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EFFECTS OF CHEMICAL TREATMENT ON CUCUMBER LIPIDS AND FLAVOR VOLATILES DURING COLD STORAGE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the degree Doctor of Philosophy in the Graduate School of The Ohio State University

By
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*****

The Ohio State University
1984

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Chapter I
INTRODUCTION

Low temperature is generally used to extend the post-harvest life of fresh fruits and vegetables. Many crops, however, are susceptible to damage on exposure to cold temperatures above freezing. This injury is usually referred to as chilling injury (CI). It is also called low temperature breakdown, chilling damage, or chilling disorder. Chilling injury can occur in the field, during transit, at the market and in the home refrigerator.

Chilling injury is often encountered with tropical or subtropical crops although some temperate fruits are also affected by low temperature. Sensitivity varies among species and on the condition and stage of development during harvest. For a particular commodity there is a critical temperature below which injury occurs. The other factor which affects the extent of CI is time of exposure to chilling temperature. Some crops like banana suffer damage after exposure of several hours to chilling temperature. On the other hand a commodity
like grapefruit requires weeks of exposure before any damage becomes apparent.

A complete understanding of what constitutes low temperature resistance or injury is presently lacking. Adaptation to low temperature is believed to be accompanied by alterations in membrane fluidity and fatty acid composition (Lyons, 1973; Lyons et al., 1979). Studies have been made relating unsaturation of fatty acids with chilling resistance or sensitivity (Lyons and Asmundson, 1965; Wilson and Crawford, 1974; St. John and Christiansen, 1976; Patterson et al., 1978a). The primary effect of chilling on membrane structure is followed by secondary physiological changes which are eventually manifested as visible symptoms of CI.

In cucumber fruits (Cucumis sativus L.) CI occurs on subjecting fruits to temperatures below 10°C. The observed symptoms of injury are surface pitting, shriveling, browning, softening and accelerated decay.

Various approaches have been used to reduce or alleviate CI. Among these are cold conditioning, intermittent warming, controlled atmosphere storage, film packaging, genetic selection and chemical treatment. This study dealt specifically with the effects of two chemicals used to reduce CI in fruits. It focused on the extent of chilling damage, changes in lipid
composition on treatment and formation of flavor volatiles derived enzymatically from unsaturated fatty acids. Calcium chloride (CaCl₂) and butylatedhydroxytoluene (BHT) were the chemicals used for treatment in this study. The objectives of this study were:

1. To determine whether application of CaCl₂ and BHT reduce CI in cucumbers stored at 4.4 C and to compare the effects of CaCl₂ and BHT on CI.

2. To study the changes in lipid classes and fatty acid composition of cucumbers after chemical treatment and refrigerated storage.

3. To follow and examine the formation of flavor volatile compounds of cucumber fruits after chemical treatment and storage at 4.4 C.
Chapter II
REVIEW OF LITERATURE

The general topic of chilling injury (CI) has been reviewed by Lyons (1973), Bramlage (1982), Couey (1982) and Morris (1982). Damage due to low temperature occurs not only in crops during germination and growth but also in fruits and vegetables after harvest.

2.1 FACTORS AFFECTING CHILLING INJURY

The extent of chilling injury depends on the following factors: 1) temperature of exposure; 2) duration of exposure to chilling temperature; 3) physiological condition at the time of chilling; and 4) plant species. For each crop there is a temperature limit below which injury will occur and for most crops this is observed to be between 10-12 C (Lyons, 1973). Cucumber, eggplant, papaya and peach fruit suffer injury below 7 C while lime, muskmelon and pineapple have thresholds of 7-10 C (Wade, 1979). The critical temperature is 5 C for apples and oranges, 10-13 C for mango and 12-13 C for banana fruit (Lutz and Hardenburg, 1968). Crop plants like
cotton, corn, rice and cowpea have also been shown to be chilling sensitive (Sellschop and Salmon, 1928). The extent of injury is dependent not only on temperature but also on time of exposure. Bananas show symptoms of CI on exposure at 5 C for 24 hours (Fidler and North, 1970; Jones et al., 1978). In mangoes severe damage occurs when fruit is stored a few days below 13-15 C (Veloz et al., 1977). Papaya, on the other hand, can be stored 2-3 weeks at 10C with only mild symptoms of CI (Nazeeb and Broughton, 1978). Apples develop CI symptoms after storage for several months at 0-4 C (Lutz and Hardenberg, 1968). Maturity, nutrition and other growth conditions affect the severity of CI. The chilling sensitivity of avocados is high at the preclimacteric stage, increases to a maximum at the climacteric and decreases as the fruit ripens (Kosiyachinda and Young, 1976). It has been observed that dry seeds of a chilling sensitive crop can be stored for extended periods at chilling temperature without injury whereas seedlings are more susceptible to CI (Wheaton, 1963).

Calcium has been implicated in the susceptibility of avocados to CI (Chaplin and Scott, 1980). In individual fruits, sections having the lowest calcium levels were most susceptible to chilling. The symptoms of CI in apples have been observed to be cultivar-specific (Bramlage, 1982). For instance, 'McIntosh' develops
CI symptoms known as browncore while another variety, 'Yellow Newton', shows a type of CI called internal breakdown. Varietal differences in chilling sensitivity have also been reported in avocado (Couey, 1982). Bramlage (1982) believed that there are large differences in chilling sensitivity among cultivars of tropical and sub-tropical plants and that storage recommendations at the present time are not yet based on the requirements of the most sensitive cultivars.

2.2 SYMPTOMS OF CHILLING INJURY

The symptoms of CI have been described for almost all important crops which are chilling sensitive. Morris (1982) listed these general symptoms: surface lesions (pitting and discoloration), water soaking of tissues, internal discoloration (browning of pulp), breakdown of tissues, failure to ripen normally, accelerated senescence, increased susceptibility to decay, compositional changes and loss of growth or sprouting capacity.

2.2.1 Tropical and Subtropical Crops

Chilling injury in avocado is commonly characterized by a grayish-brown discoloration of the flesh (Couey, 1982) which may be accompanied by uneven ripening, development of undesirable flavors and odors, pitting
and a scald-like browning. Scald-like discoloration of the skin is also observed in mangoes as well as failure to ripen, poor flavor, pitting, non-uniform ripening and increased susceptibility to decay (Sadasivam et al., 1971). Bananas are characterized by slight darkening in mild cases of injury to dull yellow skin, browning, failure to ripen, loss of flavor and hardening of the central placenta in severe CI. CI symptoms in papaya are scalding and pitting, production of off-flavors and failure to ripen (Jones and Kubota, 1940; Chan et al., 1983). In herbaceous plants like cucumber and bean CI is characterized by water loss and wilting (Wright and Simon, 1973; Wright, 1974). The most common symptoms of CI in grapefruit are pits and depressions in the rind at 4-5 C and superficial brown staining at 0-1 C (Purvis, 1980; Couey, 1982). Cucumber fruits show surface pitting, wrinkling, softening of the mesocarp, browning and accelerated decay (Eaks and Morris, 1957; Tatsumi and Murata, 1978; Fukushima and Yamazaki, 1978; Wang and Adams, 1980).

2.2.2 Temperate Zone Crops

Chilling sensitivity is usually associated with crops of tropical or sub-tropical origin. However, some temperate zone crops are also chilling-sensitive (Lutz and Hardenburg, 1968). Among these are apples, asparagus,
cranberries, nectarines, peaches, plums and potatoes. CI in asparagus is indicated by discoloration and softening of the stem tips after 3-4 weeks at 0-3 C (Ryall and Lipton, 1979). Peaches and nectarines stored from 2-4 weeks at 0-5 C develop 'wooliness', a dry, mealy texture that accompanies loss of flavor and color (Ryall and Lipton, 1979). Apples develop certain symptoms after storage for several months at 0-4 C. Browncore, internal browning, soft scald and soggy breakdown are some of the afflictions associated with CI in apples (Ryall and Lipton, 1979). In cranberries after 4-8 weeks at 3 C, the flesh becomes rubbery instead of white and crisp. Potatoes show mahogany browning.

2.3 PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS

The physiological and biochemical consequences of exposure to cold temperature in plants have been summarized in a number of reports (Lyons, 1973; Lyons et al., 1979; Wade, 1979; Christiansen and St. John, 1981; Wang, 1982; McWilliam, 1983).

2.3.1 Primary Event

Most researchers are in agreement with the proposal of Lyons and Raison (1970) that the primary response to chilling in sensitive species is a physical phase transition from a flexible liquid-crystalline to solid-gel
structure. The basis of this postulate was provided by their observation that a change occurs in the slopes of Arrhenius plots of respiratory activity in mitochondria of chilling-sensitive tissues of tomato, cucumber and sweet potato. Electron-spin resonance (ESR) studies by Raison et al. (1971) using the spin-labeling technique showed that the temperature at which the change in the ordering of the membrane lipids is detected, coincides with that at which there is an increase in activation energy of respiratory enzymes in chilling-sensitive plants. This temperature-induced phase transition has been observed not only in mitochondrial membranes but also in glyoxysomes and proplastids (Wade et al., 1974). Studies by Murata et al. (1975) showed a relationship between photosynthetic parameters and phase transitions of membrane lipids. Cold and dark storage of leaves of different chilling-sensitive plants was observed to result in a loss of Hill-reaction activity accompanied by an increase in the percentage of free fatty acids particularly linolenic acid (Kaniuga and Michalski, 1978).

Other researchers have contended that changes in membrane activity at low temperature were not necessarily correlated with chilling sensitivity since an increase in Hill-activity at low temperature was observed both with chilling-sensitive and chilling-resistant plants.
(Nolan and Smillie, 1977). Using fluorescence technique, Pike and Berry (1980) studied the phase separation temperature of phospholipids in warm and cool climate plants. Warm climate annual grasses showed separation temperatures distinctly higher than those of cool climate grasses.

2.3.2 Secondary Events

The primary change in the physical phase of membranes could lead to a number of secondary responses. These are cessation of protoplasmic streaming, changes in membrane structure and function, alterations in respiratory rates and other metabolic activities and compositional changes.

2.3.2.1 Effect on Protoplasmic Streaming

One of the earliest observations made on the effect of Cl on tissues was cessation of protoplasmic streaming. It was noted as early as 1864 by Sachs. Lewis (1956a) studied protoplasmic streaming in plants sensitive and insensitive to chilling. It was observed that in sensitive species like tomato, watermelon, tobacco and sweet potato, streaming stopped after 1 to 2 minutes at 5 or 0 C. In chilling-resistant plants like radish or carrot, streaming proceeded at 0 or 2.5 C. Wheaton (1963) observed similar effects in root hairs of
cantaloupe, pepper, tomato and watermelon. However, the studies of Patterson and Graham (1977) showed no specific critical temperature below which streaming stopped in trichome cells of plants from different climates. With Lycopersicon hirsutum, a species of wild tomato collected at different altitudes, the rate of streaming at 5°C was greatest in varieties from the highest altitudes, suggesting that streaming rates correlated with genetic adaptation to low temperature.

2.3.2.2 Changes in Membrane Structure and Function

The effects of chilling on membranes involve either a change in ordering of membrane lipids and/or subsequent loss of membrane integrity. As discussed earlier, it is believed that membrane lipids undergo a transition from flexible liquid-crystalline structure to ordered solid-gel structure on exposure to low temperature. The maintenance of fluidity within the lipid bilayer is essential for membrane function. Transitions from liquid-crystalline to gel-phase can occur within the temperature range for plant growth depending on the head groups present and the unsaturation of acyl chains (Chapman, 1975). Mazliak (1979) noticed that lowering the environmental temperature produced a higher content of polyunsaturated fatty acids as result of an increase in fatty acid desaturase activity. Electron-spin
resonance (ESR) studies on mung bean showed two phase transitions of membrane lipids of mitochondria and chloroplasts at 15°C and 28°C (Raison and Chapman, 1976). However, studies using differential thermal analysis (DTA) and $^{13}$C nuclear magnetic resonance (NMR) did not coincide with the observations that growth temperature can be directly determined by the solid-fluid phase transition temperature of membrane lipids (McElhaney and Souza, 1976; Bishop et al., 1979). Lyons et al. (1979) postulated that ESR probes do not reflect phase transitions of bulk lipids but rather some change in molecular order of discrete regions of the membrane.

Loss of membrane integrity can result in increased permeability and leakage of solutes and electrolytes. Exposure of cotton radicles to chilling at 5°C increased the loss of solutes. With increasing time of exposure to chilling temperature, there was a corresponding increase in the amounts of sugars and amino acids exuded (Christiansen et al., 1970). A recent study by Quadir (1983) showed an increase in the release of ninhydrin-reactive substances on exposure of cucumber seedlings to chilling temperature. In an earlier study with cucumber fruits a higher quantity of leakage substances was observed in fruits chilled at 0°C than at 5°C (Fukushima and Yamazaki, 1978). Work on CI in sweet potatoes (Uritani and Yamaki, 1969) indicated
a higher quantity of lipid phosphorous and acid-insoluble nitrogen in mitochondria of chilled tissues.

The effects of chilling temperature generally become more apparent if samples are brought to room temperature after chilling treatment. Furmanski and Buescher (1979) observed that electrolyte leakage in peach fruits remained constant during chilling and increased when fruits were transferred to 21 C. Eggplants, however, showed an increase in potassium ion leakage at 1 C and no change during storage at 20 C (Abe and Ogata, 1978). In mitochondria of sweet potatoes increased ion leakage, especially of potassium ions, was observed before changes in oxidative activity occurred (Lieberman et al., 1958). The experiments of Tatsumi and Murata (1978) showed that leakage of potassium ions in cucumber discs increased rapidly after chilling whereas unchilled discs remained at a constant level. From Arrhenius graphs of the rates of potassium ion leakage, the critical point of chilling was determined to be around 7 C. Similarly, Andersen and Kent (1983) measured increased ion and amino acid leakage in cucumber fruits after 6-8 days of chilling. This elevation in ion leakage has also been observed in cucumber hypocotyls (Sasson and Bramlage, 1978). Other researchers, on the other hand, found no increase in electrolyte leakage during chilling in vegetables like bell peppers and tomatoes (Murata and Tatsumi,
2.3.2.3 Effects on Metabolic Processes

Change in Respiratory Activity:

An increase in respiratory activity has been reported upon chilling of sensitive crops like cucumber (Eaks and Morris, 1956; Tatsumi and Murata, 1978), tomatoes (Lewis 1956a) and sweet potatoes (Lewis and Morris, 1956). Other researchers observed unusually high respiratory rates in avocados (Eaks, 1976) and tomatoes (Crooks and Ludford, 1983) after transfer to room temperature from a chilling treatment. Depending on the duration of exposure to chilling, respiration rates could return to normal levels after several days at room temperature (Eaks and Morris, 1956; Lewis, 1956; Watada and Morris, 1966; Eaks, 1980). This indicated that damage on brief exposure to low temperature can be reversible.

Changes in Enzyme Activity:

Research in this area has been done mainly on membrane-bound enzymes. Many investigators have reported a change in activation energy upon chilling of succinate oxidase (Lyons and Raison, 1970; Raison and Chapman, 1976; Kane et al., 1978). Duke (1977) studied soybean dehydrogenases and observed differences in Arrhenius graphs of enzymes at different temperatures. Invertase
activity in mangoes has been found to increase while that of amylase decreased on chilling (Chhatpar et al., 1971).

**Stimulation of Ethylene Production:**

Chilling has been reported to stimulate ethylene production in cucumbers (Wang and Adams, 1980; Andersen and Kent, 1982; Andersen and Kent, 1983), tomatoes (Crooks and Ludford, 1983), citrus fruits (Cooper et al., 1969), pears (Sfakiotakis and Dilley, 1974) and peaches (Wade, 1979). Elevated levels of ethylene were observed only when fruits were brought to room temperature after chilling treatment. Wang and Adams (1980) postulated that the step in ethylene biosynthesis that is affected by chilling is the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC). Other investigators, however, did not find ACC in cucumber tissues during chilling (Andersen and Kent, 1983).

**Cytological Changes:**

Ultrastructural changes in sweet potato have been reported by Yamaki and Uritani (1973). They found that vacuolar membranes were degraded and disappeared in certain parts of cells. Abe and Ogata (1978) observed swelling of mitochondria and partial disappearance of the tonoplast in eggplant slices. The same phenomenon has been reported in tomato mitochondria and plastids
after 10 days at 2°C (Moline, 1976). In sorghum plants chilled at 15°C no net synthesis of chlorophyll occurred. Electron micrographs of leaf tissue showed arrested development of thylakoid membranes (McWilliam et al., 1979). Chloroplasts of barley plants grown at 2°C showed crimping of granal thylakoids and other structural abnormalities (Smillie et al., 1978).

Changes in Formation and Amount of Metabolites:

The formation of various metabolites on chilling has been determined by many investigators. Among these are chlorogenic acid and other polyphenols (Lieberman et al., 1958; Abe and Ogata, 1978), α-farnesene (Wills et al., 1975), acetaldehyde, pyruvate and α-keto acids (Murata, 1969; Smagula and Bramlage, 1977) and acetate (Smagula and Bramlage, 1977). Decreased levels of ascorbic acid have been reported in sweet potatoes (Lieberman et al., 1958) and pineapple (Miller, 1951). Other researchers have related reducing sugar levels and resistance to chilling injury. In grapefruit peel, resistance to CI correlated with high levels of reducing sugars (Purvis et al., 1979). Total sugar levels were found to increase in leaves of certain species after chilling (Siechmann and Boe, 1978). The formation of volatiles like acetaldehyde and ethyl alcohol increased in lemons exposed to chilling temperature when fruits were brought back to 20°C (Eaks, 1980). An increase
in insoluble pectin content was observed in cucumber fruits on chilling and it has been suggested that deesterification of pectin on chilling is characteristic of chilling-sensitive plants (Fukushima and Yamazaki, 1978).

**Changes in Fatty Acid Composition:**

There is no agreement among researchers on the relationship between fatty acid composition and chilling sensitivity. However, the effect of lipid composition on membrane fluidity and function is well-documented (Kasai et al., 1976; Baldassare et al., 1977; Raison, 1980). Lyons and Asmundson (1965) observed marked differences in the freezing points of mixtures of fatty acids with variations in amounts of unsaturated fatty acids of less than 5% and concluded that this would be a factor to consider in chilling-sensitivity of plants. Studies with phospholipids of *Passiflora* species (Patterson et al., 1978a) using ESR showed that chilling-sensitive species underwent a phase change at a higher temperature than chilling-resistant species, although no differences in fatty acid composition were observed. Similar results were obtained by other investigators with leaves of cucumber varieties differing in cold resistance (Rodionov and Zakharova, 1982). Other researchers observed increased unsaturation of 18-carbon
fatty acids with increased chilling tolerance (Wang
and Baker, 1979). Chilling has also been shown to cause
an increase in the unsaturation of fatty acids in polar
lipids of chilling-sensitive plants (Wilson and Crawford,
1974; St. John and Christiansen, 1976). Linolenic
acid levels have been lowered in certain plants with
the use of pyradazinone derivatives (St. John, 1976;
St. John et al., 1979). Chilling was observed to increase
the linolenic acid content of the polar lipid fraction
and when pyradazinone was applied the low-temperature
induced increase in linolenic acid was reduced and
seedling ability to withstand chilling also lowered.
Yamaki and Uritani (1972) did not observe this response
to chilling in sweet potato and white potato. Their
findings showed that white potato, a temperate zone
crop, had a lower ratio of unsaturated fatty acids than
sweet potato, a tropical plant. A recent study suggested
that chilling sensitivity may be related to the
palmitic/palmitoleic acid ratio of the
phosphatidylglycerols of plants (Murata, 1983). Fatty
acid composition may not be the only factor affecting
chilling sensitivity and other factors which affect mem-
brane structure may need to be taken into consideration.

Effects on Plant growth:

Seeds of chilling-sensitive plants either do not
germinate or do not mature normally resulting in reduced
yields when exposed to chilling temperature. This has been observed in rice, soybean, cotton, sorghum, okra and others. Seeds are most sensitive to low temperature during the initial stages of hydration (Christiansen, 1967). The injury is believed to be due to damage to the membrane during imbibition (Priestley and Leopold, 1980). Studies with sorghum showed larger increases in activation energies compared with chilling-resistant species like barley (McWilliam et al., 1979). The rates of initial germination, seedling respiration and mesocotyl extension all decreased as the temperature was reduced from 24 to 8°C. Emerging seedlings after chilling have been reported to exhibit necrosis of the radicle (Christiansen, 1968; Creencia and Bramlage, 1971). Established seedlings subjected to low temperature showed reduced growth (Christiansen and Thomas, 1969). Chlorophyll synthesis was decreased in chilling-sensitive plants like cucumber (Van Hasselt and Strikwerda, 1976). Chlorophyll loss and chlorosis in leaves have also been reported (Muramoto et al., 1971; Bagnall, 1979). Wilting and dehydration were detected within hours of chilling in bean and cucumber (Minchin and Simon, 1973; Wright, 1974). Decrease in net photosynthetic activity after chilling was observed by many investigators (Margulies, 1960; Wright and Simon, 1973; Bagnall, 1979). Leaves exposed to chilling and returned to normal temperature
also showed decreased photosynthesis (Teeri et al., 1977; McWilliam et al., 1979).

2.4 ASSAY OF CHILLING INJURY

Measurement of CI based on severity of visual symptoms is not easy to quantify. Moreover, it is often important to know the effects of low temperature on tissues before visible symptoms appear. Assays under laboratory and field conditions have been discussed by Pauli et al., (1979). Present methods of quantitatively measuring the extent of CI are based on observed secondary responses after exposure of tissues to low temperature. Among these are leakage of ions and solute (Christiansen et al., 1970; Patterson et al., 1976; Wiest et al., 1976; Quadir, 1983), changes in respiration (Eaks and Morris, 1956; Eaks, 1960; Watada and Morris, 1966), an increase in ethylene formation (Wang and Adams, 1980) and changes in the photosynthetic system (Melcarek and Brown, 1977; Smillie and Nott, 1978; Bjorn and Forsberg, 1979). A reliable assay for chilling tolerance is needed in the selection of crops for plant breeding. In the case of fresh produce accurate assessment of the extent of CI will be useful in determining the method or treatment required to prevent or reduce injury.
2.5 METHODS OF REDUCING CHILLING INJURY

2.5.1 Temperature Conditioning or Hardening

One method of reducing CI in plants is to expose them for a certain period of time to temperatures slightly above the critical chilling temperature. This process has been beneficial in tomatoes (Wheaton and Morris, 1967), potatoes (Chen and Li, 1980), and cotton (St. John and Christiansen, 1976). Studies with a harvested produce like grapefruit (Hatton and Cubbedge, 1980) showed that a 7-day exposure at 10-15 °C reduced CI during storage at 0 °C. The hardening process is believed to involve changes in the content and metabolism of carbohydrates, proteins, nucleic acids and fatty acids (Guinn, 1971).

2.5.2 Intermittent Warming

Chilling injury has been alleviated by subjecting produce already stored at cold temperatures to short periods at warm temperatures above the chilling point. This technique has been used successfully in apples (Smith, 1959), citrus (Davis and Hofmann, 1973), cucumbers (Wang and Baker, 1979), okra (Ilker and Morris, 1975), peaches (Ben-Arie et al., 1970) and sweet peppers (Wang and Baker, 1979). The mechanism by which intermittent warming alleviates CI is not well understood. It is believed that warming allows the tissues to recover
from the effects of chilling by eliminating toxic substances or restoring metabolites destroyed by low temperatures. Changes in unsaturation of polar lipids has been observed (Wang and Baker, 1979). There has been evidence of recovery of ultrastructural features in mitochondria and plastids (Moline, 1976); Niki et al., 1979). There was restoration of rough endoplasmic reticulum and development of polysomes in the early stages of rewarming although in certain cases rewarming caused rupture of the tonoplast especially in cells chilled for 24 hours.

2.5.3 Film Packaging

Semi-permeable films have been used in packaging fruits and vegetables to reduce O$_2$ and increase CO$_2$ and maintain high humidity in the atmosphere. This process has been utilized in grapefruit (Wardowski et al., 1973) using polyvinyl chloride and cast vinyl films. It was observed that CI was prevented for one-month during storage at 4.5 C. The use of sealed polyethylene box liners has reduced scald in apples (Smock, 1957) and prevented the appearance of visual symptoms of CI in cucumber fruits (Andersen and Kent, 1983). Furthermore, fruits stored in this manner, did not show increased production of ACC and ethylene until after 8-days of chilling storage.
2.5.4 Controlled Atmospheres

Conflicting results have been reported on the effects of controlled atmosphere storage on CI. Studies in cucumber with CO\textsubscript{2} concentrations up to 23\% increased symptoms of CI in chilled compared with non-chilled fruit (Eaks and Morris, 1956). With okra, treatment with increased CO\textsubscript{2} before chilling and increased CO\textsubscript{2} during chilling reduced CI symptoms (Ilker and Morris, 1975). However, higher concentrations of CO\textsubscript{2} (20-25\%) induced damage. In avocados, controlled atmosphere storage consisting of 2\% O\textsubscript{2} and CO\textsubscript{2} for 3-4 weeks prevented development of both CI and anthracnose rot (Spalding and Reeder, 1975). Hypobaric storage has been beneficial with avocados (Spalding and Reeder, 1976).

2.5.5 Chemical Treatment

Numerous studies have been made on the use of calcium in different fruits to reduce CI. Postharvest application of calcium to cultivars of apples reduced the incidence of breakdown during cold storage (Bangerth et al., 1972; Scott, 1975). Analysis of fruit tissue after application of calcium either as a dip or spray, showed that calcium levels in the core of the fruit increased after treatment (Mason and Drought, 1975; Poovaiah, 1979; Scott and Wills, 1979; Sams and Conway, 1982). Looney (1977)
applied calcium treatment to apple trees. Others have investigated the utilization of other calcium salts in place of calcium chloride (Bramlage et al., 1983). Calcium has also been shown to affect senescence (Mason et al., 1975; Bramlage et al., 1983). Researchers have observed that respiration of apple fruits decreased with increasing calcium levels regardless of whether calcium was endogenous or applied (Faust and Shear, 1972; Bramlage et al., 1974). A study of the membrane properties of microsomal preparations indicated that calcium chloride altered the characteristic temperature limits of membranes stored at 0°C (Paliyath and Poovaiah, 1982). Murphy and Evensen (1982) noticed stimulation of ethylene production in tomatoes on addition of calcium chloride and explained this on the basis of maintenance of membrane function by calcium ion. Preharvest sprays or postharvest dips of calcium chloride decreased the extent of surface pitting in cherries on storage at 0°C (Lidster et al., 1979). Application of CaCl₂ to avocados by vacuum infiltration reduced the severity of symptoms of CI (Chaplin and Scott, 1980). On the other hand, pretreatment of cucumber seedlings with CaCl₂ had no effect on prevention of CI (Sasson and Bramlage, 1981). Utilization of calcium chloride and potassium chloride solutions together with increased CO₂ reduced CI in okra (Ilker and Morris, 1975).
Antioxidants such as diphenylamine, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ethoxyquin, which have been used to reduce soft scald in apples, increased the amount of unsaturated fatty acids in surface lipids and reduced the level of hexanol in the fruit (Smock 1957; Huelin and Coggiola, 1968; Gough et al., 1973; Wills et al., 1981). Soft scald in apples has also been alleviated by application of methyl linoleate (Wills et al., 1977). Wang and Baker (1979) experimented with two free radical scavengers, sodium benzoate and ethoxyquin, on cucumber and sweet pepper fruits and found an increase in the degree of unsaturation of 18-carbon fatty acids as well as a reduction in the extent of chilling injury. They suggested that free radical scavengers prevented the oxidation of unsaturated fatty acids in membrane lipids. Underpeel discoloration in bananas has been reduced by postharvest application of dimethylpolysiloxane, safflower oil and mineral oil (Jones et al., 1974). Chemicals like thiabendazole and benomyl which were used to reduce pitting in grapefruit peel were also reported to reduce rots during cold storage (Wardowski et al., 1957; Schiffman-Nadel et al., 1975).
2.5.6 Hormonal Control

There is no pattern observed on the effects of ethylene treatment of harvested produce. Ethylene application to sweet potatoes after chilling was found to reduce hardcore although it did not reduce its incidence (Buescher, 1977). With muskmelons, pretreatment with ethylene prior to storage at 2.5 C reduced the incidence of CI and allowed normal ripening of the fruits (Lipton and Aharoni, 1979). However, with avocados the passage of ethylene during chilling storage produced severe injury (Young and Lee, 1979).

A number of growth regulators have been used to reduce CI in plants and harvested produce. Rikin et al. (1981) examined the application of abscicic acid to cotton seedlings and cotyledon discs and found that it was effective in preventing CI or reducing electrolyte leakage and necrosis during chilling at 4 C. By using solid phase radioimmunoassay techniques it was shown that abscicic acid prevented depolymerization of the microtubules due to chilling damage. Abscicic acid has been effective in reducing CI in cucumber seedlings (Rikin and Richmond, 1976; Sasson and Bramlage, 1981; Quadir, 1983). Resistance to CI in grapefruit has been correlated with levels of growth regulators in the peel (Kawada et al., 1979). Preharvest application of benzyladenine, gibberellic acid and
2,4-dichlorophenoxyacetic acid was shown to increase resistance to CI (Ismail and Grierson, 1977). Gibberelic acid has been applied to apples to reduce low temperature breakdown (Wills and Patterson, 1971; Wills and Scott, 1974). However, its mechanism of action in reducing CI is not understood.

2.5.7 Genetic Manipulation

Chilling-sensitive species often show variation in sensitivity which can be utilized in screening of crops for chilling-tolerant lines. This variation has been confirmed in *Passiflora* (Patterson et al., 1976), *Zea mays* and tomato, *Lycopersicon hirsutum* (Patterson et al., 1978b; Vallejos, 1979). A number of screening techniques utilizing tissue culture and plant tissue tests has been discussed by Pauli et al. (1979). Chilling resistant cell lines of *Nicotiana* and *Capsicum* have been isolated using cell culture (Dix and Street, 1976; Dix, 1979). If problems in plant regeneration can be overcome, this technique shows great promise as a tool for selection and cultivation of crops with genetic resistance to chilling stress.

2.6 FLAVOR VOLATILE COMPOUNDS FROM FATTY ACIDS

Although present in a small amount in plant tissues, lipids play an important role in the generation of the
characteristic flavor compounds of fruits and vegetables. Aldehydes, ketones and alcohols have been shown to be derived from linoleic and linolenic acids on the disruption of tissues of apples, melons, tomatoes, cucumbers, mushrooms and others. The volatile compounds formed are characteristic for each particular commodity and depend on the nature and properties of the enzymes responsible for the oxidation of lipids. In many cases the initial reaction involves the enzyme lipoxygenase to form hydroperoxides, followed by degradation of the hydroperoxides to shorter chain volatiles by the action of other enzymes like hydrolases, isomerases and reductases.

Lipids in cucumbers occur only to the extent of 0.10% (Salunkhe, 1974; Yamaguchi, 1983). Kinsella (1971) reported that cucumbers for fresh table use contained 39% of neutral lipids, 49% of phospholipids and 15% of glycolipids. Pickling cucumbers were found to contain 9.6% of neutral lipids, 60.0% of glycolipids and 10.4% of phospholipids (Peng and Geisman, 1976). The major fatty acids in all lipid classes were observed to be palmitic, linoleic and linolenic acids.

The total amount of aldehydes obtained from cucumbers is 2-20 parts per million (0.0002-0.002%). The major volatile component, trans-2-cis-6-nonadienal, has an odor threshold of 0.001-0.01 parts per million (Grosch,
The volatiles which have been reported in cucumber essence can be classified into two groups: alkenals and alkadienals of 6 or 9 carbon atoms and long chain aldehydes of 12 up to 17 carbon atoms. In the first group are hexanal, 2-hexenal, trans-2-cis-6-nonadienal, cis,cis-3,6-nonadienal, trans-2-nonenal and 2-pentylfuran (Forss et al., 1962; Fleming et al., 1968; Tressl et al., 1981). Other researchers have identified alcohols like 1-nonanol, 2-nonen-1-ol, 3-nonen-1-ol, 6-nonen-1-ol, trans-2-cis-6-nonadien-1-ol and cis,cis-3,6-nonadien-1-ol as minor constituents of cucumber essence. In the second group comprising the long-chain volatiles, dodecanal, tridecanal, tetradecanal, pentadecanal, cis-7-hexadecenal, cis-8-heptadecenal, cis,cis-7,10-hexadecadienal, cis,cis-8,11-heptadecadienal, cis,cis,cis,-7,10,13-hexadecatrienal, and cis,cis,cis-8,11,14-heptadecatrienal have been reported (Kemp et al., 1974; Kemp, 1975). Studies have shown that negligible amounts of these volatiles are present in intact cucumber tissue (Fleming et al., 1968) and that the carbonyl compounds are generated on disruption of the tissue in the presence of oxygen. It has been observed that maximum volatile formation occurs at a pH of 5.5 (Hatanaka et al., 1975).

The enzyme system responsible for the conversion of linoleic and linolenic acids to 6- and 9-carbon carbonyl compounds is composed of lipoxygenase, a
hydroperoxide cleavage enzyme and an isomerase (Tressl et al., 1981). The lipoxygenase system in cucumbers forms 9- and 13-hydroperoxides (Galliard and Phillips, 1976) which are then acted upon by a hydroperoxide cleavage enzyme to form C₆ and C₉ aldehydes (Galliard et al., 1976; Phillips et al., 1978). Cis-enals are converted to trans-enals by an isomerase (Hatanaka et al., 1975; Tressl et al., 1981). It has also been shown that hexanal (2-pentylfuran, and trans-2-nonenal are derived mainly from linoleic acid while trans-2-cis-6-nonadienal, trans-2-hexenal and pentenylfuran are generated from linolenic acid (Grosch and Schwarz, 1971; Tressl et al., 1981). The formation of long-chain aldehydes has been attributed to an enzyme system that catalyzes the α-oxidation of fatty acids to the C₁₂ to C₁₇ aldehydes that have been observed (Galliard and Matthew, 1976). This α-oxidation system is active on saturated and unsaturated fatty acids (Kemp, 1975).
Chapter III
MATERIALS AND METHODS

3.1 MATERIALS

European cucumber cv. 'V-5' (Cucumis sativus L.) from De Ruiter Seeds, Inc., Columbus, OH., were grown in the Ohio State University Horticultural greenhouses. Plants were grown using standard horticultural practice and harvested when mature green and 10-12 inches long.

3.2 TREATMENT OF SAMPLES

3.2.1 Chemical Application

3.2.1.1 Calcium Chloride (CaCl₂).

Fruits were dipped in 6% calcium chloride solution (w/v) for 30 minutes. Control fruits were dipped in distilled water only.

3.2.1.2 Butylatedhydroxytoluene (BHT).

BHT (CAO-3 antioxidant, Ashland Chemical Co., Columbus, OH.) solution in acetone (1%, w/v) with 0.1% Tween 20 was applied as a spray. Each fruit was sprayed uniformly with approximately 20 ml of solution. Fruits sprayed
3.2.2 Chilling Storage

Fruits were allowed to air-dry at room temperature after chemical treatment, placed in polyethylene film liners and stored in the refrigerator at 4.4°C for 7-days. They were then removed from cold storage and transferred to 20°C for another week. Unchilled, untreated fruits were maintained at 20°C for two weeks as control.

3.3 EVALUATION OF CHILLING INJURY. NINHYDRIN-REACTIVE COMPOUNDS (NRC)

Chilling injury was assayed by measuring ninhydrin-reactive compounds in samples as the percentage of the maximal release after complete disruption of the plasmalemma. Cucumber fruits were peeled, sliced into 2-cm thick sections and 100 mg samples obtained from each section. Ten measurements were made for each treatment. The procedure of Rosen (1957) as modified by Wiest et al. (1976) was followed to measure the release of ninhydrin-reactive compounds. This procedure can be briefly described as follows: Samples were shaken with 15 ml distilled water at 120 rpm for 16 hours at 4°C. A one ml aliquot was then taken and to this was added 0.5 ml buffer (2.645 M sodium acetate, 2 X 10^{-4} M sodium cyanide in 6.6% (w/v) glacial acetic acid) with acetone in 0.1% Tween 20 served as control.
and 0.5 ml of 3% (w/v) ninhydrin in methyl cellosolve. Samples were heated in a boiling water bath for 15 minutes. Immediately after removal from the bath 10 ml of isopropyl alcohol:water (1:1,v/v) was added. After thorough mixing and cooling to room temperature absorbance was read at 570 nm using a Bausch and Lomb Spectronic 20 spectrophotometer. The remaining tissue samples were then placed in the boiling water bath for 25 minutes. After samples were brought back to room temperature another 1 ml aliquot was taken and the total amount of NRC in sample was determined as before. Results were then expressed as percent released comparing the ratio of unboiled sample with the boiled sample.

3.4 LIPID ANALYSIS

Fruits were peeled (1-2 mm), cut into 2-cm sections and mixed thoroughly. Moisture content was determined by weight difference after heating in a Precision-Thelco circulating oven at 100-105 C for 20 hours.

3.4.1 Extraction

Triplicate samples (200 g each) of cucumber tissue were boiled 5 minutes in isopropyl alcohol before blending (Kates, 1972). Distilled water (200 ml) was then added and samples blended for 2 minutes in a Waring Blendor. The slurry was mixed thoroughly with 20 g silicic acid
(SiliCAR cc-7, Special, 100-200 mesh, Mallinckrodt Chemical Works, St. Louis, MO.) and 10 g Celite (Johns-Manville, New York, NY.). The mixture was filtered with Whatman No. 1 paper in a Buchner funnel under reduced pressure until no continuous water drop was observed. The sample pad was extracted in a Waring Blender with 200 ml Folch reagent (Folch et al., 1957), which consisted of chloroform-methanol (2:1 v/v), for 2 minutes at room temperature and filtered by suction. The residue was reextracted with another portion of 200 ml solvent, filtered, washed twice with 25 ml solvent and 25 ml chloroform. The combined extract was quantitatively transferred to a separatory funnel and allowed to stand 5-10 minutes. The lower chloroform phase was collected, the upper alcohol phase was extracted with 30 ml chloroform and combined with the lower phase. The extract was left in a refrigerator overnight for complete separation and the aqueous layer removed by siphoning. The extract was then concentrated in a rotary evaporator at reduced pressure at 45 C and stored in a vacuum desiccator until constant weight was obtained. All samples were protected under nitrogen atmosphere throughout extraction and subsequent fatty acid analysis from oxidation.
3.4.2 Column Chromatography

Extracted lipids were separated into three classes by silicic acid column chromatography. A 1.1 cm diameter glass column was used with a 250 ml reservoir flask at a sample loading ratio of 2-100 g adsorbent. Neutral lipids were eluted by chloroform at an elution ratio of 25 ml/g adsorbent and a flow rate of 0.5 ml/minute. Glycolipids were eluted by acetone at 40 ml/g adsorbent and phospholipids by methanol at 25 ml/g both at the same flow rate. Each fraction was collected in bulk and the solvent was removed by rotary vacuum evaporation. The purity of each lipid class was monitored by thin-layer chromatography using glass plates (20 X 10 cm) coated with silica Gel G (Brinkman Instruments, Westbury, NY.), 250 micron thickness. The developing system was chloroform for neutral lipids and chloroform-acetone-methanol-acetic acid-water (65 :20 :10 :10 :3, v/v) for glycolipids and phospholipids (Lepage, 1967). Phosphomolybdic acid solution (5% in ethanol) was used for detecting lipids in each class.

3.4.3 Gas Chromatography

Analyses of fatty acids were performed by a Hewlett-Packard Model 5880A gas chromatograph equipped with a flame ionization detector and electronic integrator. Methyl esters were prepared according to
Metcalfe et al. (1966) by using boron trifluoride-methanol. A coiled stainless steel column (10 ft X 1/8 in outside diameter) was packed by Supelco (Supelco Park, Bellefonte, PA.) with 10% by weight diethyleneglycolsuccinate (DEGS) on acid-washed Chromosorb W, 80-100 mesh as the support phase. Operating conditions were oven temperature 190 C, injection temperature 200 C, detector temperature 250 C and carrier gas (nitrogen) at a flow rate of 20 ml/minute. Fatty acids were identified by comparing retention times vs. carbon number on semi-log paper for those other than reference compounds run on the same column under the same conditions (Peng, 1974). Fatty acids were expressed as area percentage of total areas of all methyl esters.

3.5 ANALYSIS OF BHT RESIDUE

The method of Gough et al. (1973) was used. Cucumbers were peeled and cut into sections as before. Samples of 40-50 g were weighed, blended with 50 ml of dichloromethane in a Waring Blender and filtered by suction through Celite. The dichloromethane layer was concentrated in a rotary evaporator and passed through a short Florisil column to remove pigments. It was further concentrated to 1 ml and the BHT residue was measured in a Becker Model 409 gas chromatograph fitted with a flame ionization detector using a 6 ft X 1/8
inch stainless steel column packed with 15% by weight of DEGS on 80/100 Chromosorb W. The carrier gas was nitrogen with a flow rate of 20 ml/minute, isothermal oven temperature at 170°C, detector temperature at 220°C and injection temperature of 240°C. The same BHT reagent (CAO-3 antioxidant) used for treatment of cucumber fruits was used as standard.

3.6 FLAVOR VOLATILES

Flavor volatiles were obtained as follows: Cucumber fruits were peeled, sliced and duplicate samples of 300 g were frozen immediately in liquid nitrogen. For extraction of volatiles, frozen samples were blended with 300 ml distilled water for 2 minutes and distilled in a Likens-Nickerson apparatus with 50 ml of hexane (Fisher Scientific Co., Fair Lawn, NJ.) for 1.5 hours. The hexane extract was then concentrated to 0.5 ml in a rotary evaporator. Volatiles were tentatively identified by gas-chromatography with a Hewlett-Packard Model 5880A gas chromatograph with flame ionization detector and electronic integrator. A 10% DEGS column as described previously was used under the following operating conditions: programmed oven temperature from 100-180°C, program rate 4°C/minute, injection temperature 200°C and detector temperature 250°C with carrier gas at 20 ml/minute. Aldehyde standards
trans-2-cis-6-nonadienal, 2-nonenal and cis-6-nonenal (ICN Pharmaceuticals, Inc., Plainview, NY.) were used to compare retention times. Further identification of peaks was obtained using gas chromatography-mass spectrometry (GC-MS) with a Finnigan 4021 GC-MS data system at the Campus Chemical Instrument Center of The Ohio State University.

3.7 STATISTICAL ANALYSIS

Analysis of variance and Duncan's Multiple Range test at the 5% level were used to compare means of controls and treatments. The statistical analyses were performed at The Ohio State University using the Statistical Analysis System (SAS Institute, Cary, NC.) program.
Chapter IV
RESULTS

4.1 CHILLING INJURY. NINHYDRIN-REACTIVE COMPOUNDS

There were no visible symptoms of CI in cucumber fruits immediately after chilling. On transfer to 20 C, pitting and shriveling appeared after two days. Fruits which were treated with BHT exhibited less surface pitting than CaCl₂-treated fruits. Figure 1 shows the release of ninhydrin-reactive compounds (NRC) in untreated, unchilled cucumber fruits kept at 20 C for two weeks. It is seen that NRC remained relatively constant at 0 and 7 days and increased at 12 and 14 days. The mean percentages of NRC observed were 40.85% at 7 days, 63.70% at 12 days and 57.31% at 14 days. A storage period of 7, 12 and 14 days at 20 C for unchilled, untreated fruits corresponded to 0, 5 and 7 days at 20 C for chilled and/or treated samples. The release of NRC in BHT-treated fruits is shown in Figure 2 and that for CaCl₂-treated fruits is shown in Figure 3. A comparison of the means obtained at various time periods after chilling is given in Table 1. NRC was greater in chilled samples (controls
Figure 1: PERCENT OF NINHYDRIN-REACTIVE COMPOUNDS IN UNCHILLED, UNTREATED CUCUMBER FRUIT
Figure 2: PERCENT OF NINHYDRIN-REACTIVE COMPOUNDS IN A) CONTROL AND B) BHT-TREATED CUCUMBER FRUIT AFTER CHILLING AT 4.4 C
Figure 3: PERCENT OF NINHYDRIN-REACTIVE COMPOUNDS IN A) CONTROL AND B) CaCl₂-TREATED CUCUMBER FRUIT AFTER CHILLING AT 4.4 °C
and treatment) compared with unchilled samples. Immediately after chilling (0 days), fruits had significantly higher CI than unchilled fruits regardless of treatment. The means for release of NRC in control fruits were 62.38% and 74.25%, and 66.20% and 64.40% for BHT and CaCl$_2$ treatment. It was 40.85% for unchilled fruits. After 5 and 7 days at room temperature differences were observed in the CI of various treatments. At 5 days after chilling CaCl$_2$-control fruits which had been dipped in distilled water showed higher NRC than the unchilled sample. The extent of CI in CaCl$_2$- and BHT-treated fruits was the same as that in unchilled samples. However, if treatments are compared, CaCl$_2$-treated fruits suffered greater CI than BHT-treated fruits. At 7 days after chilling CaCl$_2$-control fruits still showed higher NRC compared with unchilled fruits. Samples subjected to BHT or CaCl$_2$ treatment exhibited the same degree of CI as unchilled, untreated fruits. A significant difference was observed in CI between CaCl$_2$-treated and control fruits. Throughout the entire period of storage at room temperature after chilling CaCl$_2$ treatment reduced CI compared with control fruits. However, CI in CaCl$_2$-control fruits was greater than BHT-control and unchilled fruits. BHT treatment did not reduce CI compared with BHT-control fruits but CI in these samples was initially lower than that in
Table 1

EFFECT OF CHILLING AND CHEMICAL TREATMENT ON
RELEASE OF NINHYDRIN-REACTIVE COMPOUNDS¹ IN
CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days at 20° C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Unchilled, Untreated</td>
<td>40.85 b</td>
</tr>
<tr>
<td>BHT Control</td>
<td>62.38 a</td>
</tr>
<tr>
<td>1 % BHT</td>
<td>66.20 a</td>
</tr>
<tr>
<td>CaCl₂ Control</td>
<td>74.25 a</td>
</tr>
<tr>
<td>6 % CaCl₂</td>
<td>64.40 a</td>
</tr>
</tbody>
</table>

¹ - Mean of 20 Determinations Expressed as % of Total Ninhydrin-Reactive Compounds.

² - Means Within the Same Column Followed By the Same Letter Are Not Significantly Different (P > .05).
CaCl$_2$-control fruits.
4.2 LIPID COMPOSITION

The total lipid content of peeled cucumber fruit was 0.11%. In unchilled, untreated tissue phospholipids (44.13%) and glycolipids (37.47%) were the major lipid classes while neutral lipids were present in the smallest amount (17.50%). The moisture and lipid content of unchilled and chilled and/or treated fruits are given in Table 2 and Table 3. The F values from the analysis of variance are shown in Table 15 of the Appendix. It is seen that there was no significant difference in the total lipid content of samples regardless of chilling or treatment. However, there were significant changes in the amounts of the different lipid classes. Chilling caused an increase in neutral lipids in control fruits and treated samples. Treatment with 6% CaCl₂ increased neutral lipids by 6% compared with chilled controls. In the glycolipid fraction chilling had an effect only in BHT-control fruits where the percentage increased from 37.47% to 52.80% then decreased to 36.50% on treatment. The same pattern was observed on CaCl₂ treatment. There was a decreasing trend in phospholipid content on chilling and treatment. BHT restored the level of phospholipids in its treated fruits from 20.50% to 36.73%, however, CaCl₂ caused a decrease in phospholipids. In general there was an increase in neutral lipids at the expense of phospholipids on chilling and/or treatment.
Table 2
MOISTURE CONTENT OF TREATED AND UNTREATED CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unchilled, Untreated</td>
<td>96.50</td>
</tr>
<tr>
<td>Chilled, 0 BHT</td>
<td>96.44</td>
</tr>
<tr>
<td>Chilled, 1 % BHT</td>
<td>97.05</td>
</tr>
<tr>
<td>Chilled, 0 CaCl₂</td>
<td>96.69</td>
</tr>
<tr>
<td>Chilled, 6 % CaCl₂</td>
<td>96.54</td>
</tr>
</tbody>
</table>

* Mean of Three Determinations
Table 3

EFFECT OF CHILLING AND CHEMICAL TREATMENT ON LIPID CONTENT\(^1\) OF CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lipid Class (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Lipids</td>
<td>Neutral Lipids</td>
<td>Glycolipids</td>
<td>Phospholipids</td>
</tr>
<tr>
<td>Control, Unchilled</td>
<td>0.11 a</td>
<td>17.50 c</td>
<td>37.47 b</td>
<td>44.13 a</td>
</tr>
<tr>
<td>Control, BHT</td>
<td>0.11 a</td>
<td>25.20 b</td>
<td>52.80 a</td>
<td>20.50 c</td>
</tr>
<tr>
<td>1 % BHT</td>
<td>0.11 a</td>
<td>25.83 b</td>
<td>36.50 b</td>
<td>36.73 ab</td>
</tr>
<tr>
<td>Control, CaCl(_2)</td>
<td>0.09 a</td>
<td>24.03 b</td>
<td>42.03 b</td>
<td>33.57 b</td>
</tr>
<tr>
<td>6 % CaCl(_2)</td>
<td>0.09 a</td>
<td>30.10 a</td>
<td>39.83 b</td>
<td>28.57 bc</td>
</tr>
</tbody>
</table>

\(^1\) Mean of Three Determinations

Means within the column followed by the same letter are not significantly different (P > .05).
4.3 FATTY ACID COMPOSITION

Only the major fatty acids are given in this study. The predominant fatty acids were found to be palmitic (C16:0), linoleic (C18:2), linolenic (C18:3), oleic (C18:1) and stearic (C18:0) acids. These fatty acids are of particular interest in relation to chilling and chilling injury.

4.3.1 Total Lipids

The fatty acid composition of total lipids is shown in Table 4. Lauric (C12:0), myristic (C14:0), stearic and oleic acids decreased on chilling and treatment; palmitic acid content remained unchanged, while linoleic and linolenic acids increased. If we examine the effects of treatment it is apparent that CaCl$_2$ treatment decreased stearic acid and increased linoleic and linolenic acids.
Table 4

EFFECT OF CHILLING AND CHEMICAL TREATMENT ON
FATTY ACID COMPOSITION\(^1\) OF TOTAL LIPIDS OF
CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Fatty Acid (^2)</th>
<th>Treatment</th>
<th>Control Unchilled</th>
<th>Control BHT</th>
<th>1% BHT</th>
<th>Control CaCl(_2)</th>
<th>6% CaCl(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td></td>
<td>1.35 a</td>
<td>0.49 bc</td>
<td>0.58 bc</td>
<td>0.64 b</td>
<td>0.09 c</td>
</tr>
<tr>
<td>14:0</td>
<td></td>
<td>2.02 a</td>
<td>1.86 a</td>
<td>0.88 b</td>
<td>1.65 a</td>
<td>0.15 c</td>
</tr>
<tr>
<td>16:0</td>
<td></td>
<td>34.20 ab</td>
<td>34.17 ab</td>
<td>27.27 ab</td>
<td>38.27 a</td>
<td>34.00 b</td>
</tr>
<tr>
<td>18:0</td>
<td></td>
<td>6.68 a</td>
<td>3.01 b</td>
<td>3.63 b</td>
<td>3.26 b</td>
<td>1.65 c</td>
</tr>
<tr>
<td>18:1</td>
<td></td>
<td>5.89 a</td>
<td>3.50 b</td>
<td>4.58 b</td>
<td>3.62 b</td>
<td>3.75 b</td>
</tr>
<tr>
<td>18:2</td>
<td></td>
<td>23.73 b</td>
<td>30.53 a</td>
<td>33.93 a</td>
<td>25.07 b</td>
<td>29.70 a</td>
</tr>
<tr>
<td>18:3</td>
<td></td>
<td>18.37 c</td>
<td>23.50 b</td>
<td>22.93 b</td>
<td>21.47 bc</td>
<td>30.57 a</td>
</tr>
</tbody>
</table>

1  Mean of Three Determinations Expressed as % of Total Fatty Acids

2  Carbon Number: Number of Double Bonds

Means Within the Same Row Followed By The Same Letter Are Not Significantly Different (P > .05).
4.3.2 Neutral Lipids

Table 5 shows the fatty acid composition of neutral lipids after chilling and treatment. Analysis of this lipid class illustrated the same decreasing pattern in lauric and myristic acids observed in total lipids. Palmitic acid did not change significantly on chilling and BHT treatment, but decreased on CaCl₂ application. Chilling increased oleic acid content for BHT-control fruits then decreased on BHT treatment although it was still significantly higher than unchilled fruits. On the other hand, CaCl₂ controls had about the same level of oleic acid as unchilled fruits and it was elevated from 4.72% to 10.61% after treatment. Linoleic acid content was increased by chemical application, especially of 6% CaCl₂. There was a significant decrease in linolenic acid content on chilling and none of the treatments alleviated this change.
Table 5

EFFECT OF CHILLING AND CHEMICAL TREATMENT ON FATTY ACID COMPOSITION^ OF NEUTRAL LIPIDS OF CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control Unchilled</th>
<th>Control BHT</th>
<th>1 % BHT</th>
<th>Control</th>
<th>6 % CaCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>4.09 a</td>
<td>0.62 b</td>
<td>0.66 b</td>
<td>0.60 b</td>
<td>1.09 b</td>
</tr>
<tr>
<td>14:0</td>
<td>4.14 a</td>
<td>3.33 a</td>
<td>1.88 b</td>
<td>1.40 b</td>
<td>1.49 b</td>
</tr>
<tr>
<td>16:0</td>
<td>39.63 ab</td>
<td>50.47 a</td>
<td>42.70 ab</td>
<td>49.07 a</td>
<td>34.37 b</td>
</tr>
<tr>
<td>18:0</td>
<td>4.45 a</td>
<td>6.02 a</td>
<td>5.00 a</td>
<td>4.25 a</td>
<td>3.87 a</td>
</tr>
<tr>
<td>18:1</td>
<td>4.06 c</td>
<td>10.61 a</td>
<td>7.55 b</td>
<td>4.72 c</td>
<td>10.61 a</td>
</tr>
<tr>
<td>18:2</td>
<td>11.42 b</td>
<td>7.92 b</td>
<td>14.55 b</td>
<td>12.40 b</td>
<td>24.00 a</td>
</tr>
<tr>
<td>18:3</td>
<td>13.67 a</td>
<td>3.41 c</td>
<td>7.08 bc</td>
<td>8.00 b</td>
<td>8.04 b</td>
</tr>
</tbody>
</table>

1 Mean of Three Determinations Expressed as % of Total Fatty Acids

2 Carbon Number: Number of Double Bonds

Means Within the Same Row Followed By The Same Letter Are Not Significantly Different (P > .05).
4.3.3 Glycolipids

The fatty acid composition of glycolipids is shown in Table 6. Lauric and myristic acids also decreased in this class after chilling and treatment. An increase in palmitic acid was observed on chilling and treatment. In the 18-carbon fatty acids no regular trend could be observed in stearic acid. It increased on chilling for BHT-control fruits but decreased on BHT treatment. There was a decrease in stearic acid levels with CaCl$_2$ application. The amount of oleic acid was not altered by CaCl$_2$ when compared with both chilled and unchilled fruits. Calcium chloride treatment increased linoleic acid compared with unchilled fruits (10.98% to 20.56%). Chilling was observed to decrease linolenic acid levels in BHT- and CaCl$_2$- control fruits, however, application of BHT restored it to a certain extent.
Table 6

EFFECT OF CHILLING AND CHEMICAL TREATMENT ON FATTY ACID COMPOSITION\(^{1}\) OF GLYCOLIPIDS OF CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Fatty Acid(^{2})</th>
<th>Treatment</th>
<th>Control Unchilled</th>
<th>Control BHT</th>
<th>1% BHT</th>
<th>Control CaCl(_2)</th>
<th>6% CaCl(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td></td>
<td>2.26 a</td>
<td>1.38 b</td>
<td>0.24 c</td>
<td>0.33 c</td>
<td>0.43 c</td>
</tr>
<tr>
<td>14:0</td>
<td></td>
<td>3.82 a</td>
<td>0.62 b</td>
<td>0.32 b</td>
<td>0.40 b</td>
<td>0.36 b</td>
</tr>
<tr>
<td>16:0</td>
<td></td>
<td>48.93 b</td>
<td>64.87 a</td>
<td>53.73 ab</td>
<td>64.10 a</td>
<td>64.87 a</td>
</tr>
<tr>
<td>18:0</td>
<td></td>
<td>9.73 b</td>
<td>13.30 a</td>
<td>7.17 c</td>
<td>7.55 c</td>
<td>4.28 d</td>
</tr>
<tr>
<td>18:1</td>
<td></td>
<td>4.75 ab</td>
<td>2.79 bc</td>
<td>1.96 c</td>
<td>5.32 a</td>
<td>4.91 ab</td>
</tr>
<tr>
<td>18:2</td>
<td></td>
<td>10.98 bc</td>
<td>3.27 c</td>
<td>12.07 abc</td>
<td>13.97 ab</td>
<td>20.56 a</td>
</tr>
<tr>
<td>18:3</td>
<td></td>
<td>16.10 a</td>
<td>3.58 b</td>
<td>12.47 a</td>
<td>5.50 b</td>
<td>3.55 b</td>
</tr>
</tbody>
</table>

1 Mean of Three Determinations Expressed as % of Total Fatty acids
2 Carbon Number: Number of Double Bonds

Means within the same row followed by the same letter are not significantly different (P>0.05)
4.3.4 Phospholipids

Table 7 gives the fatty acid composition of this lipid class. Chilling increased palmitic acid content slightly in both BHT control (52.53%) and BHT treatment (53.85%). On the other hand, this acid decreased in CaCl₂-treated fruits. The amount of stearic acid decreased significantly in both chilled and treated fruits. There was no significant change in the content of oleic acid. Control fruits for BHT treatment had lower linoleic acid levels than unchilled sample and BHT application restored it. Chilling did not change linoleic acid content in CaCl₂-control fruits but CaCl₂ treatment increased it. BHT treatment did not affect linolenic acid content. The amount of this fatty acid was decreased on chilling of CaCl₂-control fruits but increased on CaCl₂ treatment (11.37% to 20.77%).
Table 7
EFFECT OF CHILLING AND CHEMICAL TREATMENT ON FATTY ACID COMPOSITION\textsuperscript{1} OF PHOSPHOLIPIDS OF CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Fatty Acid\textsuperscript{2}</th>
<th>Control Unchilled</th>
<th>Control BHT</th>
<th>1 % BHT</th>
<th>Control CaCl\textsubscript{2}</th>
<th>6% CaCl\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>1.34 a</td>
<td>0.43 bc</td>
<td>0.69 b</td>
<td>0.13 c</td>
<td>0.12 c</td>
</tr>
<tr>
<td>14:0</td>
<td>1.40 ab</td>
<td>1.46 ab</td>
<td>1.54 a</td>
<td>0.34 c</td>
<td>0.95 b</td>
</tr>
<tr>
<td>16:0</td>
<td>47.13 ab</td>
<td>52.53 a</td>
<td>53.85 a</td>
<td>52.05 a</td>
<td>42.20 b</td>
</tr>
<tr>
<td>18:0</td>
<td>5.78 a</td>
<td>3.57 b</td>
<td>3.47 b</td>
<td>2.87 b</td>
<td>3.62 b</td>
</tr>
<tr>
<td>18:1</td>
<td>4.04 ab</td>
<td>4.06 ab</td>
<td>3.87 ab</td>
<td>3.15 b</td>
<td>4.73 a</td>
</tr>
<tr>
<td>18:2</td>
<td>20.30 b</td>
<td>16.43 c</td>
<td>21.70 b</td>
<td>20.57 b</td>
<td>25.80 a</td>
</tr>
<tr>
<td>18:3</td>
<td>15.67 b</td>
<td>15.13 b</td>
<td>14.17 bc</td>
<td>11.37 c</td>
<td>20.77 a</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Mean of Three Determinations Expressed as % of Total Fatty acids
\textsuperscript{2} Carbon Number: Number of Double Bonds
Means Within The Same Row Followed By The Same Letter Are Not Significantly Different (P>0.05)
4.4 UNSATURATION RATIOS OF FATTY ACIDS

Three types of unsaturation ratios were considered. Table 8 gives unsaturation ratios of fatty acids in total lipids in cucumber fruits. It was observed that the ratio of linoleic to linolenic acid, $R_1$, was decreased with 6% CaCl$_2$ treatment. With $R_2$ and $R_3$, the ratios of unsaturated to saturated fatty acids, there was a significant increase in $R_2$ for CaCl$_2$ treatment compared with chilled control and unchilled fruits and a significant increase in $R_3$ if we compare BHT and CaCl$_2$ treatments respectively with their control.

Unsaturation ratios in each lipid class were also studied. In neutral lipids, as shown in Table 9, no differences were observed in $R_1$ between treatments and corresponding chilled controls. $R_2$ rose significantly in CaCl$_2$-treated fruits but did not change with BHT treatment. On the other hand, $R_3$ decreased on chilling and increased slightly on BHT treatment. Application of 6% CaCl$_2$ increased $R_3$ to a level higher than chilled and unchilled fruit.

No definite trend could be drawn for $R_1$ in the glycolipid fraction as seen in Table 10. There was an increase in this ratio in CaCl$_2$-control fruits which was enhanced by CaCl$_2$ treatment. There were changes in the ratios $R_2$ and $R_3$. $R_3$ remained unchanged between CaCl$_2$ treatment
Table 8  
EFFECT OF CHILLING AND CHEMICAL TREATMENT ON  
UNSATURATION RATIOS$^1$ OF FATTY ACIDS OF TOTAL  
LIPIDS OF CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Unsaturation Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_1$</td>
</tr>
<tr>
<td>Unchilled, Untreated</td>
<td>1.31 ab</td>
</tr>
<tr>
<td>Control BHT</td>
<td>1.30 ab</td>
</tr>
<tr>
<td>1 % BHT</td>
<td>1.49 a</td>
</tr>
<tr>
<td>Control CaCl$_2$</td>
<td>1.17 b</td>
</tr>
<tr>
<td>6 % CaCl$_2$</td>
<td>0.97 c</td>
</tr>
</tbody>
</table>

$^1 R_1 = 18:2/18:3$

$R_2 = \frac{18:1 + 18:2 + 18:3}{18:0}$

$R_3 = \frac{18:1 + 18:2 + 18:3}{16:0 + 18:0}$

Means Within The Same Column Followed By The Same Letter Are Not Significantly Different (P > .05)
Table 9

EFFECT OF CHILLING AND CHEMICAL TREATMENT ON UNSATURATION RATIOS\(^1\) OF FATTY ACIDS IN NEUTRAL LIPIDS OF CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Unsaturation Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(R_1)</td>
</tr>
<tr>
<td>Unchilled, Untreated</td>
<td>0.84 b</td>
</tr>
<tr>
<td>Control BHT</td>
<td>3.84 a</td>
</tr>
<tr>
<td>1 % BHT</td>
<td>2.04 ab</td>
</tr>
<tr>
<td>Control CaCl(_2)</td>
<td>1.83 ab</td>
</tr>
<tr>
<td>6 % CaCl(_2)</td>
<td>3.05 ab</td>
</tr>
</tbody>
</table>

\(1 \quad R_1 = \frac{18:2}{18:3}\)

\(R_2 = \frac{18:1 + 18:2 + 18:3}{19:0}\)

\(R_3 = \frac{18:1 + 18:2 + 18:3}{16:0 + 18:0}\)

Means Within The Same Column Followed By The Same Letter Are Not Significantly Different (\(P>.05\))
Table 10
EFFECT OF CHILLING AND CHEMICAL TREATMENT ON UNSATURATION RATIOS$^1$ OF FATTY ACIDS IN GLYCOLIPIDS OF CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Unsaturation Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_1$</td>
</tr>
<tr>
<td>Unchilled, Untreated</td>
<td>0.72 c</td>
</tr>
<tr>
<td>Control, BHT</td>
<td>1.04 c</td>
</tr>
<tr>
<td>1 % BHT</td>
<td>0.96 c</td>
</tr>
<tr>
<td>Control CaCl$_2$</td>
<td>2.56 b</td>
</tr>
<tr>
<td>6 % CaCl$_2$</td>
<td>7.43 a</td>
</tr>
</tbody>
</table>

$^1 R_1 = \frac{18:2}{18:3}$

$R_2 = \frac{18:1 + 18:2 + 18:3}{18:0}$

$R_3 = \frac{18:1 + 18:2 + 18:3}{16:0 + 18:0}$

Means Within The Same Column Followed By The Same Letter Are Not Significantly Different (P>.05)
and control. $R_2$ and $R_3$ increased between BHT treatment and control. Moreover, in both $R_2$ and $R_3$ there was a decrease on chilling and an increase on BHT treatment. CaCl$_2$ treatment increased $R_2$ in comparison with chilled fruits.

The unsaturation ratio, $R_1$, in the phospholipid class (Table 11) showed a significant increase on chilling which was decreased on application of CaCl$_2$. Considering treatments separately, $R_2$ for BHT-control fruits was not different from unchilled and treated fruits. For CaCl$_2$ treatment $R_2$ was also not significantly different between chilled and treated fruits, but was significantly higher for treatment compared with unchilled fruit. $R_3$ decreased on chilling of BHT-control fruits but increased to the original level on BHT application. It decreased on chilling and increased on CaCl$_2$ application.
Table 11

EFFECT OF CHILLING AND CHEMICAL TREATMENT ON UNSATURATION RATIOS\(^{1}\) OF FATTY ACIDS IN PHOSPHOLIPIDS OF CUCUMBER FRUIT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Unsaturation Ratio</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(R_1)</td>
<td>(R_2)</td>
<td>(R_3)</td>
</tr>
<tr>
<td>Unchilled, Untreated</td>
<td>1.31 b</td>
<td>7.00 b</td>
<td>0.74 b</td>
</tr>
<tr>
<td>Control, BHT</td>
<td>1.14 b</td>
<td>10.52 ab</td>
<td>0.63 c</td>
</tr>
<tr>
<td>1 % BHT</td>
<td>1.53 ab</td>
<td>11.67 ab</td>
<td>0.71 b</td>
</tr>
<tr>
<td>Control CaCl(_2)</td>
<td>1.83 a</td>
<td>12.76 a</td>
<td>0.64 c</td>
</tr>
<tr>
<td>6 % CaCl(_2)</td>
<td>1.24 b</td>
<td>14.50 a</td>
<td>1.12 a</td>
</tr>
</tbody>
</table>

\(^{1}\) \(R_1 = \frac{18:2}{18:3}\)

\(R_2 = \frac{18:1 + 18:2 + 18:3}{18:0}\)

\(R_3 = \frac{18:1 + 18:2 + 18:3}{16:0 + 18:0}\)

Means Within The Same Column Followed By The Same Letter Are Not Significantly Different (\(P>.05\))
4.5 FLAVOR VOLATILE COMPOUNDS OF CUCUMBER FRUIT

The volatile compounds obtained by steam distillation in the Likens-Nickerson apparatus using hexane as the solvent were studied using GC-MS and identified by matching their mass spectra with the library of mass spectral data in the Finnigan 4021 system or by comparison with the mass spectra of reference compounds run under the same conditions in the GC-MS.

Figure 4 shows the gas chromatogram from steam distillation of untreated, unchilled cucumbers. The major volatiles were trans-2-cis-6-nonadienal and 2-nonenal. Figure 5 gives the mass spectrum of trans-2-cis-6-nonadienal compound from cucumber extract. The molecular ion (M+) peak was observed at m/e 138 with major peaks at 70, 69, 67, and 53. A mixture of 2-nonenal, trans-2-cis-6-nonadienal, cis-6-nonenal and BHT were distilled in the Likens-Nickerson apparatus under the same conditions as cucumber tissue samples. The mass spectrum of trans-2-cis-6-nonadienal from this mixture appears in Figure 6. The M+ peak at m/e 138 was not obtained in this sample although the mass spectral pattern is similar to that of trans-2-cis-6-nonadienal from the cucumber extract. The mass spectrum of a reference sample of trans-2-cis-6-nonadienal is given in Figure 7 and the mass spectral peaks are the same as those in Figure 5.
Figure 4: GAS CHROMATOGRAM FROM STEAM DISTILLATION OF UNCHILLED, UNTREATED CUCUMBER FRUIT
Figure 5: MASS SPECTRUM OF TRANS-2-CIS-6-NONADIENAL IN FLAVOR VOLATILES OF CUCUMBER FRUIT
Figure 6: MASS SPECTRUM OF TRANS-2-CIS-6-NONADIENAL FROM DISTILLATION OF REFERENCE MIXTURE OF BHT AND ALDEHYDES
Figure 7: MASS SPECTRUM OF TRANS-2-CIS-6-NONADIENAL REFERENCE SAMPLE
and Figure 6. The mass spectrum of 2-nonenal is shown in Figures 8 to 11. Figure 8 and Figure 9 show the mass spectra of 2-nonenal in sample extract while Figure 10 and Figure 11 show the mass spectra of 2-nonenal from mixed aldehydes and reference sample. Another 9-carbon alkenal was tentatively identified as 6-nonenal, the mass spectra of which are given in Figures 12 to 14. Figure 12 shows the mass spectrum from the volatile extract while Figure 13 and Figure 14 give the spectra of the reference sample and mixed aldehydes.

The lower boiling volatiles hexanal, 2-pentylfuran and 2-hexenal were identified primarily from matching the experimental spectra with the mass spectral library of the Finnigan 4021 GC-MS system. In Figure 15 the molecular ion (M⁺) at m/e 100 was obtained for hexanal with major peaks at 56, 55, 44, 43, and 41. The volatile 2-pentylfuran with mass spectrum shown in Figure 16 yielded M⁺ at m/e 138 and major peaks at 81, 80 and 53. Another low boiling aldehyde, 2-hexenal, shown in Figure 17 was identified after 2-pentylfuran with M⁺ at m/e 98 and major mass spectral peaks at 42, 55 and 69.

There were three large peaks of higher boiling compounds in the extract from fresh cucumber fruit. One of them was identified as tetradecanal by matching with the spectral library of the GC-MS system used. The mass spectrum of this compound is shown in Figure 18. Two other high boiling
Figure 8: MASS SPECTRUM OF 2-NONENAL IN FLAVOR VOLATILES OF CUCUMBER FRUIT
Figure 9: MASS SPECTRUM OF 2-NONENAL IN FLAVOR VOLATILES OF CUCUMBER FRUIT
Figure 10: MASS SPECTRUM OF 2-NONENAL FROM DISTILLATION OF REFERENCE MIXTURE OF BHT AND ALDEHYDES
Figure 11: MASS SPECTRUM OF 2-NONENAL REFERENCE SAMPLE
Figure 12: MASS SPECTRUM OF 6-NONENAL IN FLAVOR VOLATILES OF CUCUMBER FRUIT
Figure 13: MASS SPECTRUM OF CIS-6-Nonenal FROM DISTILLATION OF REFERENCE MIXTURE OF BHT AND ALDEHYDES
Figure 14: MASS SPECTRUM OF CIS-6-NONENAL REFERENCE SAMPLE
Figure 15: MASS SPECTRUM OF HEXANAL IN FLAVOR VOLATILES OF CUCUMBER FRUIT
Figure 16: MASS SPECTRUM OF 2-PENTYLFLURAN IN FLAVOR VOLATILES OF CUCUMBER FRUIT
Figure 17: MASS SPECTRUM OF 2-HEXENAL IN FLAVOR VOLATILES OF CUCUMBER FRUIT
Figure 18: MASS SPECTRUM OF TETRADECANAL IN FLAVOR VOLATILES OF CUCUMBER FRUIT
compounds came after tetradecanal which the spectral library identified as 9, 17-octadecadienal and 9, 12, 15-octadecatrienal (Figure 19 and Figure 20).

Figure 21, Figure 22 and Figure 23 show the gas chromatograms from the volatile extract of BHT-treated and control fruits at 0, 5 and 7 days after chilling at 4.4°C. The lower boiling compounds in these chromatograms are essentially the same as those in fresh extract. Hexanal, 2-pentylfuran, 2-hexenal, 2-nonenal, and trans-2-cis-6-nonadienal peaks were observed. The higher boiling portion of the chromatogram yielded two new peaks. Peak 6 in Figures 21 to 23 was positively identified as BHT steam-distilling with the cucumber volatiles. The mass spectrum of this peak is given in Figure 24 and has a molecular ion (M⁺) of 220. It matched the mass spectrum of BHT obtained from the mixture of aldehydes and BHT described earlier as well as the mass spectrum of BHT reference sample (Figure 25 and Figure 26). The presence of BHT residue in cucumber tissue was verified by extracting BHT from the flesh of BHT-treated fruit and analyzing by gas chromatography. BHT-treated fruits at 0 and 5 days after chilling were found to contain 423 and 394 ppm of BHT respectively. Peak 7 in Figures 21 to 23 which did not appear to be present in unchilled, untreated sample was tentatively identified as dodecanal. The mass spectrum of this compound is shown in Figure 27.
Figure 19: MASS SPECTRUM OF 9, 17-OCTADECADIENAL IN FLAVOR VOLATILES OF CUCUMBER FRUIT
Figure 20: MASS SPECTRUM OF 9, 12, 15-OCTADECATRIENAL IN FLAVOR VOLATILES OF CUCUMBER FRUIT
Figure 21: GAS CHROMATOGRAMS FROM STEAM DISTILLATION OF A) CONTROL AND B) BHT-TREATED CUCUMBER FRUIT AFTER CHILLING AT 4.4°C

1 Hexanal
2 2-Pentylfuran
3 2-Hexenal
4 2-Nonenal
5 Trans-2-cis-6-Nonadienal
6 Butylatedhydroxytoluene
7 Dodecanal
8 (?)
Figure 22: GAS CHROMATOGRAMS FROM STEAM DISTILLATION OF A) CONTROL AND B) BHT-TREATED CUCUMBER FRUIT 5 DAYS AFTER CHILLING AT 4.4 C

1 Hexanal
2 2-Pentylfuran
3 2-Hexenal
4 2-Nonenal
5 Trans-2-cis-6-Nonadienal
6 Butylatedhydroxytoluene
7 Dodecanal
8 (?)
Figure 23: GAS CHROMATOGRAMS FROM STEAM DISTILLATION OF A) CONTROL AND B) BHT-TREATED CUCUMBER FRUIT 7 DAYS AFTER CHILLING AT 4.4 C

1 Hexenal
2 2-Pentylfuran
3 2-Hexenal
4 2-Nonenal
5 Trans-2-cis-6-Nonadienal
6 Butylatedhydroxytoluene
7 Dodecanal
8 (?)
Figure 24: MASS SPECTRUM OF BUTYLATEDHYDROXYTOLUENE (BHT) IN FLAVOR VOLATILES OF BHT-TREATED CUCUMBER FRUIT
Figure 25: MASS SPECTRUM OF BUTYLATEDHYDROXYTOLUENE (BHT)
FROM DISTILLATION OF REFERENCE MIXTURE OF BHT
AND ALDEHYDES
Figure 26: MASS SPECTRUM OF BUTYLATEDHYDROXYTOLUENE (BHT) REFERENCE SAMPLE
Figure 27: MASS SPECTRUM OF DODECANAL IN FLAVOR VOLATILES OF BHT-TREATED CUCUMBER FRUIT
The volatiles in CaCl\textsubscript{2}-treated cucumber fruits at 0, 5 and 7 days after chilling are shown in Figure 28, Figure 29 and Figure 30. The compounds formed were the same as those in fresh fruit. The notable difference in the gas chromatogram of CaCl\textsubscript{2}-treated fruit was the increase in the intensity of an unidentified peak (Peak 5 in Figure 28) immediately before the peak of 2-nonenal. This peak diminished in intensity at 5 and 7 days after chilling.

A quantitative assessment of the volatiles in fresh fruit is shown in Table 12. The major component trans-2-cis-6-nonadienal comprised 39.05% of total volatiles followed by 2-nonenal, tetradecanal, 6-nonenal and 2-pentylfuran. Looking at changes in volatile levels in chilled fruit versus fresh fruit it was found that there were significant changes in the amounts of 2-nonenal and tetradecanal (Table 16 of the Appendix). There was a decrease in 2-nonenal on chilling. After 5 and 7 days 2-nonenal had decreased substantially from the level immediately after chilling. There was no clear trend in the formation of tetradecanal. It appeared to increase immediately after chilling then decrease on storage at room temperature (Table 17 of the Appendix).

The volatile compounds in CaCl\textsubscript{2}-treated fruits are shown in Table 13. Treatment with CaCl\textsubscript{2} compared with unchilled fruit caused significant changes in hexanal, 2-hexenal, 2-pentylfuran and trans-2-cis-6-nonadienal.
Table 12

<table>
<thead>
<tr>
<th>Compound</th>
<th>% of Total Volatiles$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexanal</td>
<td>0.44</td>
</tr>
<tr>
<td>2-Pentylfuran</td>
<td>1.58</td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>0.63</td>
</tr>
<tr>
<td>6-Nonenal</td>
<td>1.06</td>
</tr>
<tr>
<td>2-Nonenal</td>
<td>15.90</td>
</tr>
<tr>
<td>Trans-2-cis-6-Nonadienal</td>
<td>39.05</td>
</tr>
<tr>
<td>Tetradecanal</td>
<td>7.12</td>
</tr>
<tr>
<td>9,17-Octadecadienal</td>
<td>9.42</td>
</tr>
<tr>
<td>9,12,15-Octadecatrienal</td>
<td>13.05</td>
</tr>
</tbody>
</table>

$^1$ Mean of Two Determinations
<table>
<thead>
<tr>
<th>Compound</th>
<th>Days</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Control</td>
<td>6% CaCl₂</td>
<td>Control</td>
<td>6% CaCl₂</td>
<td>Control</td>
<td>6% CaCl₂</td>
</tr>
<tr>
<td>Hexanal</td>
<td>3.47</td>
<td>2.22</td>
<td>7.75</td>
<td>17.10</td>
<td>5.84</td>
<td>7.05</td>
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<tr>
<td>2-Pentylfuran</td>
<td>2.17</td>
<td>2.01</td>
<td>5.95</td>
<td>7.51</td>
<td>5.58</td>
<td>6.93</td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>1.94</td>
<td>1.11</td>
<td>7.10</td>
<td>5.05</td>
<td>5.11</td>
<td>5.84</td>
</tr>
<tr>
<td>6-Nonenal</td>
<td>0.97</td>
<td>2.32</td>
<td>0.81</td>
<td>1.09</td>
<td>0.94</td>
<td>0.75</td>
</tr>
<tr>
<td>2-Nonenal</td>
<td>13.20</td>
<td>9.10</td>
<td>6.49</td>
<td>7.08</td>
<td>7.09</td>
<td>8.31</td>
</tr>
<tr>
<td>Trans-2-cis-6-Nonadienal</td>
<td>20.70</td>
<td>26.40</td>
<td>19.40</td>
<td>14.70</td>
<td>18.45</td>
<td>21.75</td>
</tr>
<tr>
<td>Tetradecanal</td>
<td>11.20</td>
<td>1.63</td>
<td>3.89</td>
<td>8.09</td>
<td>5.75</td>
<td>5.12</td>
</tr>
</tbody>
</table>

1 Mean of Two Determinations Expressed As % of Total Volatiles.
Figure 28: GAS CHROMATOGRAMS FROM STEAM DISTILLATION OF A) CONTROL AND B) CaCl₂-TREATED CUCUMBER FRUIT AFTER CHILLING AT 4.4 °C

1 Hexanal
2 2-Pentylfuran
3 2-Hexenal
4 6-Nonenal
5 (?)
6 2-Nonenal
7 Trans-2-cis-6-Nonadienal
8 Tetradecanal
9 (?)
Figure 29: GAS CHROMATOGRAMS FROM STEAM DISTILLATION OF A) CONTROL AND B) CaCl₂-TREATMENT CUCUMBER FRUIT 5 DAYS AFTER CHILLING AT 4.4 C

1 Hexanal
2 2-Pentylfuran
3 2-Hexenal
4 6-Nonenal
5 2-Nonenal
6 Trans-2-cis-6-Nonadienal
7 Tetradecanal
Figure 30: GAS CHROMATOGRAMS FROM STEAM DISTILLATION OF A) CONTROL AND B) CaCl$_2$-TREATED CUCUMBER FRUIT 7 DAYS AFTER CHILLING AT 4.4 C
(Table 18 of the Appendix). Hexanal, 2-hexenal and 2-pentylfuran increased at 5 and 7 days after transfer of fruits to 20 C. The major cucumber volatile trans-2-cis-6-nonadienal decreased in chilled CaCl$_2$-treated fruits. (Table 17 of the Appendix.)

Table 14 shows the volatiles in BHT-treated fruits. BHT residue which steam-distilled with the volatile compounds became the major component of the extract. New peaks which were not positively identified were observed. The mass spectra of these peaks are shown in Figure 31 and Figure 32. Due to the presence of BHT and possibly other compounds coming from BHT in the volatile extract it was not possible to quantitatively compare the formation of volatiles in BHT-treated fruits.
Table 14

FLAVOR VOLATILES\(^1\) OF BHT-TREATED CUCUMBER FRUIT AT 0, 5 AND 7 DAYS AFTER CHILLING

<table>
<thead>
<tr>
<th>Compound</th>
<th>Days At 20° C</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1 % BHT</td>
<td>Control</td>
</tr>
<tr>
<td>Hexanal</td>
<td>0.90</td>
<td>1.90</td>
<td>3.96</td>
</tr>
<tr>
<td>2-Pentylfuran</td>
<td>2.44</td>
<td>0.44</td>
<td>3.29</td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>2.48</td>
<td>1.51</td>
<td>3.47</td>
</tr>
<tr>
<td>2-Nonenal</td>
<td>11.82</td>
<td>6.85</td>
<td>9.34</td>
</tr>
<tr>
<td>Trans-2-cis-6-Nonadienal</td>
<td>30.15</td>
<td>14.20</td>
<td>21.85</td>
</tr>
<tr>
<td>BHT</td>
<td>---</td>
<td>27.25</td>
<td>---</td>
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<tr>
<td>?</td>
<td>11.29</td>
<td>8.60</td>
<td>15.90</td>
</tr>
<tr>
<td>?</td>
<td>---</td>
<td>11.37</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^1\) Mean of Two Determination Expressed as % of Total Volatiles.
Figure 31:  MASS SPECTRUM OF UNIDENTIFIED COMPOUND IN FLAVOR VOLATILES OF BHT-TREATED CUCUMBER FRUIT
Figure 32: MASS SPECTRUM OF UNIDENTIFIED COMPOUND IN FLAVOR VOLATILES OF BHT-TREATED CUCUMBER FRUIT
5.1 CHILLING INJURY

Ninhydrin-reactive compounds (NRC) in unchilled fruit remained at the same level during the first week of storage at 20 C. Deterioration of the fruits during the second week occurred in the form of yellowing and shrinking. Chilling increased solute leakage as assayed by the measurement of NRC. Chilled fruits, including those which had been treated with 6% CaCl₂ and 1% BHT had higher mean NRC over the entire period at 20 C compared with unchilled fruits. This assay of CI, which was originally used with root tissues, was observed to be applicable also to fruits. The values obtained were consistent with the visible symptoms of CI observed in the various samples. Experiments by Kozukue et al. (1984) on amino acid changes during chilling showed marked increases in alanine during chilling in cucumbers, okra and eggplant.

There were no visible symptoms of CI in fruits immediately after chilling. Upon transfer to 20 C, pitting and shriveling appeared after two days. By the fifth day, CI was moderate to severe in the samples. Fruits
which were treated with BHT had less surface pitting compared with CaCl₂-treated fruits. Measurements of NRC verified this observation. Over the period of 7 days at 20 C BHT-treated fruits had a mean NRC of 61.25% while CaCl₂-treated fruits had a significantly higher mean of 69.30% (Table 1). Control fruits for CaCl₂ treatment which had been dipped in distilled water had high CI (82.64%) while BHT-control fruits had NRC of 64.02%. BHT-control fruits had been sprayed with a solution of BHT in acetone with Tween 20 as a wetting agent and it is possible that the lesser degree of NRC in BHT-control fruits was due to the film of Tween 20 on the skin of the fruits preventing severe pitting and shrinking. However, the BHT-control and -treated fruits showed the same extent of CI at all time periods, therefore, BHT-treatment did not reduce CI in treated samples. CaCl₂, on the other hand, reduced CI compared with control fruits. CaCl₂-control fruits suffered the highest degree of CI. After 5 and 7 days at 20 C CaCl₂ treatment had decreased CI 8.7 to 24.2% from the control. These results are reasonable on the basis of observations by other investigators that solute loss due to chilling can be prevented or reduced by calcium (Ca²⁺) or magnesium (Mg²⁺) ions (Christiansen et al., 1970). The results of CI evaluation in this study were also consistent with the changes observed in the lipid and fatty acid composition of the fruit. The severe CI in CaCl₂-control fruits which
was reduced by CaCl₂ treatment was accompanied by changes in the linoleic and linolenic acid content of the fruits.

5.2 LIPID CONTENT

Phospholipids, glycolipids and neutral lipids were present in the amounts of 44.13%, 37.47% and 17.50% respectively. Kinsella (1971) reported 39%, 46% and 15% of neutral lipids, phospholipids and glycolipids respectively for field-grown cucumbers. Studies on pickling cucumbers (Peng and Geisman, 1976) showed 29.6% neutral lipids, 60.0% glycolipids and 10.4% phospholipids. Discrepancies in the lipid classes in this study compared with other researchers could be due to varietal differences since this research used greenhouse European cucumbers. Neutral lipids are composed of glycerides, waxes, sterols, sterol esters, hydrocarbons, carotenoid and chlorophyll pigments. The glycolipids are made up of mono- and di-galactosyl-diglycerides and steryl glycosides. Phospholipids are either glycerophosphatides or sphingosylphosphatides. Phospholipids and glycolipids have been found to be the major lipids of fruit tissues (approximately 75%) while neutral lipids, mainly triglycerides, comprised the remaining 25% (Hitchcock and Nichols, 1971).
The total lipid content of cucumber fruit remained the same on chilling and treatment (Table 3) although there were changes in the amounts and composition of each lipid class. It is to be expected that the adaptation to low temperature stress and chemical treatment on the fruits would be manifested in changes in the lipid classes and/or fatty acid composition since these could consequently affect membrane structure and function.

Neutral lipids increased on chilling and CaCl₂ treatment. There was a slight change in glycolipid content for CaCl₂ treatment and control. Chilling increased glycolipids in BHT-control fruits and BHT treatment reduced it to the same level as unchilled fruits. Phospholipids were decreased on chilling. Studies on the lipid composition of chilling-sensitive commodities have dealt primarily with changes in content and composition of polar lipids especially phospholipids. Wilson and Crawford (1974) noticed a decrease in the weights of phospholipids and glycolipids in non-hardened, chill-sensitive *Gossypium hirsutum* and *Phaseolus vulgaris* plants on chilling. They attributed the loss of phospholipids to increased phospholipase activity. In this study the decrease in phospholipids was compensated by an increase in neutral lipids content. Hardening of cucumbers by low temperature has been shown to result in a higher phospholipid level and more unsaturated phospholipid (Horvath et al., 1983). If the chemical treatments could be considered as a type
of hardening process an increase in phospholipid content would be expected on such treatment. This effect was observed with BHT but not with CaCl₂. The effect of CaCl₂ application on lipid content was found in neutral lipids which were increased on treatment. Glycolipid content in this study remained unchanged except for an unexplainable increase on chilling with BHT-control fruits.

Fatty acid composition and degree of unsaturation of polar lipids have been studied more extensively than lipid content. In this research lauric and myristic acids decreased in total lipids and in each lipid class. Analysis of palmitic acid in total lipids showed no significant changes on chilling and treatment. However, the results of analysis of each lipid class showed an increasing trend in this fatty acid in glycolipids on chilling. A study of polar lipids in cucumber fruit by Wang and Baker (1979) reported a similar increase in the level of palmitic acid on application of ethoxyquin and sodium benzoate. An explanation of this observation is not known. Work by Kasai et al. (1978) indicated that a decrease in the molar ratio of palmitoleic to palmitic acid occurred on induction of chilling injury. A recent study by Murata (1983) of phosphatidylglycerols suggested that chilling sensitivity is associated with the dipalmitoyl and 1-palmitoyl-2-(trans-3-hexadecenoyl) species of phosphatidylglycerols. He proposed that differences in the amounts of these two compounds in chilling-sensitive
and chilling-resistant plants is due to preferential activity of acyltransferases during biosynthesis.

Other studies have related the effects of chilling and chemical treatment to the 18-carbon fatty acids and the degree of unsaturation of these fatty acids (Wilson and Crawford, 1974; St. John and Christiansen, 1976; Wang and Baker, 1979). It has been reported by these researchers that unsaturation of fatty acids increases on chilling. However, Yamaki and Uritani (1972) observed no significant change in unsaturated fatty acids of sweet potato as a result of chilling storage. The results of this study did not find a general pattern in unsaturation after chilling. In total lipids (Table 4) chilling of BHT-control fruits increased linoleic and linolenic acid content and BHT treatment maintained these levels after chilling. Chilling of CaCl₂ controls did not change the amounts of linoleic and linolenic acids but CaCl₂ treatment increased their content. In the neutral lipid (Table 5) fraction chilling did not increase linoleic acid content but application of CaCl₂ did. Linolenic acid, however, decreased on chilling but not on treatment. The same trend was observed for linolenic acid in the glycolipid fraction (Table 6). Linolenic acid in this fraction was decreased on chilling but restored by BHT treatment. In the phospholipid class CaCl₂ treatment increased linoleic and linolenic acid, whereas BHT treatment increased only linoleic acid (Table 7). These observations were supported
by unsaturation ratios of the unsaturated and saturated fatty acids (Table 8). The increase in linoleic acid content with CaCl₂ treatment was especially reflected in $R_2$, the ratio of all unsaturated fatty acids to the corresponding saturated fatty acid. For BHT treatment the increase in linoleic acid content in phospholipids was shown by increasing $R_3$ in that fraction. These results are comparable with the observations of Wang and Baker (1979) who found an increase in unsaturated fatty acids on application of free radical scavengers. Changes in the unsaturation of fatty acids are controlled by fatty acid desaturase activity (Kasai et al., 1976). Fluorescence studies by Martin and Thompson (1978) pointed out that lipid modifications first occur in the endoplasmic reticulum where enzyme desaturase systems are associated.

CaCl₂ treatment was observed to produce more changes in unsaturated fatty acid content than BHT (Tables 4, 5, 6 and 7). Studies have shown that calcium ion reduced CI in apples (Scott and Wills, 1979; Conway, 1982). Paliyath and Poovaiah (1982) reported that CaCl₂ application in microsomal preparations altered the temperature of the phase transitions and made it more abrupt. Calcium ion has also been observed to induce phase separation of phospholipids (Jacobson and Papahadjopoulos, 1975). Ingram (1977) applied calcium propionate to E. coli cells and found an increase in the amount of phosphatidylglycerol. He postulated that the effect of this compound was probably
at the level of fatty acid synthesis. Calcium has been shown to have diverse roles in biochemical regulation. Its effect in altering lipid and fatty acid composition could also be due to its interaction with enzymes responsible for desaturation or biosynthesis.

BHT increased linoleic acid in the phospholipid fraction and linolenic acid in the glycolipid fraction. BHT has antioxidant properties and has been shown to interact with membranes (Snipes et al., 1975). It has been used to reduce scald in apples although its mechanism of action is not known (Gough et al., 1973). Studies with a closely related compound butylatedhydroxyanisole (BHA), revealed that the antioxidant inhibited the synthesis of polar lipids (Surak and Singh, 1980). Wang and Baker (1979) working with the free radical scavengers ethoxyquin and sodium benzoate believed that although lipid unsaturation increased on treatment the inconsistency in the ratio of linoleic to linolenic acid in sweet pepper and cucumber fruits indicated that the compounds used did not specifically influence synthesis of linolenic acid. It was observed in this study that visible symptoms of CI were less severe in BHT-treated fruits although the treatment did not significantly lower NRC compared with control fruits. Bramlage (1972) proposed that the action of antioxidants is specific to the development of symptoms and not to a basic effect on chilling through its action on membrane lipids.
5.3 FLAVOR VOLATILES OF CUCUMBER FRUIT

The pH of fresh cucumber homogenate was 5.25. Studies by Hatanaka et al. (1975) reported optimum volatile formation at pH 5.50. Cucumber volatiles are generated when the tissue is cut or ruptured. Fleming et al. (1968) observed that negligible amounts of alkenals and alkadienals were present in intact fruit. Volatile formation requires the presence of oxygen. Linoleic and linolenic acids have been identified as the fatty acids from which the characteristic flavor components are derived (Grosch and Schwarz, 1971). The volatiles observed in this study were hexanal, 2-pentylfuran, 2-hexenal, 6-nonenal, 2-nonenal, trans-2-cis-6-nonadienal and tetradecanal which have been reported by other investigators (Forss et al., 1962; Fleming et al., 1968; Kemp et al., 1974; Hatanaka et al., 1975; Tressl et al., 1981). Mass spectral data for each of these compounds are shown in Figures 5 to 18 and published spectral data are reported in Figures 34 to 46 of the Appendix.

It has been proposed that the volatile components of cucumber flavor can be divided into two types: C₉ alkenals, alkadienals and other shorter chain aldehydes formed by enzymic breakdown of linoleic and linolenic acid and long chain aldehydes (C₁₂ and higher) from α-oxidation of saturated and unsaturated fatty acids (Galliard and Matthew, 1976). The enzyme system which generates the first group of volatiles is believed to be composed of lipoxygenase,
a hydroperoxide cleaving enzyme, an isomerase and possibly an oxidoreductase (Galliard and Phillips, 1976; Galliard et al., 1976; Phillips and Galliard, 1978; Tressl et al., 1981). Hexanal, 2-pentylfuran and 2-nonenal have been related primarily to linoleic acid while trans-2-cis-6-nonadienal and 2-hexenal come from linolenic acid (Grosch and Schwarz, 1971). Pentenylfuran from linolenic acid has also been reported (Tressl et al., 1981). Figure 33 shows the pathway proposed by Tressl et al. (1981) for the formation of aroma constituents of cucumber. In this scheme, cis-3-nonenal (I) formed from the 9-hydroperoxide by $E_2$, is converted to trans-2-nonenal (II), alcohols (IV and V) and 2-pentylfuran (III) by an isomerase ($E_3$), an oxidoreductase ($E_4$) and an uncharacterized enzyme ($E_?$), respectively. The lactones VI, VII, VIII and IX, are believed to be derived also from the reactive cis-3-nonenal. Trans-2-cis-6-nonadienal is formed from trans-3-trans-6-nonadienal by an analogous reaction.

Long chain aldehydes cis-7-hexadecenal, dis-8-heptadecenal, cis,cis-8,11-heptadecadienal, cis,cis,cis-7,10,13-hexadecatrienal and cis,cis,cis-8,11,14-heptadecatrienal have been isolated and identified by Kemp et al. (1974) using mass spectrometry, infrared spectrophotometry (IR) and nuclear magnetic resonance (NMR) spectroscopy. The α-oxidation system believed to be responsible for these reactions
Figure 33: PROPOSED PATHWAY FOR THE FORMATION OF VOLATILE COMPOUNDS OF CUCUMBER FRUIT (FROM TRESSL ET AL., 1981)
was observed in acetone powders of cucumber tissue by Galliard and Matthew (1976). In this study two long chain aldehydes, 9, 17-octadecadienial and 9,12,15-octadecatrienial were tentatively identified by a match of experimental spectra with the spectral library of the Finnigan 4021 GC-MS system (Figures 19 and 20). These compounds can obviously be formed from reduction of linoleic acid and linolenic acids. There is a question, therefore, as to whether these were correctly identified or were actually formed from the original fatty acids by a chemical or enzymatic reduction process.

Forss et al. (1962) reported the following average composition of cucumber volatiles: 10% ethanal, 10% propanal, 15% hexanal, 15% 2-hexenal, 20% 2-nonenal and 30% trans-2-cis-6-nonadienial. However, this study found 0.44% hexanal, 1.58% 2-pentylfuran, 0.63% 2-hexenal, 1.06% 6-nonenal, 15.9% 2-nonenal, 39.05% trans-2-cis-6-nonadienial and 7.12% tetradecanal (Table 12). Differences in these figures are probably due to the fact that this study used European variety cucumbers whereas other researchers used field-grown cucumbers. It is to be noted also that 2-pentylfuran was not reported in the study by Forss (1962) but mentioned by German researchers (Tressl et al., 1981).

In this research chemical treatment was observed to cause changes in the amounts of linoleic and linolenic acids in cucumber tissue. CaCl₂ increased linoleic acid in all lipid fractions and linolenic acid in phospholipids.
It was noticed that at 5 and 7 days after chilling CaCl$_2$-treated fruits had higher levels of hexanal than controls. When formation of volatiles is compared between fresh fruit and CaCl$_2$-treated fruit the amounts of hexanal, 2-hexenal and 2-pentylfuran increased on storage at room temperature. Since CaCl$_2$ application increased linoleic acid and linolenic acid it is reasonable that the formation of the lower boiling compounds would also be increased. The decrease in trans-2-cis-6-nonadienal with storage time at 20°C compared with fresh fruit was probably due to some deteriorative changes in linolenic acid from which it is mainly derived. The observations regarding the effect of CaCl$_2$ on formation of low molecular weight volatiles can also be explained on the basis of the effect of calcium ion on lipoxygenase activity. Calcium has been observed to act either as inhibitor or activator of different isozymes of lipoxygenase (Eskin et al., 1977). It is not known whether there are several isozymes of lipoxygenase in cucumber fruit. It is also possible that calcium ion affects the other enzymes involved in volatile formation. Much still remains to be studied in this area of enzymatic aroma generation in fruits and vegetables.

BHT would be expected to be an inhibitor of lipoxygenase. Its antioxidant activity would prevent the formation of hydroperoxides of lipoxygenase. It has been reported as one of the most powerful antioxidants to inhibit
lipoxygenase activity Eskin et al., 1977). The results of this research showed an increase in linoleic acid in phospholipids on treatment with BHT. BHT residue from the samples steam distilled with the flavor volatiles and contributed to the peak areas detected by gas chromatography. It was positively identified in the extracted volatile constituents. Two other high boiling compounds which appeared to be derived from BHT were also obtained and these made quantitative comparison of volatiles impossible. However, if the counts for the BHT peak are omitted from the total counts and the percentage of the volatiles recalculated (Table 19 of the Appendix), lower levels of hexanal would be observed compared with CaCl₂ treatment.
Chapter VI
SUMMARY AND CONCLUSIONS

European variety cucumbers were grown in the Ohio State University Horticulture greenhouses for this study. Fruits were stored at 4.4 C and the effects of CaCl₂ and BHT on chilling injury, lipid content and formation of flavor volatiles were determined. Three types of controls and two types of chemical treatments were studied. Control fruits consisted of 1) those unchilled and untreated, 2) those dipped in distilled water as control for CaCl₂ treatment, and 3) those sprayed with acetone with 0.1% Tween 20 as control for BHT treatment. Chemical treatments consisted of 6% CaCl₂ dipping and 1% BHT spraying.

Chilling injury was assayed by measuring leakage of ninhydrin-reactive compounds (NRC). This was expressed as the ratio of NRC in intact samples to total NRC in boiled samples. Chilling injury in cucumber fruits was characterized by pitting and shriveling. Visible symptoms of CI were not apparent in chilled fruit immediately after cold storage. On transfer to 20 C pitting and shrinking
appeared after two days. BHT-treated fruits showed less surface pitting than CaCl$_2$-treated fruits. Over the 7-day period at 20 C chilled samples had higher NRC than unchilled control fruits. There was no significant difference between NRC of BHT-treated and control fruits. CaCl$_2$ treatment resulted in significantly lower NRC compared with control fruits.

It is generally believed that the primary effect of chilling is on the fluidity of cell membranes. Lipid content and fatty acid composition were analyzed to determine whether chilling and chemical treatment caused alterations in fatty acid unsaturation which could affect membrane function. Lipids were extracted with chloroform-methanol (2:1, v/v) then separated into neutral lipids, glycolipids and phospholipids by silicic acid column chromatography. Fatty acids were determined by gas chromatography as their methyl ester derivatives. Fresh cucumber tissue contained 0.11% total lipids and this level remained unchanged by chilling and treatment. Total lipids in unchilled, untreated fruit consisted of 17.50% neutral lipids, 37.47% glycolipids and 44.13% phospholipids. Neutral lipid content increased on chilling and CaCl$_2$ treatment. Phospholipids tended to decrease on chilling and increase with BHT treatment. The increase in glycolipids due to chilling was reversed by BHT treatment.
The major fatty acids of interest in CI were palmitic, linoleic, linolenic, oleic and stearic acids. CaCl$_2$ treatment increased linoleic and linolenic acids in total lipids. Oleic and stearic acids decreased on chilling and treatment. In neutral lipids chilling decreased linolenic acid, stearic acid content remained unchanged, while oleic acid increased with CaCl$_2$ application. CaCl$_2$ treatment increased linoleic acid and BHT treatment maintained linoleic acid levels. In the glycolipid fraction, chilling decreased linolenic acid, CaCl$_2$ increased linoleic acid and BHT increased linolenic acid. In the phospholipids chilling and chemical treatment decreased stearic acid, CaCl$_2$ increased linoleic and linolenic acids while BHT increased only linoleic acid.

Unsaturation ratios involving the 16- and 18-carbon saturated and unsaturated fatty acids were studied. It was found that CaCl$_2$ which caused more changes in fatty acid content also showed changes in unsaturation in total lipids and various lipid classes. Lipid and fatty acid analyses showed results that were consistent with the observations on the extent of Cl in the two chemical treatments.

This study also examined the volatile constituents of cucumber flavor which are derived from enzymatic action on linoleic and linolenic acids. Cucumber volatiles were extracted by steam distillation with hexane. The
composition of the extract was analyzed by gas chromatography and compounds identified by gas chromatography-mass spectrometry. The identified volatiles were hexanal, 2-pentylfuran, 2-hexenal, 6-nonenal, 2-nonenal, trans-2-cis-6-nonadienal and tetradecanal. Two high boiling compounds were tentatively identified as 9, 17-octadecadienal and 9,12,15-octadecatrienal. The major component of the volatile extract was trans-2-cis-6-nonadienal which comprised 39.05% of total volatiles followed by 2-nonenal (15.90%), tetradecanal (7.12%), 2-hexenal (0.63%), 2-pentylfuran (1.58%), 6-nonenal (1.06%) and hexanal (0.44%). CaCl₂ treatment increased the amounts of the low boiling volatiles hexanal, 2-pentylfuran and 2-hexenal, and decreased 2-nonenal and trans-2-cis-6-nonadienal after chilling and during storage at 20 C. This could be related to the increase in linoleic acid on CaCl₂ treatment and possibly to the effects of calcium ion on lipoxygenase activity. The major component trans-2-cis-6-nonadienal which is derived mainly from linolenic acid decreased on storage at 20 C. This could be due to decomposition of linolenic acid. It was difficult to quantitatively evaluate the formation of volatiles in BHT-treated fruit, however, the levels of low boiling compounds like hexanal decreased on BHT application, an observation consistent with the inhibitory effect of BHT on lipoxygenase.
The findings of this study could be summarized as follows:

1. Chilled fruits (BHT and CaCl$_2$ controls) had higher NRC than unchilled fruits.
2. BHT-treated fruits had the same degree of NRC as its chilled controls.
3. CaCl$_2$ treatment reduced CI as shown by significantly lower NRC in treated fruits than control fruits. The most severe CI was observed in CaCl$_2$ control fruits.
4. BHT-treated fruits showed less surface pitting than CaCl$_2$-treated fruits and showed lower mean NRC during the entire period at 20 C.
5. The total lipid content of cucumbers (0.11%) was unaffected by chilling and treatment.
6. Neutral lipids increased on chilling and CaCl$_2$ treatment while phospholipids decreased on chilling but was restored by BHT treatment. Chilling increased glycolipids in BHT-control fruits. It decreased to the same level as unchilled fruit after treatment.
7. CaCl$_2$ treatment increased linoleic and linolenic acids and decreased palmitic and stearic acids in total lipids while BHT treatment maintained the levels of these fatty acids.
8. In neutral lipids CaCl$_2$ treatment increased oleic and linoleic acids and decreased palmitic acid. BHT decreased oleic acid.
9. In glycolipids CaCl₂ increased linoleic acid to a certain extent while BHT increased linolenic acid. Both treatments decreased stearic acid.

10. In phospholipids CaCl₂ increased oleic, linoleic and linolenic acids and decreased palmitic acid. BHT increased only linoleic acid.

11. The trend to increasing linoleic acid in all classes due to CaCl₂ treatment was reflected in increasing unsaturation ratios in each lipid class.

12. The volatile components identified in cucumber extract were hexanal, 2-pentylfuran, 2-hexenal, 6-nonenal, 2-nonenal, trans-2-cis-6-nonadienal and tetradecanal. The three major volatiles were trans-2-cis-6-nonadienal, 2-nonenal and tetradecanal.

13. CaCl₂ treatment increased the levels of the low boiling volatiles hexanal, 2-pentylfuran and 2-hexenal.

14. BHT treatment appeared to inhibit the formation of the low boiling components of cucumber volatiles.
Table 15
F VALUES FROM ANALYSIS OF VARIANCE OF LIPID CONTENT AND CHILLING INJURY OF CUCUMBER FRUITS

<table>
<thead>
<tr>
<th>Compound</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Lipids</td>
<td>1.84 n.s.</td>
</tr>
<tr>
<td>Neutral Lipids</td>
<td>27.99 **</td>
</tr>
<tr>
<td>Glycolipids</td>
<td>12.60 **</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>18.32 **</td>
</tr>
<tr>
<td>Ninhydrin-Reactive</td>
<td>16.04 **</td>
</tr>
</tbody>
</table>

n.s.  not significant (P>.05)
** highly significant (P>.01)
<table>
<thead>
<tr>
<th>Compound</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexanal</td>
<td>6.07 n.s.</td>
</tr>
<tr>
<td>2-Pentylfuran</td>
<td>4.00 n.s.</td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>3.99 n.s.</td>
</tr>
<tr>
<td>6-Nonenal</td>
<td>2.45 n.s.</td>
</tr>
<tr>
<td>2-Nonenal</td>
<td>12.48 *</td>
</tr>
<tr>
<td>Trans-2-cis-6-Nonadienal</td>
<td>5.43 n.s.</td>
</tr>
<tr>
<td>Tetradecanal</td>
<td>11.66 *</td>
</tr>
</tbody>
</table>

* Significant Difference (P<.05)

n.s. Not Significant
<table>
<thead>
<tr>
<th>Compound</th>
<th>Unchilled, Untreated Fruit</th>
<th>Treatment (Days at 20°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Chilled Fruit:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Nonenal</td>
<td>15.90 a</td>
<td>13.20 a</td>
</tr>
<tr>
<td>Tetradecanal</td>
<td>5.75 b</td>
<td>11.20 a</td>
</tr>
<tr>
<td><strong>Chilled, CaCl(_2) Treated Fruit:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexanal</td>
<td>0.45 C</td>
<td>2.22 c</td>
</tr>
<tr>
<td>2-Pentylfuran</td>
<td>1.58 b</td>
<td>2.01 b</td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>0.63 b</td>
<td>1.11 b</td>
</tr>
<tr>
<td>Trans-2-cis-6-Non-adienal</td>
<td>39.10 a</td>
<td>26.40 b</td>
</tr>
</tbody>
</table>

Means Within The Same Row Followed By The Same Letter Are Not Significantly Different (P > .05)
<table>
<thead>
<tr>
<th>Compound</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexanal</td>
<td>64.9 **</td>
</tr>
<tr>
<td>2-Pentylfuran</td>
<td>12.68 *</td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>17.9 **</td>
</tr>
<tr>
<td>6-Nonenal</td>
<td>0.54 n.s.</td>
</tr>
<tr>
<td>2-Nonenal</td>
<td>1.08 n.s.</td>
</tr>
<tr>
<td>Trans-2-cis-6-Nonadienal</td>
<td>11.2 *</td>
</tr>
<tr>
<td>Tetradecanal</td>
<td>5.54 n.s.</td>
</tr>
</tbody>
</table>

* Significant Difference (P<.05)
** Highly Significant (P<.01)
n.s. Not Significant
<table>
<thead>
<tr>
<th>Compound</th>
<th>Days At 20° C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Hexanal</td>
<td>0.90</td>
</tr>
<tr>
<td>2-Pentylfuran</td>
<td>2.44</td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>2.48</td>
</tr>
<tr>
<td>2-Nonenal</td>
<td>11.82</td>
</tr>
<tr>
<td>Trans-2-cis-6-Nonadienal</td>
<td>30.15</td>
</tr>
</tbody>
</table>

1 Mean of Two Determinations Expressed as % of Total Volatiles.
**Figure 34:** MASS SPECTRUM OF TRANS-2-TRANS-6-NONADIENAL (FROM HELLER AND MILNE, 1980).
Figure 35: MASS SPECTRUM OF 2-NONENAL (FROM HELLER AND MILNE, 1978).
Figure 36: MASS SPECTRUM OF TRANS-2-NONENAL (FROM HELLER AND MILNE, 1980).
Figure 37: MASS SPECTRUM OF CIS-6-NONENAL
140
6-Nonenal, (E)-

C_{9}H_{16}O

2277-20-5

Figure 38: MASS SPECTRUM OF TRANS-6-NONENAL
(FROM HELLER AND MILNE, 1980).
Figure 39: MASS SPECTRUM OF HEXANAL (FROM HELLER AND MILNE, 1980).
Figure 40: MASS SPECTRUM OF 2-PENTYL FurAn (FROM Heller and Milne, 1980).
Figure 41: Mass spectrum of 2-hexenal (from Heller and Milne, 1978).
Figure 42: MASS SPECTRUM OF TRANS-2-HEXENAL (FROM HELLER AND MILNE, 1980).
Figure 43: MASS SPECTRUM OF TETRADECANAL (FROM HELLER AND MILNE, 1978).
Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-

Figure 45: MASS SPECTRUM OF BUTYLATEDHYDROXYTOLUENE (BHT) (FROM HELLER AND MILNE, 1978).
Figure 46: MASS SPECTRUM OF DODECANAL (FROM HELLER AND MILNE, 1978).
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