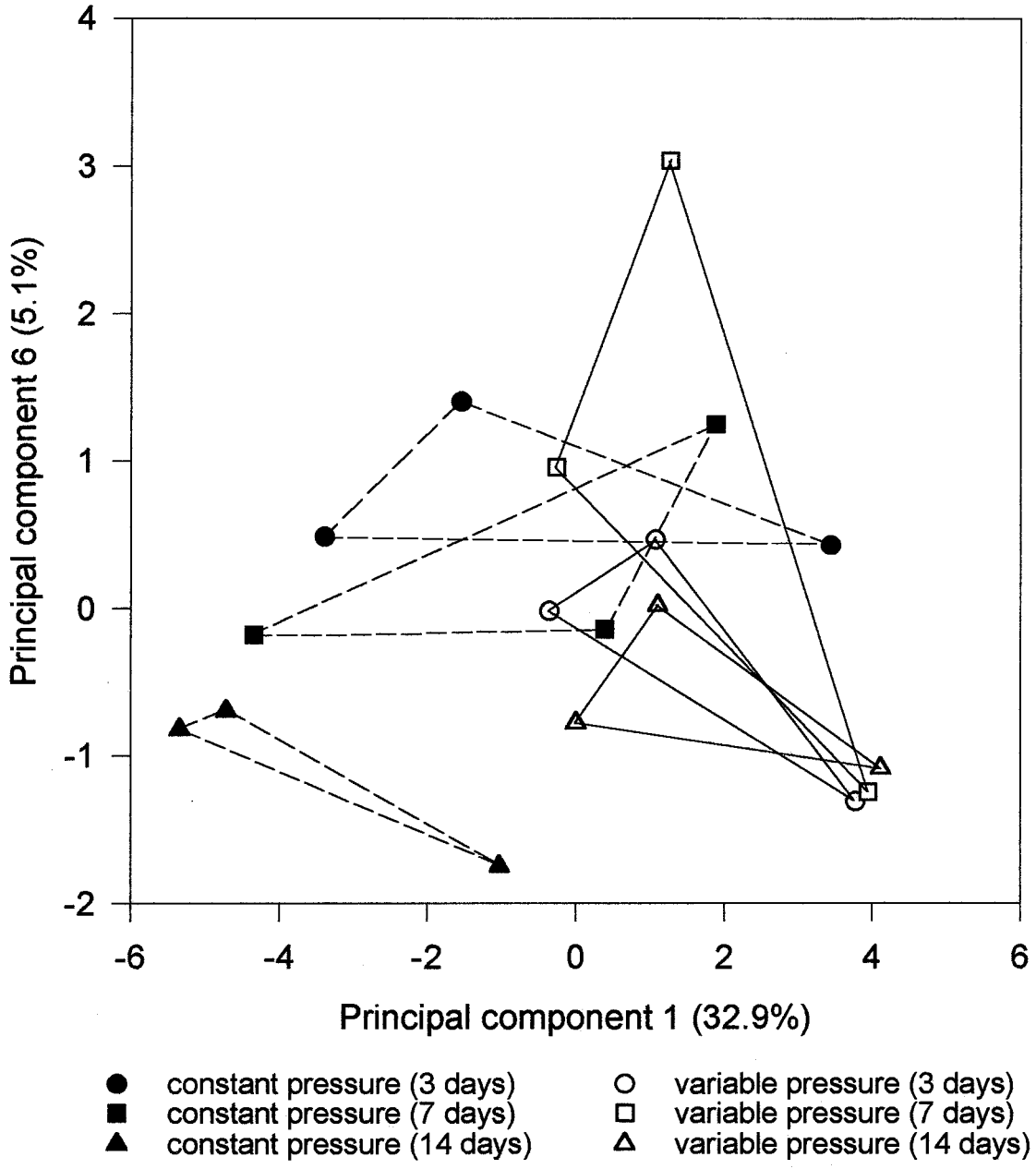

Fig. 2.18. Principal component analysis of aroma compounds from peppers stored under constant atmospheric pressure and variable pressure treatments (n=3). A two-dimensional plot of the first and sixth eigenvectors.

pressure, indicated that oxygen concentration may affect the production of E-2-hexenal and Z-3-hexen-1-ol (Table 2.14). Presumably, reducing the concentration of O_2 in the atmosphere surrounding the peppers resulted in lower rates of lipid oxidation hence a lower production of E-2-hexenal. Further investigations are required to confirm this observation since the trial lacked adequate control samples, which were not included due to time constraints.

F. Total Water Loss

Water loss from the plant material in the treatment vessel of the gas exchange circuit was a concern because of the high flow rate of the circulating gases (1.5 L/min) and the presence of the fan in the treatment vessel. Studies with leaves (Nobel, 1974) showed that, in still air, relative humidity near the evaporating surface was higher than that of the ambient air and thus there is little water vapour movement from the leaf to the air. Air currents greater than 84 cm s^{-1} disturb this boundary layer and decrease its relative humidity so that transpiration increases (Shive and Brown, 1978). Shive and Brown (1978) suggested that mass flows of gases (including water vapour) may be driven by external air movement. There is little information in the literature about the importance of air flow rate in transpiration rate of fruit and vegetables. One study revealed that, at a constant air flow rate and relative humidity, air currents accounted for less than a 5% increase in transpiration rate in apples (Pantastico, 1975). Other research indicated that stored apples showed a 30-100% acceleration of transpiration rates when air currents of increasing velocities were used (Sastry et al. 1978).

The benefit of high humidity storage (98-100% RH) of vegetables has been recognized for nearly 20 years (van den Berg and Lentz, 1977). A comprehensive mathematical analysis was performed by van den Berg and

Table 2.14. Means[1] of volatile compounds identified in sweet pepper subjected to variable pressures at different oxygen concentrations.

		Relative amounts of compounds[3]	
Peak Number	Component	VAR(3%)[2]	VAR(21%)
1	1-penten-3-one	0.05	0.11
2	unidentified	0.12	0.07
3	3-pentanone	0.08	0.08
4	E-2-pentenal	0.06	0.15
5	hexanal	3.92	4.62
6	E-2-hexenal	10.17[b]	24.42[a]
7	Z-3-hexen-1-ol	0.88[a]	0.73[b]
8	1-hexanol	0.41	0.21
9	1,3-dimethylbenzene	0.23	0.18
10	2-heptanone	0.21	0.12
11	2,4-hexadienal	0.10	0.09
12	Cis-2-heptenal	0.14	0.14
13	dimethyl-trisulfide	0.28	0.15
14	5-ethyl-2-(5H)-furanone	0.04	0.11
15	cyclobutanone oxime	1.59	2.36
16	D-limonene	0.04	0.08
17	E-3,7-dimethyl-1,3,6-octatriene	0.63	0.50
18	E-3,7-dimethyl-1,3,6-octatriene	3.29	2.65
19	3,7,7-trimethyl-bicyclo[4.1.0]hept-2-ene	0.13	0.08
20	3,7-dimethyl-1,6-octadien-3-ol	0.30	0.15
21	2-isobutyl-3-methoxypyrazine	0.92	0.78
22	methylsalicylate	0.23	0.29
23	dimethyl tetrasulfide	0.28	0.19
24	2-methyltridecane	0.01	trace
25	3-hydroxy-2,4,4-trimethylpentyl ester	0.03	0.05
26	copaene	0.03	0.01
27	unidentified	1.24	2.00

[1] means of two observations.

[2] VAR = variable pressure treatment only, oxygen concentration (%) in parenthesis.

[3] Amounts are relative to 2-octanone (internal standard) peak.

[a,b] Means within rows with different letters are significantly ($P<0.05$) different.

Lentz (1978) in which they noted that an increase in air velocity lowered the rate of moisture loss in stored produce. However, this effect was only apparent at distances of at least 1 m between the air intake and the produce.

In the present study, the distance between the air intake and the produce was only 2-7 cm, allowing gases to flow over the produce. The results of total weight loss measurements in Figure 2.19. suggest that variable pressure treatments did not accelerate water loss in tomatoes and peppers compared to those fruits stored at constant pressure. Moisture loss in onions was unaffected by treatment probably due to the low transpiration rate and protective dry scales.

In conclusion, data collected from GC/MS analyses indicate that exposing vegetables to variable atmospheric pressure changes may subtly alter the profile of aroma compounds. Application of multicomponent analysis assisted in discriminating among constant and variable pressure treatments and identified the compounds which were important in making that distinction. Variable pressure treatments did not promote water loss in onions, tomatoes or sweet peppers.

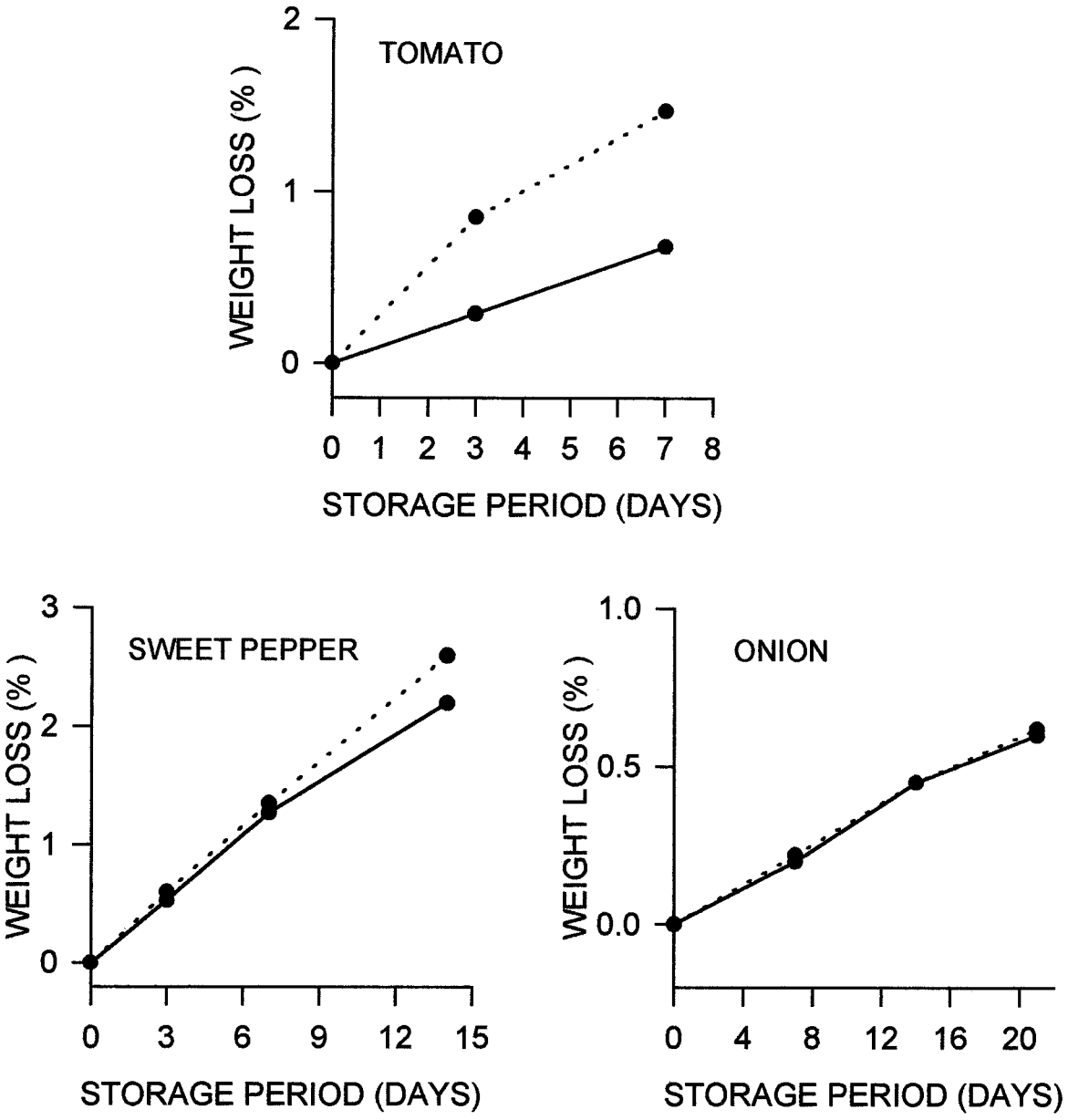

Fig. 2.19. Comparison of water loss in pressure treated and control vegetables. Treatments were: variable pressure treatment at 6.9 kPa ——— and the control treatment at constant pressure - - - - - - - .

GENERAL SUMMARY AND CONCLUSIONS

Although gas exchange in bulky tissues has been studied for many years, most of the research effort has concentrated on fruits, rather than vegetables, and on diffusive gas flow, rather than bulk gas flow. Bulk gas flow has been reported to occur in aquatic plants (Dacey, 1981, 1987; Grosse et al, 1991; Armstrong and Armstrong, 1991) and in leaves (Day and Parkinson, 1979). There is also some indication that temperature-driven (Corey and Tan, 1990) and pressure-driven (Jolliffe and Dyck, 1988) bulk gas flow can be induced in fruit tissues. The main objective of this study was to determine whether flow of CO_2 could be enhanced by varying the atmospheric pressure surrounding bulky tissues. Since temperature and O_2 concentration affect respiration rate, a secondary objective was to determine the effect of other environmental factors on the expression of the response to atmospheric pressure. Longer term exposures to varying atmospheric pressures (3 days to 3 weeks) were used in an attempt to determine the effect of pressure-driven gas flow on water loss and changes in volatile aroma compounds. The vegetables used in this study were deliberately chosen because of their widely diverse morphological types and internal matrices. The major findings and accomplishments of the current study were:

1. The acquisition of new information regarding mass gas flow in bulky tissues in an environment of fluctuating external gas pressures. Small changes in atmospheric gas pressure induced large increases in CO_2 emissions in those plant organs possessing relatively low resistance to gas exchange (onions and sweet peppers). Following pressure treatments, net CO_2 efflux rates returned to the same rate, or slightly lower than pretreatment values, thus there was no evidence of enhanced metabolic rate due to treatment. Increases in the level of

pressure variations resulted in a corresponding increase in CO_2 efflux rate which is in agreement with Darcy's law for fluid flow through a porous media (Nobel, 1983). Gas pressure variations had the greatest effect on net CO_2 efflux rate when sufficient time was allowed between treatments for maximum gas flow along the gas transport pathways. It was evident that due to their anatomical structure, sweet peppers could exchange gases at a very rapid rate and were unaffected by time lag between the series of gas pressure pulses (Fig 1.6).

In an oxygen limiting environment the magnitude of the response to variable gas pressure was diminished for onions, but net CO_2 efflux rates of pressure-treated organs still exceeded that of onions held at constant pressure. The lack of response to variable pressure treatments by cucumbers and tomatoes is consistent with available information on the barriers to diffusion in these organs.

Fluctuating gas pressure storage may have an application as a pretreatment or intermittent treatment for bulky organs in which diffusive gas flow does not satisfy the potential of those tissues to emit respiration gases. Small external pressure variations may potentially be used to establish optimum internal gas concentrations in produce placed in CA storage or to remove harmful buildup of CO_2 during rewarming of plant tissue.

2. The development of a purge and trap sampling method for the collection and concentration of volatile vegetable aroma compounds. This method was inexpensive, rapid and easy to perform. Activated charcoal captures a broad range of organic compounds which, when eluted with a polar solvent such as dichloromethane, yield definitive chromatograms with little peak tailing. At the time of developing the method, use of a charcoal trap technique had not been reported in studies of vegetable aroma compounds. The main advantage of the

new technique, compared to steam distillation and solvent extraction, is the collection of trace volatiles with a minimum of degradation and interference from other compounds.

3. The novel use of multicomponent analysis to determine differences in aroma profiles of onions, sweet pepper and tomatoes subjected to variable pressure treatments. Using a trained panel to discriminate between experimental treatments may not always be practical or yield results that are easily interpreted. Compared to other statistical methods, multicomponent analysis of chromatographic results provided an improved technique for segregating vegetable responses to storage treatments.

4. The design and construction of a device to vary the atmospheric pressure surrounding the test material while maintaining a constant temperature. Pressure was cycled inside a vessel for a predetermined length of time before returning to constant conditions. The period of the pressure cycle could be varied as desired. Carbon dioxide measurements were simplified by computer-controlled stream sampling of respiration gases, which contributed to the accuracy of the gas measurements by gas chromatography.

Further experiments to substantiate the feasibility of using variable atmospheric pressures in storage should include: measurement of the response to variable pressures at high O_2 atmospheres and ambient O_2 concentrations in order to ascertain any metabolic effects due to a supra-optimal flow of O_2, measurement of internal atmospheric compositions of different vegetables following variable pressure treatments (although this is difficult), and long-term storage for several weeks or months depending on the vegetable type.

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Appendix 1. Contrast statements used in analysis of variance.

contrast 'temp(lin)' temperature -1 0 1

contrast 'temp (quad)' temperature 1 -2 1

contrast 'P*T(lin)' temperature*pressure -1 0 1 1 0 -1

contrast 'P*T(quad)' temperature*pressure -1 -2 1 -1 2 -1

contrast 'D*T(lin)' temperature*duration -1 0 1 1 0 -1

contrast 'D*T(quad)' temperature*duration 1 -2 1 -1 2 -1

contrast 'P*D*T(lin)' temperature*pressure*duration -1 0 1 1 0 -1 1 0 -1 -1 0 1

contrast 'P*D*T(quad)' temperature*pressure*duration 1 -2 1 -1 2 -1 -1 2 -1 1 -2 1

Appendix 2. Mean net carbon dioxide emission rates in onions, cucumbers, sweet peppers and tomatoes stored under variable and constant atmospheric pressure.

Table 1.4. Effect of temperature, level of pressure variation and DCAP on net carbon dioxide efflux rate ($\mu L\ kg^{-1}\ s^{-1}$) of onion during constant and variable pressure treatments.

			Variable	Constant
°C	kPa[1]	DCAP[2]	Mean[a] SD	Mean SD
0	3.5	5	0.43 ± 0.10	0.33 ± 0.09
0	3.5	15	1.69 ± 0.85	0.22 ± 0.01
0	6.9	5	0.68 ± 0.16	0.26 ± 0.09
0	6.9	15	3.56 ± 0.75	0.25 ± 0.05
10	3.5	5	0.88 ± 0.28	0.50 ± 0.18
10	3.5	15	3.18 ± 0.69	0.36 ± 0.06
10	6.9	5	1.82 ± 0.23	0.44 ± 0.15
10	6.9	15	5.49 ± 1.10	0.29 ± 0.06
20	3.5	5	1.80 ± 1.19	0.94 ± 0.59
20	3.5	15	5.57 ± 1.09	0.75 ± 0.12
20	6.9	5	3.74 ± 0.73	1.45 ± 0.31
20	6.9	15	8.91 ± 1.61	0.78 ± 0.06

[a] n = 3

[1] Increase in pressure above ambient atmospheric pressure.

[2] Duration of the interval of constant atmospheric pressure between 5 min variable pressure treatments.

Table 1.5. Effect of level of pressure variation, temperature and oxygen concentration on net carbon dioxide efflux rate ($\mu L\ kg^{-1}\ s^{-1}$) of onion during constant and variable pressure treatments.

			Variable	Constant
°C	kPa[1]	O_2(%)	Mean[a] SD	Mean SD
0	3.5	1	0.76 ± 0.29	0.35 ± 0.11
0	3.5	21	1.70 ± 0.85	0.22 ± 0.01
0	6.9	1	0.78 ± 0.36	0.32 ± 0.04
0	6.9	21	3.56 ± 0.75	0.25 ± 0.05
10	3.5	1	0.64 ± 0.22	0.41 ± 0.11
10	3.5	21	3.18 ± 0.69	0.36 ± 0.06
10	6.9	1	0.83 ± 0.14	0.37 ± 0.11
10	6.9	21	5.49 ± 1.10	0.29 ± 0.06
20	3.5	1	0.80 ± 0.22	0.54 ± 0.15
20	3.5	21	5.57 ± 1.09	0.75 ± 0.12
20	6.9	1	1.12 ± 0.23	0.52 ± 0.07
20	6.9	21	8.91 ± 1.61	0.78 ± 0.06

[a] n = 3

[1] Increase in pressure above ambient atmospheric pressure.

Table 1.6. Effect of temperature, level of pressure variation and DCAP on net carbon dioxide efflux rate ($\mu L\ kg^{-1}\ s^{-1}$) of cucumber during constant and variable pressure treatments.

			Variable	Constant
°C	kPa[1]	DCAP[2]	Mean[a] SD	Mean SD
10	3.5	5	3.67 ± 0.82	3.89 ± 1.21
10	3.5	15	3.84 ± 0.08	3.43 ± 0.51
10	6.9	5	3.65 ± 1.82	3.86 ± 1.98
10	6.9	15	4.30 ± 0.13	3.69 ± 0.58
20	3.5	5	5.75 ± 2.18	7.00 ± 1.24
20	3.5	15	8.58 ± 0.10	8.13 ± 0.25
20	6.9	5	7.00 ± 2.52	7.50 ± 2.05
20	6.9	15	8.76 ± 0.69	8.48 ± 0.97

[a] n = 3

[1] Increase in pressure above ambient atmospheric pressure.

[2] Duration of the interval of constant atmospheric pressure between 5 min variable pressure treatments.

Table 1.7. Effect of temperature, level of presssure variation and oxygen concentration on net carbon dioxide efflux rate ($\mu L\ kg^{-1}\ s^{-1}$) of cucumber during constant and variable pressure treatments.

			Variable	Constant
°C	kPa[1]	O_2(%)	Mean[a] SD	Mean SD
10	3.5	3	3.25 ± 1.45	2.93 ± 1.37
10	3.5	21	3.84 ± 0.08	3.43 ± 0.51
10	6.9	3	3.09 ± 1.33	2.73 ± 1.23
10	6.9	21	4.30 ± 0.13	3.69 ± 0.58
20	3.5	3	5.31 ± 2.18	4.55 ± 2.03
20	3.5	21	8.58 ± 0.10	8.13 ± 0.25
20	6.9	3	6.77 ± 1.95	6.11 ± 1.44
20	6.9	21	8.76 ± 0.69	8.48 ± 0.97

[a] n = 3

[1] Increase in pressure above ambient atmospheric pressure.

Table 1.8. Effect of temperature, level of pressure variation and DCAP on net carbon dioxide efflux rate ($\mu L\ kg^{-1}\ s^{-1}$) of sweet pepper during constant and variable pressure treatments.

			Variable	Constant
°C	kPa[1]	DCAP[2]	Mean[a] SD	Mean SD
10	3.5	5	3.16 ± 1.54	1.59 ± 0.89
10	3.5	15	2.69 ± 0.73	1.05 ± 0.26
10	6.9	5	5.48 ± 1.35	1.91 ± 0.46
10	6.9	15	4.97 ± 0.94	0.97 ± 0.38
20	3.5	5	8.97 ± 1.85	3.64 ± 1.01
20	3.5	15	10.36 ± 2.15	4.63 ± 1.09
20	6.9	5	14.25 ± 5.75	4.13 ± 0.99
20	6.9	15	13.04 ± 1.89	4.64 ± 0.95

[a] n = 3

[1] Increase in pressure above ambient atmospheric pressure.

[2] Duration of the interval of constant atmospheric pressure between 5 min variable pressure treatments.

Table 1.9. Effect of temperature, level of pressure variation and oxygen concentration on net carbon dioxide efflux rate ($\mu L\ kg^{-1}\ s^{-1}$) of sweet pepper during constant and variable pressure treatments.

			Variable	Constant
°C	kPa[1]	O_2(%)	Mean[a] SD	Mean SD
10	3.5	3	3.36 ± 0.62	1.30 ± 0.45
10	3.5	21	2.69 ± 0.74	1.05 ± 0.26
10	6.9	3	5.34 ± 3.17	1.34 ± 0.85
10	6.9	21	4.97 ± 0.94	0.97 ± 0.38
20	3.5	3	8.68 ± 2.06	4.06 ± 1.16
20	3.5	21	10.36 ± 2.15	4.63 ± 1.09
20	6.9	3	12.78 ± 1.75	3.91 ± 0.90
20	6.9	21	13.04 ± 1.19	4.64 ± 0.95

[a] n = 3

[1] Increase in pressure above ambient atmospheric pressure.

Table 1.10. Effect of temperature, level of pressure variation and DCAP on net carbon dioxide efflux rate ($\mu L\ kg^{-1}\ s^{-1}$) of tomato during constant and variable pressure treatments.

			Variable	Constant
°C	kPa[1]	DCAP[2]	Mean[a] SD	Mean SD
10	3.5	5	1.43 ± 0.54	1.66 ± 0.16
10	3.5	15	1.70 ± 0.54	1.79 ± 0.66
10	6.9	5	1.58 ± 0.02	1.49 ± 0.14
10	6.9	15	2.09 ± 0.61	1.53 ± 0.35
20	3.5	5	3.82 ± 0.39	2.59 ± 0.17
20	3.5	15	3.80 ± 0.21	4.27 ± 0.54
20	6.9	5	3.40 ± 0.57	3.85 ± 0.61
20	6.9	15	4.48 ± 0.56	4.64 ± 0.58

[a] n = 3

[1] Increase in pressure above ambient atmospheric pressure.

[2] Duration of the interval of constant atmospheric pressure between 5 min variable pressure treatments.

Table 1.11. Effect of temperature, level of pressure variation and oxygen concentration on net carbon dioxide efflux rate ($\mu L\ kg^{-1}\ s^{-1}$) of tomato during constant and variable pressure treatments.

			Variable	Constant
°C	kPa[1]	O_2 (%)	Mean[a] SD	Mean SD
10	3.5	3	2.34 ± 0.34	2.28 ± 0.43
10	3.5	21	1.70 ± 0.54	1.79 ± 0.66
10	6.9	3	2.16 ± 0.34	1.90 ± 0.52
10	6.9	21	2.09 ± 0.61	1.53 ± 0.35
20	3.5	3	3.46 ± 1.23	3.35 ± 1.23
20	3.5	21	3.80 ± 0.21	4.27 ± 0.54
20	6.9	3	3.77 ± 1.19	2.89 ± 0.45
20	6.9	21	4.48 ± 0.56	4.64 ± 0.58

[a] n = 3

[1] Increase in pressure above ambient atmospheric pressure.

Appendix 3. Total ion chromatograms of volatiles extracted from onion, tomato and sweet pepper stored under variable and constant atmospheric pressure.

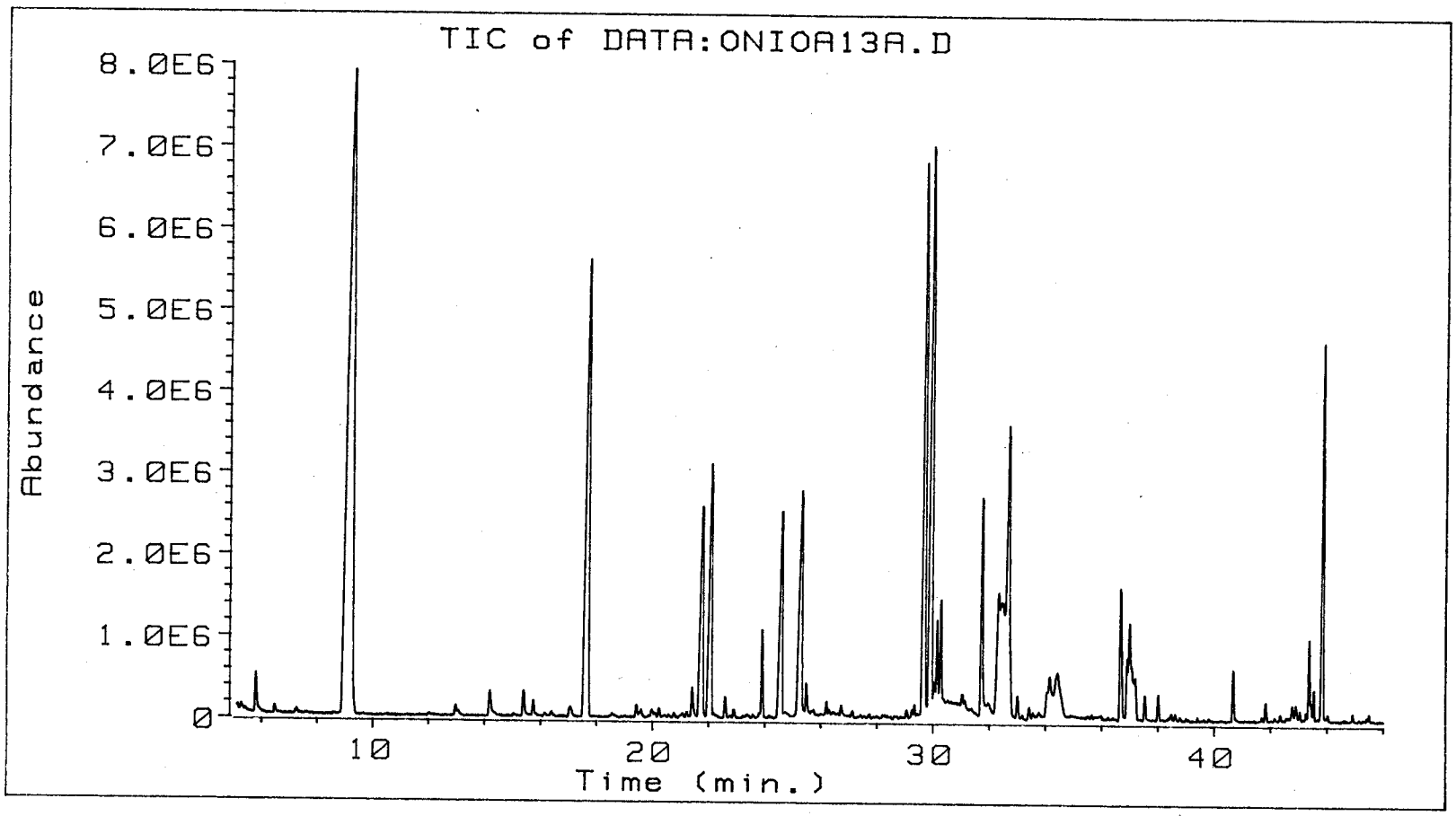

Fig. 2.20. Total ion chromatogram of volatiles extracted from onion stored under constant pressure at 15°C for 14 days.

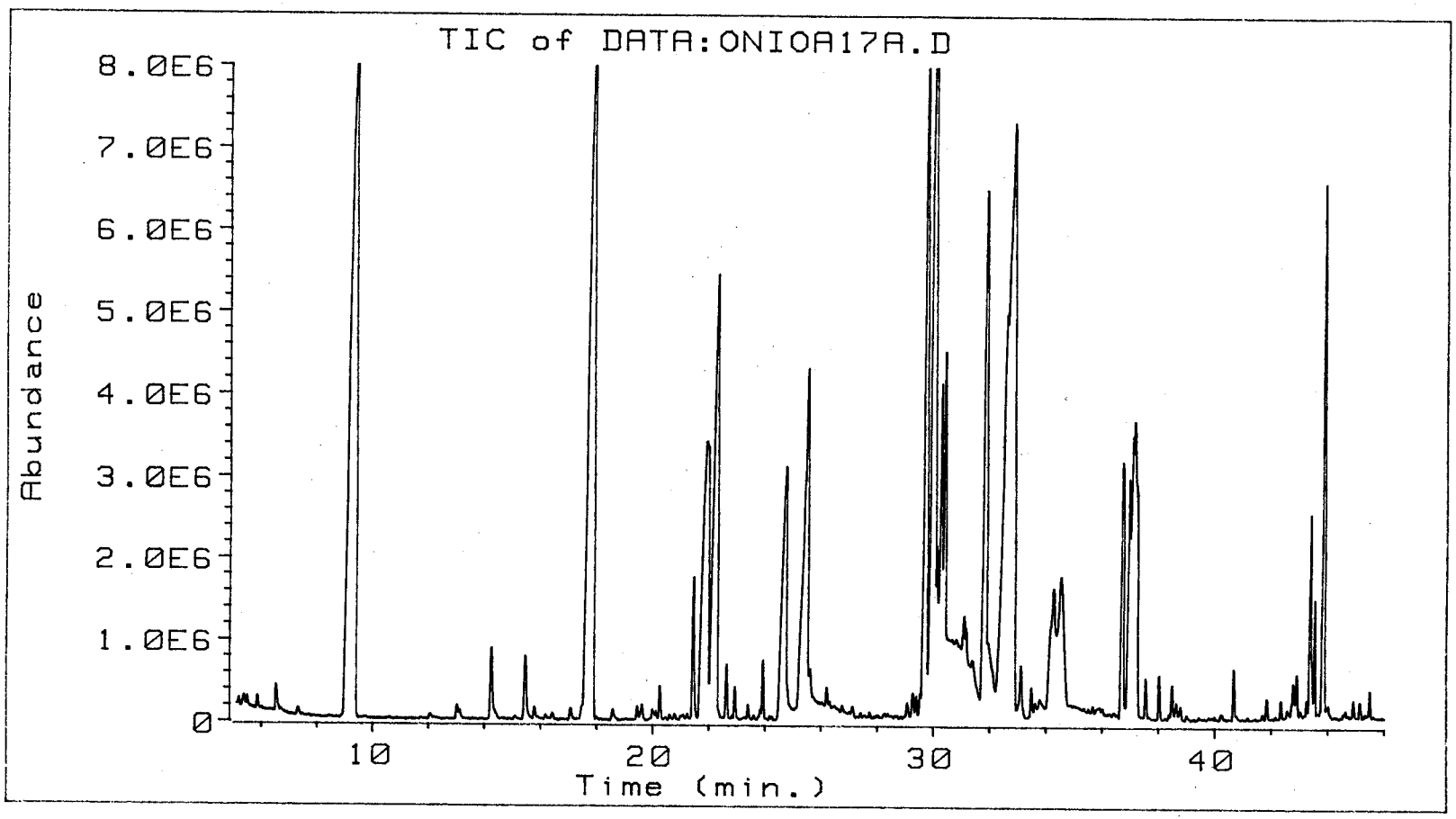

Fig. 2.21. Total ion chromatogram of volatiles extracted from onion stored under constant pressure at 15°C for 21 days.

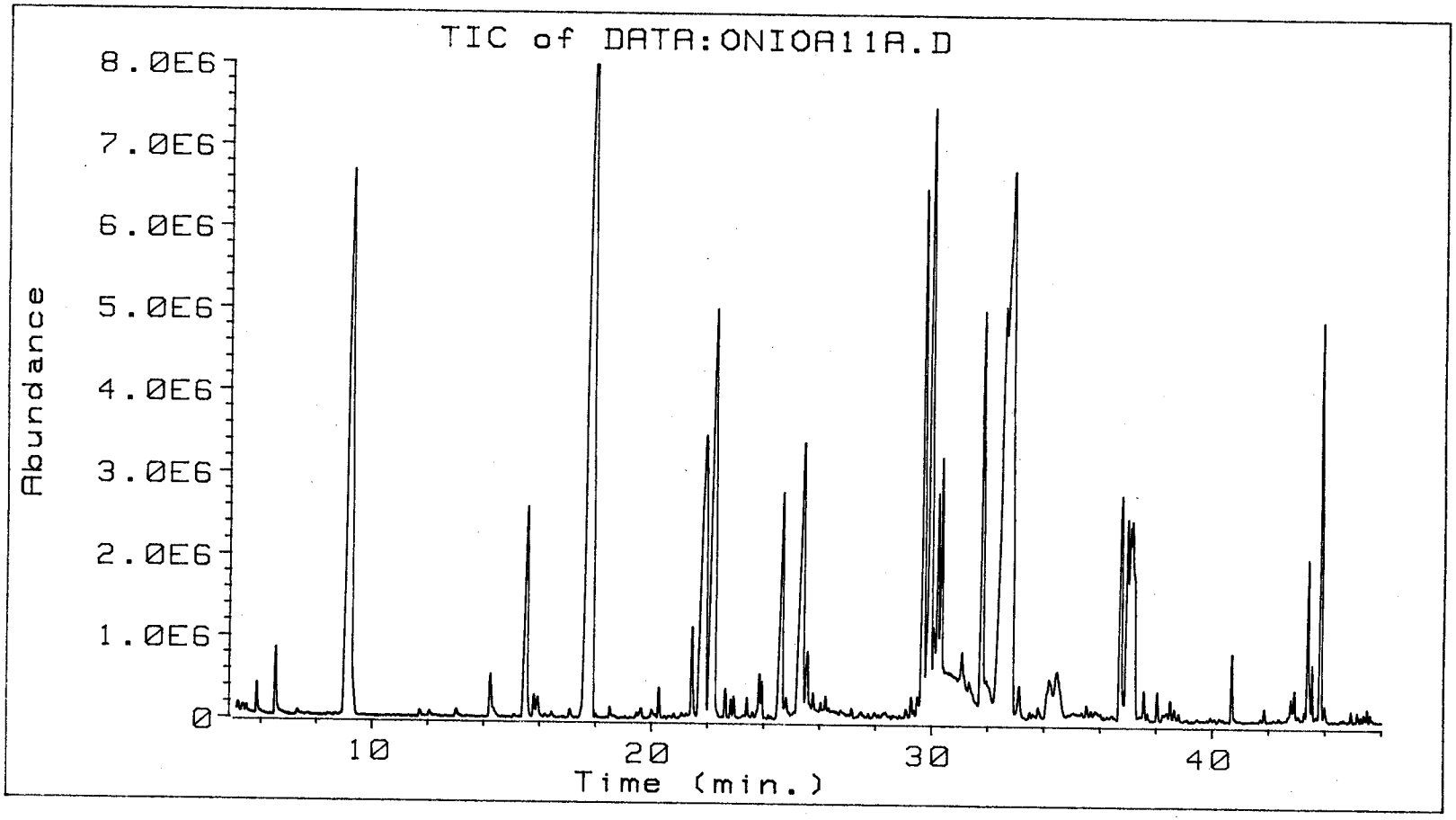

Fig. 2.22. Total ion chromatogram of volatiles extracted from onion stored under variable pressure at 15°C for 7 days.

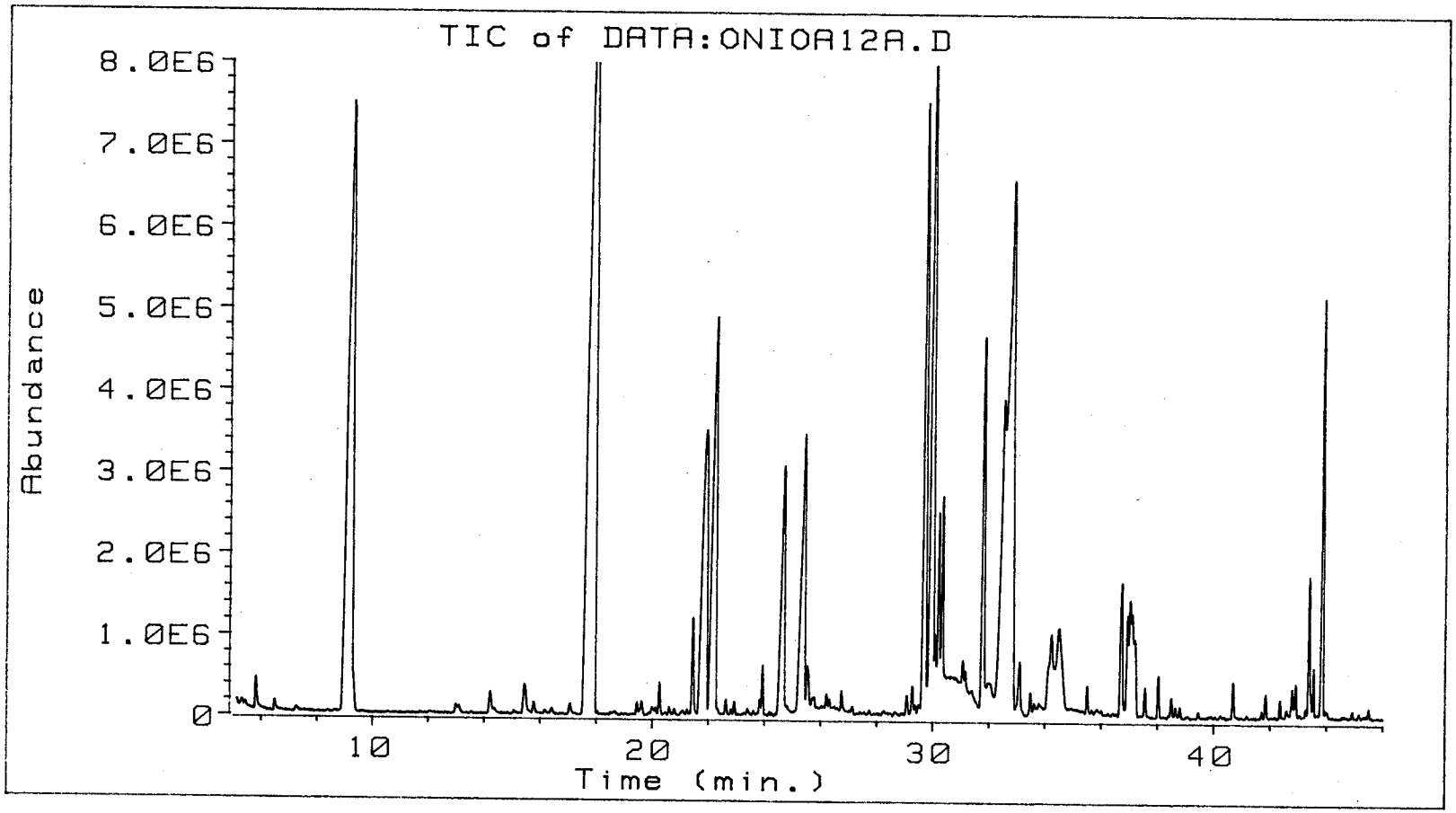

Fig. 2.23. Total ion chromatogram of volatiles extracted from onion stored under variable pressure at 15°C for 14 days.

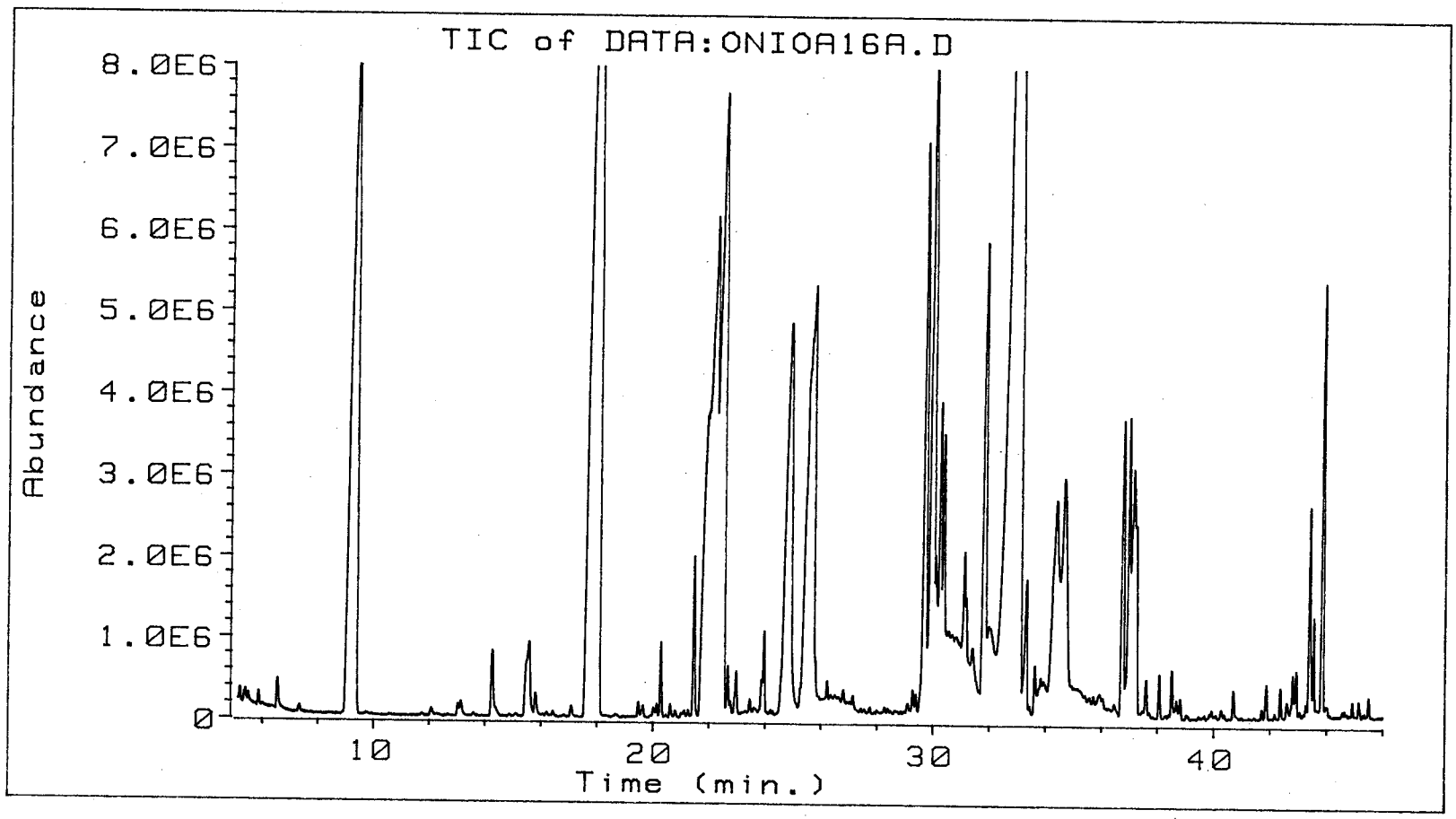

Fig. 2.24. Total ion chromatogram of volatiles extracted from onion stored under variable pressure at 15°C for 21 days.

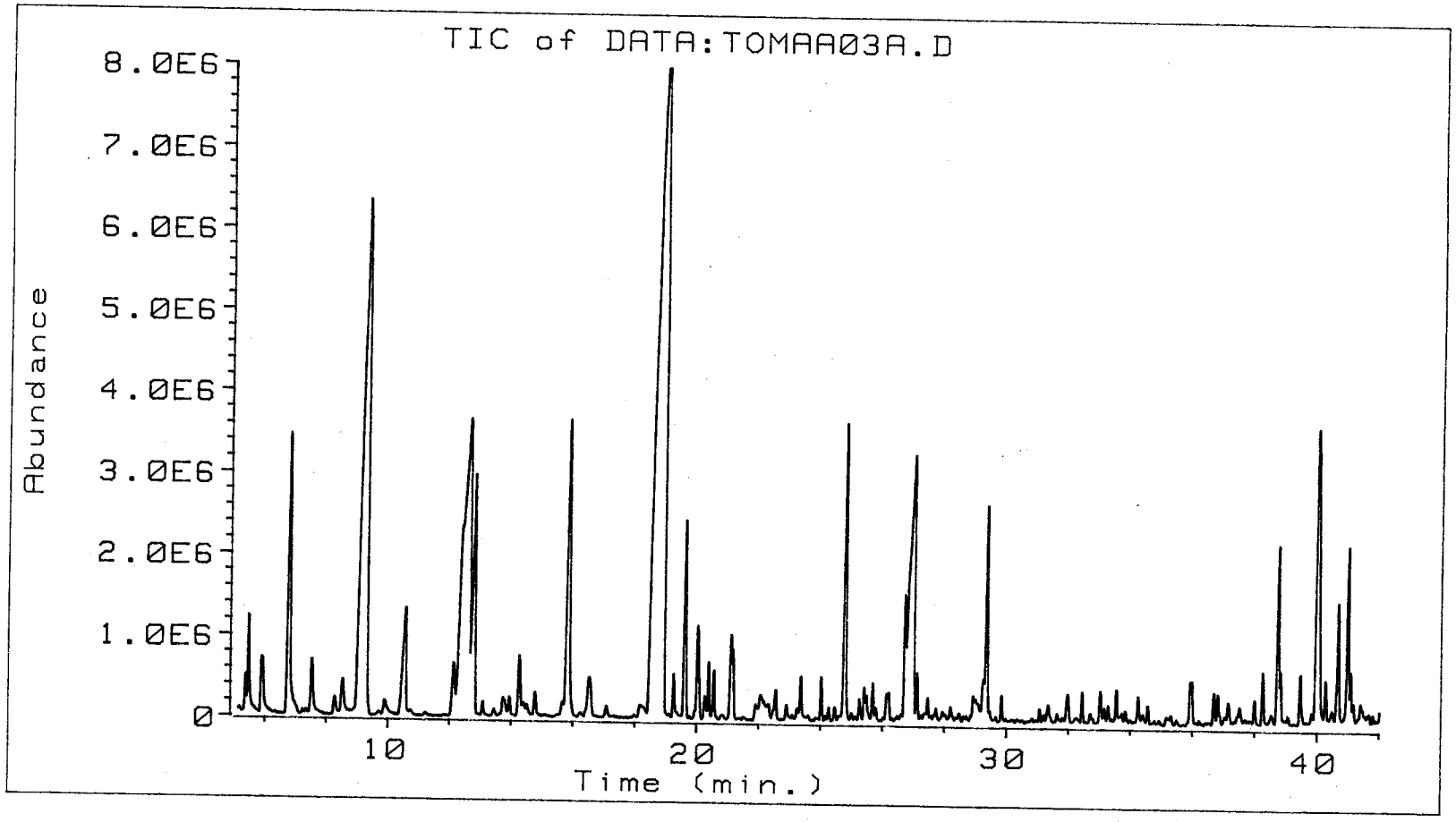

Fig. 2.25. Total ion chromatogram of volatiles extracted from tomato stored under constant pressure at 15°C for 7 days.

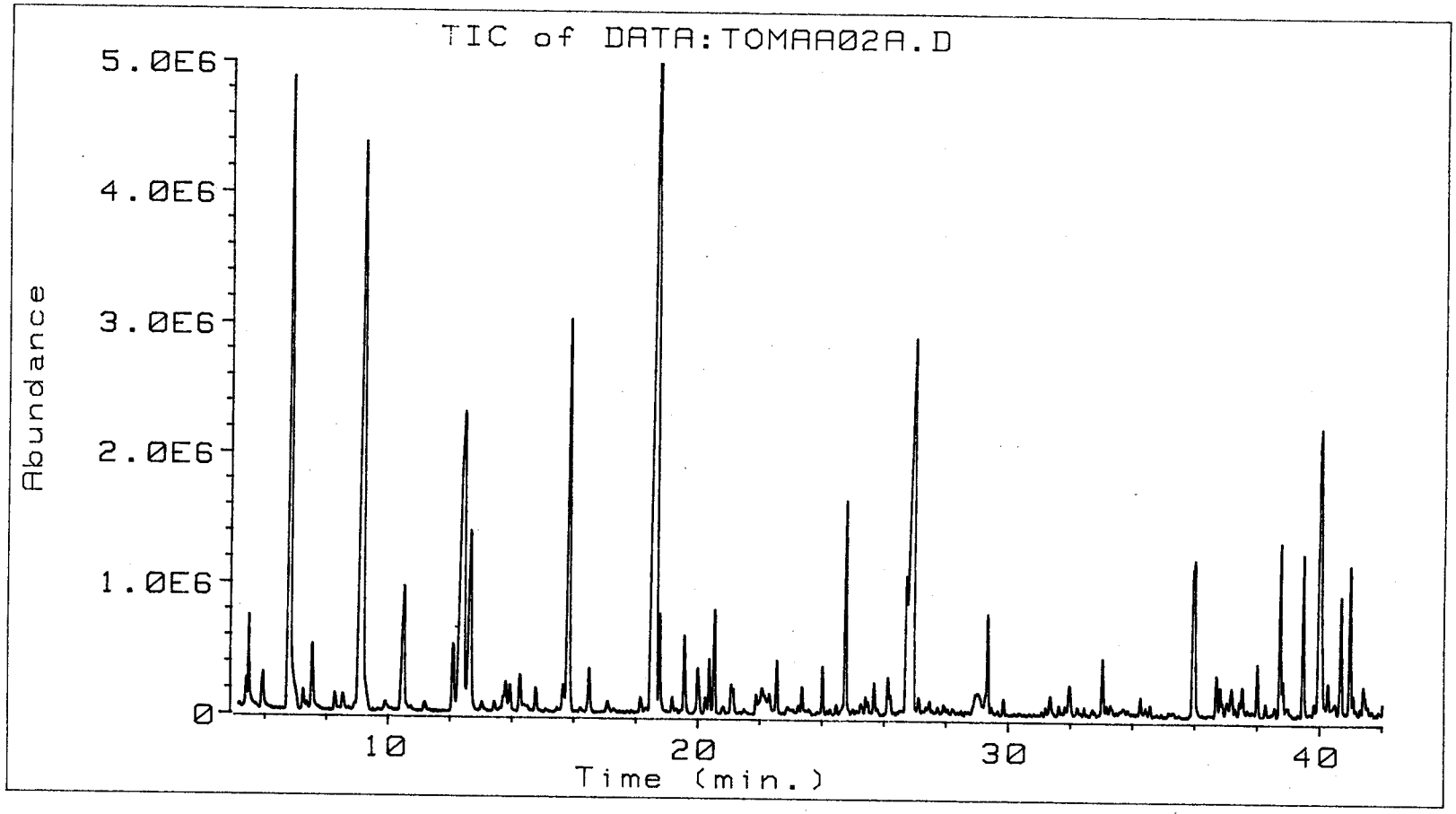

Fig. 2.26. Total ion chromatogram of volatiles extracted from tomato stored under variable pressure at 15°C for 3 days.

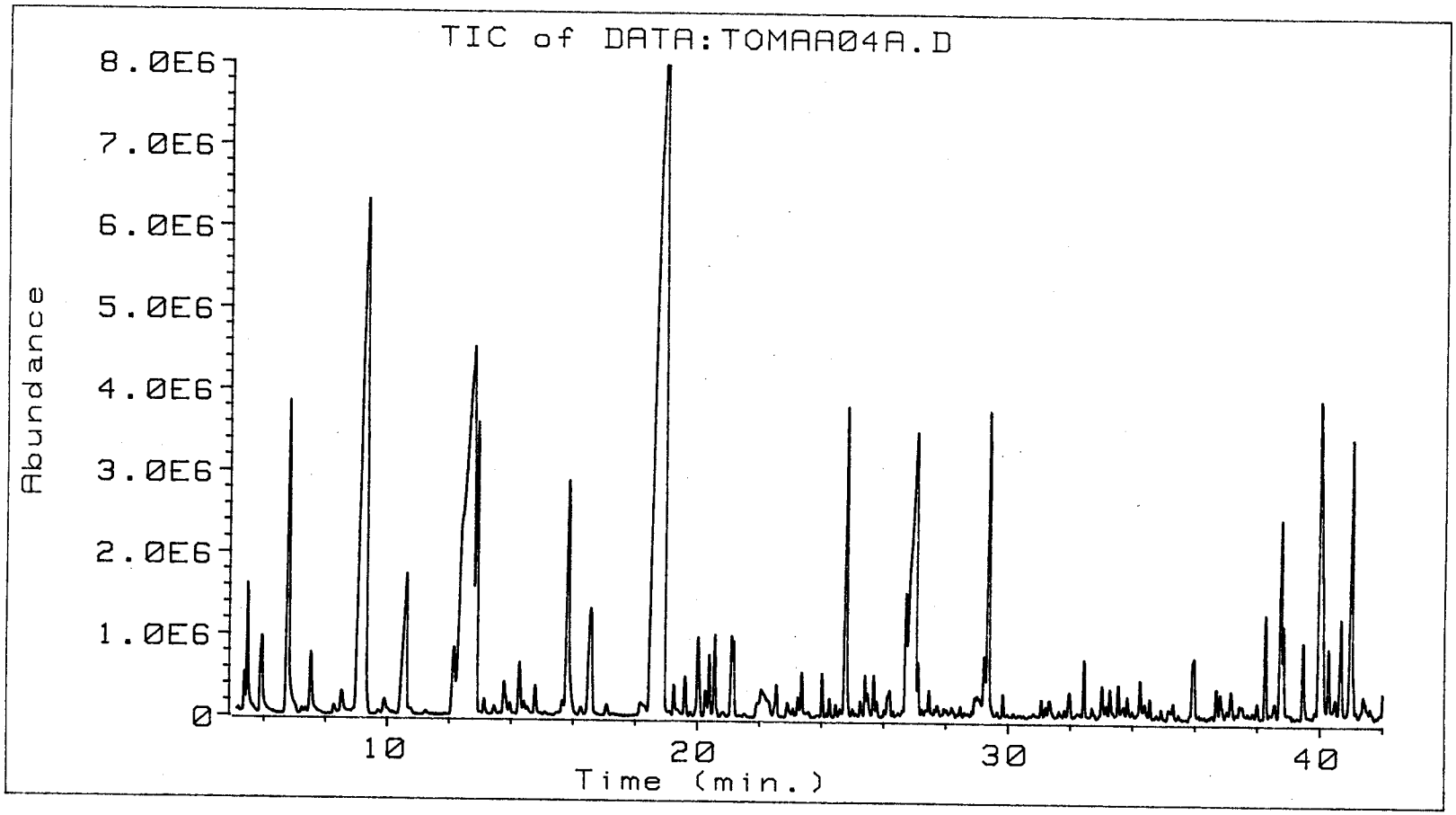

Fig. 2.27. Total ion chromatogram of volatiles extracted from tomato stored under variable pressure at 15°C for 7 days.

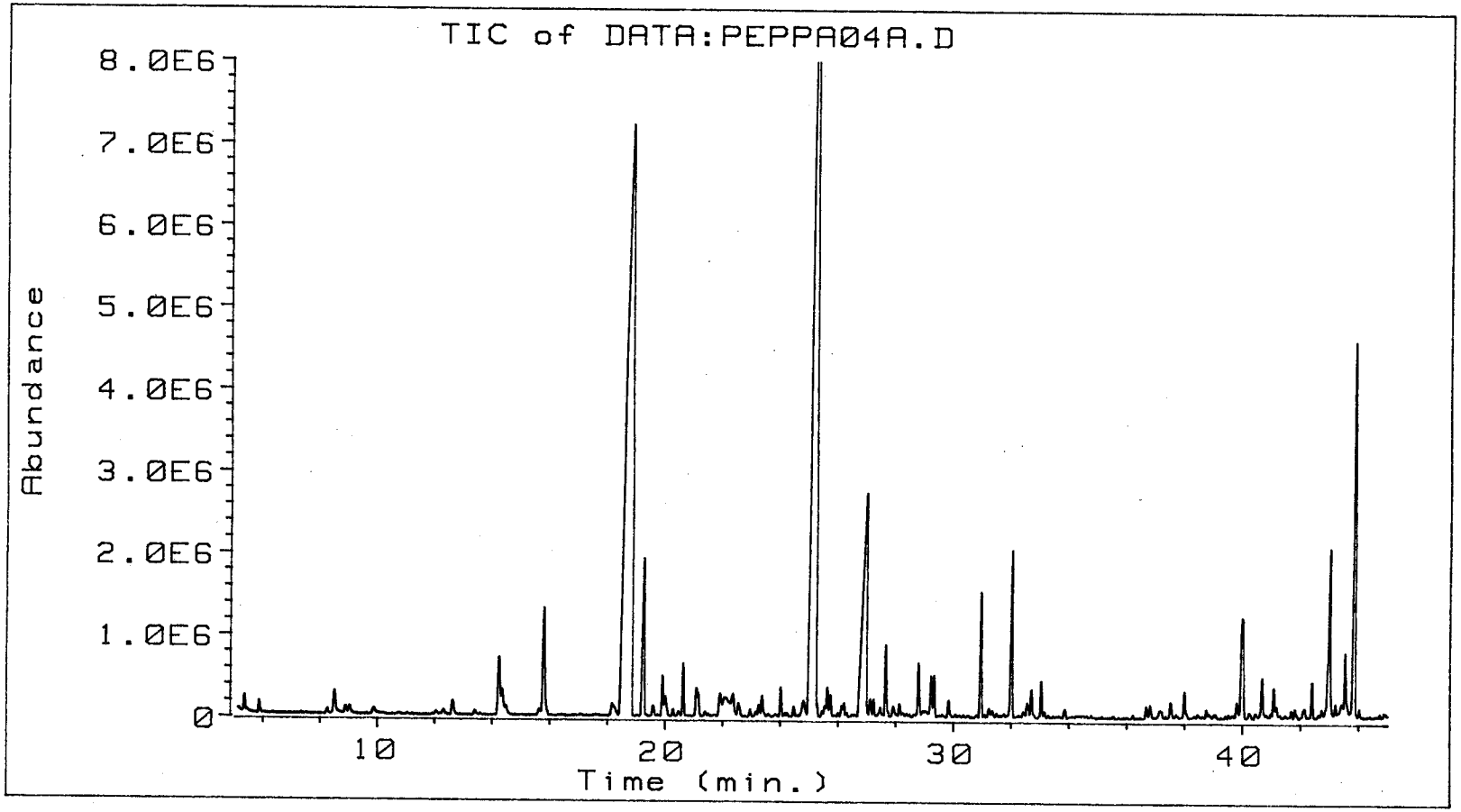

Fig. 2.28. Total ion chromatogram of volatiles extracted from sweet pepper stored under constant pressure at 15° C for 7 days.

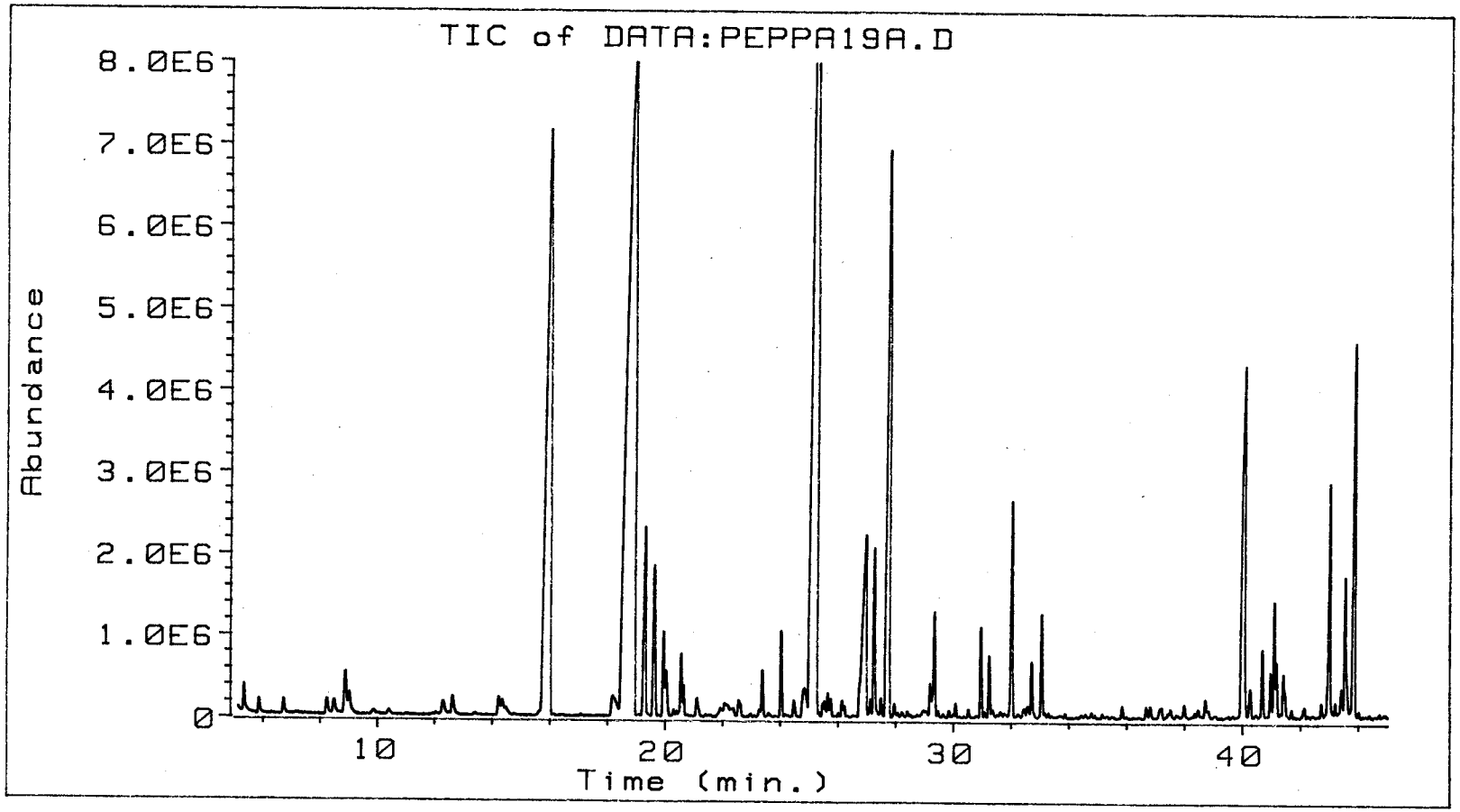

Fig. 2.29. Total ion chromatogram of volatiles extracted from sweet pepper stored under constant pressure at 15° C for 14 days.

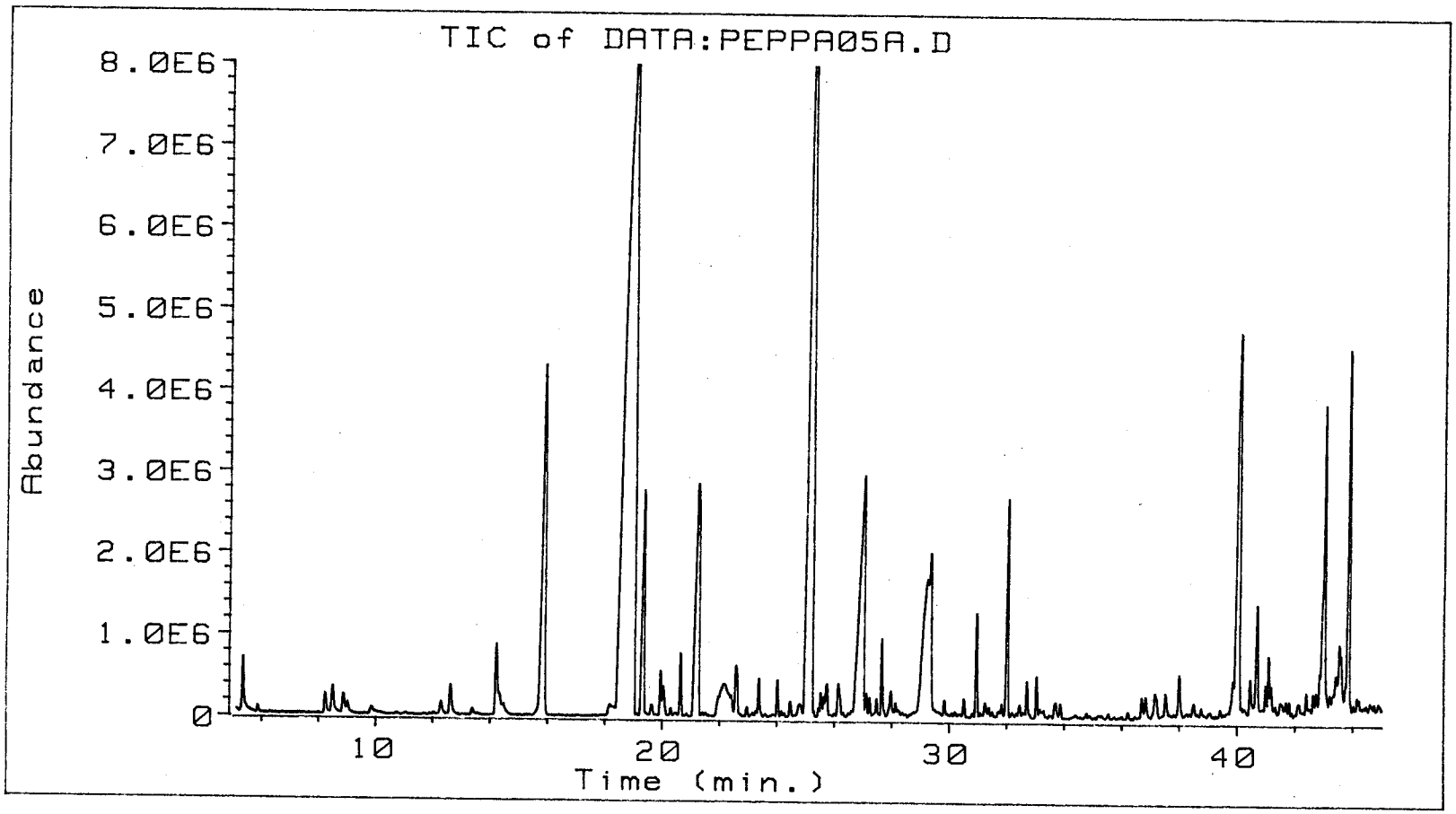

Fig. 2.30. Total ion chromatogram of volatiles extracted from sweet pepper stored under variable pressure at 15°C for 3 days.

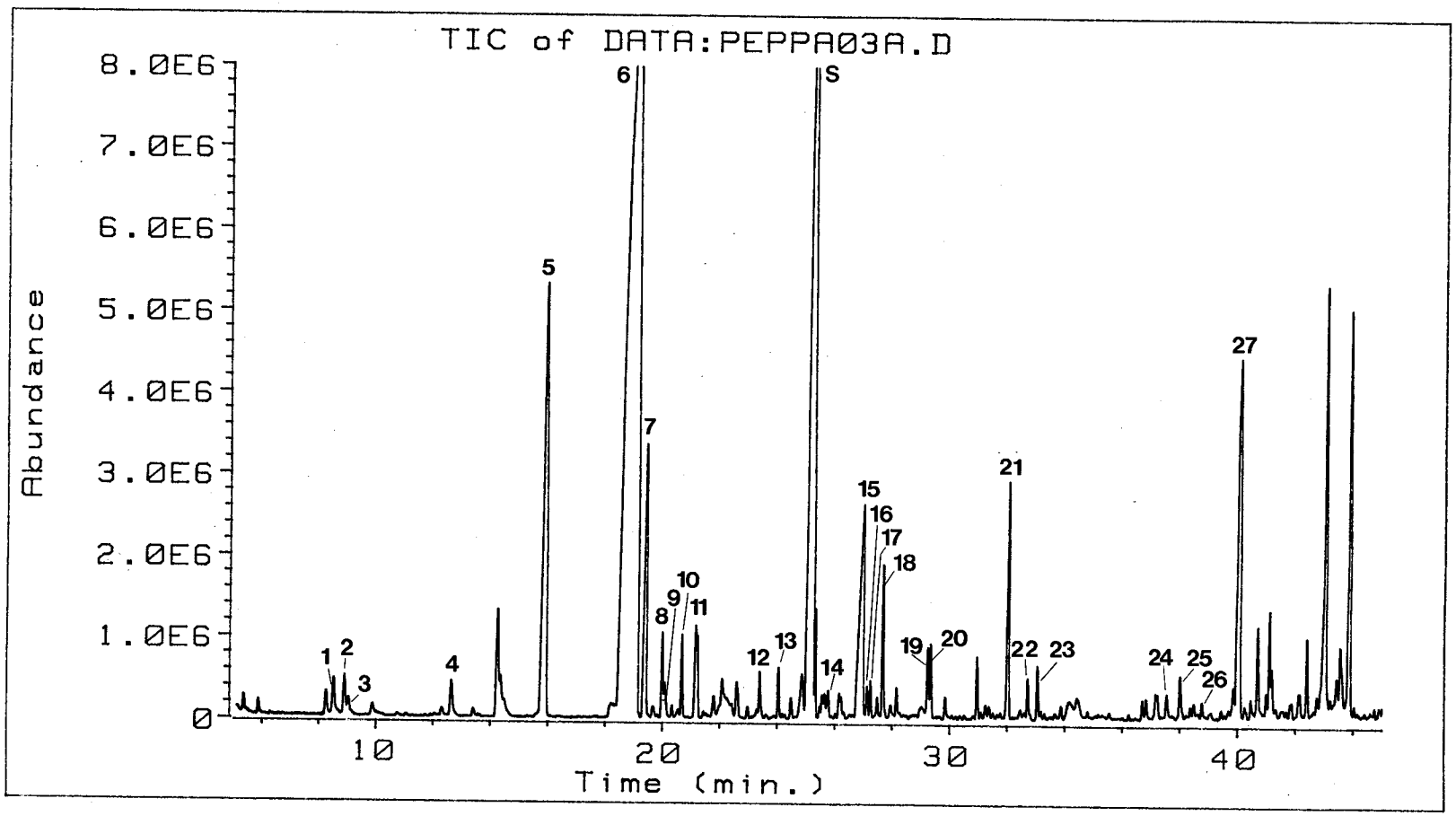

Fig. 2.31. Total ion chromatogram of volatiles extracted from sweet pepper stored under variable pressure at 15°C for 7 days.

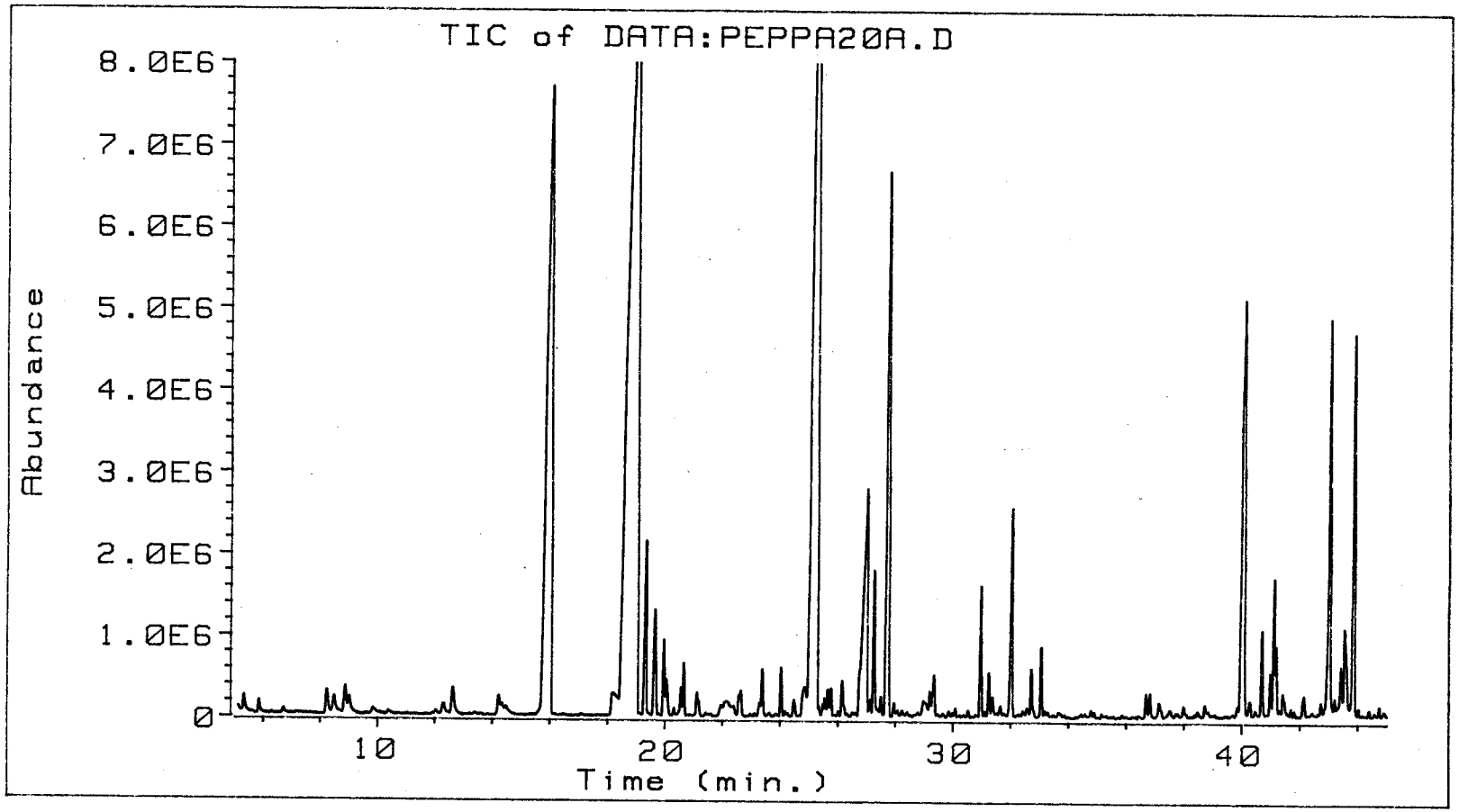

Fig. 2.32. Total ion chromatogram of volatiles extracted from sweet pepper stored under variable pressure at 15°C for 14 days.