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Effects of temperature and maturity on fatty acid composition, lipid related flavor compounds and lipoxygenase activity of strawberries

Yang, Chun-Yung Steve, Ph.D.
The Ohio State University, 1994
EFFECTS OF TEMPERATURE AND MATURITY ON FATTY ACID COMPOSITION, LIPID RELATED FLAVOR COMPOUNDS AND LIPoxyGENASE ACTIVITY OF STRAWBERRIES

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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*****

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1994

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To My Parents
ACKNOWLEDGMENTS

I would like to express my sincere appreciation to Dr. Andrew C. Peng, my research advisor for his guidance and supervision throughout the research. Thanks also go to the other members of my committee, Drs. M. Scott Biggs, Grady W. Chism and Daniel K. Struve, for their valuable suggestions and comments. Gratitude is expressed to Drs. Pablo S. Jourdan, Michael Knee, Mark Lagrimini and Charles V. Morr for generously lending me their laboratory equipment. Special thanks go to Dr. Ewan Ha and Isabelle Laye for their technical assistance on the subject of flavor analysis. I offer sincere gratitude to my fellow graduate students, especially Mary Stuart and Bruce Searles for their remarkable friendship and support. The technical assistance of Joe Takayama and Sara Swain is gratefully acknowledged. To my wife, Feili, I offer sincere thanks for your unshakable faith and unfailing encouragement. To my children, Rachael and Elizabeth, I thank you for understanding my lengthy absence.
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Chapter 1
INTRODUCTION

Lipids play critical roles in fruits, although they are not a major component in most fruits. Lipids give fruits a significant portion of their characteristic flavor as they are precursor of certain volatile compounds. Lipids lessen the chilling damage in fruits by changing the fluidity of cell membranes by altering the composition of fatty acids. Many fruits also contain lipids that are essential to human health.

Studies of lipids in fruits have been carried out in a variety of fruits. For example, Feys et al., (1980) studied the changes in fatty acid composition of apples during post harvest storage. Wade and Bishop (1978) studied changes in lipid composition of ripening banana fruits.

Strawberries (\textit{Fragaria x ananassa }), which have attractive color, aroma and flavor, are highly valued as dessert fruit. The fresh market is the fruit's primary market, followed by the frozen preserves and jam (Mitchell, 1985). Strawberry is one of the most perishable fruits. Generally, it is harvested ripe or almost ripe. In this stage, spoilage of the berry is rapid
(Woodard, 1972). This process, however, can be delayed by rapid cooling (Luoto, 1984). During the process of strawberry harvest, temperature in the container can rise as high as 40 °C for up to 2 hours (Shoemaker, 1975). For each rise of 8.3 °C, the life of berry is reduced 50 % (Shoemaker, 1975). Normally, a five to seven day market life is required when the market is at a great distance from the area of production.

The development of flavor and aroma in fruits is one of the most prominent changes that occur during maturation and storage (Yamashita et al., 1975). For frozen strawberry, deterioration of aroma occurs immediately after freezing (Guadagni et al., 1961). The formation of a number of aroma compounds can be traced back to lipid degradation. Aroma development occurs in a variety of foods both of high and low fat contents. Strawberry is a low fat fruit.

The biogenesis of many flavors in plants involves lipid oxidation (Supran, 1978). These lipids, mainly linoleic and linolenic acids, are oxidized to their hydroperoxides by enzymatic or non-enzymatic transformation, which in turn, yields specific aldehydes and other secondary compounds. These aldehydes, together with a corresponding alcohol, are responsible for many of the characteristic flavors as well as off-flavors in fruits. Generally, lipoxygenase (LOX) catalyses the oxidation of polyunsaturated fatty acids, namely, linoleic and linolenic acids, to hydroperoxides (Galliard and Chan, 1980). LOX activity in
strawberry during fruit growth and storage has not yet been investigated. The qualitative composition of the volatile constituents, sterols, total sugars, and organic acids of strawberry fruit have been investigated extensively for several decades (Shaw, 1988; Couture et al., 1989; Yamashita et al., 1977). Basic lipid analyses of strawberries have been reported (Couture et al., 1988, ). Yet, none of them explores lipid classes and the changes of lipid composition during growth and storage. In this study, lipids and LOX activity of strawberry were analyzed from fruits at different stages of maturity, different conditions of refrigeration and freezing, and mild heat treatment. In addition, antioxidant and control sprays were applied to the fruits to determine the effect of antioxidant spray on fatty acid content of strawberries. Formation of flavor compounds possibly derived from unsaturated fatty acids was also studied.

The objectives of this study were:

1. To provide the basic information of lipid classes and fatty acid composition of strawberry fruits qualitatively and quantitatively at different stages of maturity.
2. To study the quantitative changes in lipid classes and fatty acid composition of strawberry after temperature and antioxidant spray treatments.
3. To determine and examine the formation of possible lipid-derived flavor compounds.
4. To study the changes of LOX activity of strawberries during fruit maturity and after temperature and antioxidant spray treatments.
Strawberry plant

The predominant cultivated strawberry, *Fragaria* x *ananassa*, is a herbaceous perennial (Maas, 1984; Funt et al., 1985). After pollination, the fertilized ovule develops into a seed within the dry, hard single-seeded fruit or achene (Dana, 1981). Each of the "seeds" on the strawberry is in fact a fruit (achene) and the edible structure is an enlarged receptacle upon which many individual fruits are borne (Dana, 1981). Generally, a strawberry fruit purchased from market consists of stem (peduncle), cap (calyx, sepals) and fruit (receptacle plus achenes).

Classification of strawberry. Currently, there are three types of strawberries, based on their photoperiodic responses in flower bud induction; they are June-bearers, everbearers, and day-neutrals (Hamelrick, 1984). Flower buds of June-bearers are initiated on short days (less than 10 hours light per day). Everbearers are long day plants, which initiate flowers when days exceed twelve hours of light. For day-neutrals, flower buds
are generally induced under all normal day lengths, but may be directly modified by temperature (Hamelrick, 1984).

**Lipids**

Lipids from foods can be consumed either in the form of "visible" or "invisible" fats. Visible fats are the lipids separated from the original plant or animal source, such as lard, salad oil, or shortening. Invisible fats are consumed along with other basic components of many foods, such as the fats in milk, meat, fruit, or vegetables.

Generally, lipids are hydrophobic, non polar compounds which either exist naturally as liquids (oils) or solids (fats) at 25 °C. Lipids are mainly composed of long chains of hydrocarbon, and their properties may be changed by the presence of a small number of more reactive and polar groups in the molecule. The hydrocarbon chains are provided by the saturated or unsaturated monocarboxylic aliphatic acids, which are generally termed "fatty acids".

Each lipid class contains a wide selection of fatty acids depending on the lipid and its source; therefore, each lipid is represented by a large number of possible molecular species. In their natural state, lipids occur as compounds containing alcohols (especially glycerol), bases, phosphate esters, sugars, sterols or their combinations (Hitchcock, 1975). As the result of intensive research in lipid chemistry over the past few decades, these lipid
classes can be separated and analyzed by techniques of liquid chromatography and gas chromatography.

By using column chromatography, lipids, in general, can be separated into three major classes according to chemical structure and chromatographic resolution on silica gel columns (Rouser et al., 1967). In general, they can be grouped into: (1) neutral lipids (NL); (2) glycolipids (GL); and phospholipids (PL).

Neutral lipids are mainly composed of triglycerides, which are the fatty acid esters of glycerols. Free fatty acids and diglycerides are also included in this class but to a lesser extent. Triglycerides are the major lipid component in plant tissue. Glycolipids are triglycerides where one of the fatty acid molecules is replaced by a sugar. Monogalactosyldiglyceride and digalactosyldiglyceride occur in a wide range of plant tissues. Phospholipids can be defined as any lipid containing phosphoric acid as a mono- or diester (Nawar, 1985).

Phospholipids are integral components of cell membranes in human, animal and plant tissues (Weihrauch and Son, 1983). They are functional lipids due to their involvement in the function of cell membranes and their ability to interact with metabolites, ions, hormones, antibodies and other cells. Off-flavors are often related to the deterioration of the functional lipids in foods (Pun et al., 1980). Weihrauch and Son (1983) also indicated that most leafy vegetables, fruiting parts, roots and
tubers have lower phospholipid contents than seeds of the legumes or oil seeds.

Over two hundred different fatty acids have been isolated from plants, but the majority of these may be classified as "minor" since they occur in only a few plant species. But the few that are widely distributed in most plants can be termed "major" fatty acids (Hitchcock, 1975). The major fatty acids are either saturated or unsaturated monocarboxylic acids with a straight even-numbered carbon chain.

The saturated major fatty acids, such as lauric (dodecanoic), myristic (tetradecanoic), palmitic (hexadecanoic) and stearic (octadecanoic) acids all occur in plants, but unsaturated fatty acids, oleic (cis-9-octadecenoic), linoleic (cis-9, cis-12-octadecadienoic) and linolenic (cis-9, cis-12, cis-15-octadecatrienoic) acids are much more abundant. The unsaturated fatty acids carry the general structure of the cis-double bond in the 9-position and of the cis-, cis-, 1,4-pentadiene or "methylene-interrupted" structure (Hitchcock and Nichols, 1971).

_Lipids in fruits._ The "flesh" tissue of most fruits, such as plums, pears, peaches and apples, has a relatively low content of lipid. But in rare cases, such as in oil palm (*Elaeis guineensis*), the flesh does contain a lipid content as high as 70% of the fresh tissue weight (Hitchcock and Nichols, 1971). In spite of their
comparatively low concentrations in fruits, lipids perform important functions conferring stability upon storage, organoleptic properties, and nutritional and biological value (Kolesnik et al., 1987).

Studies of lipids in fruits have been carried out in different fruits based on the above noted aspects. Nicolosi-Asmundo et al. (1987), suggested that the lipid content in citrus fruit has an effect on stability of juice and significantly changes the flavor of juice after pasteurization and storage. In addition, Nordby and Nagy (1969, 1971), and Nagy and Nordby (1974) proposed to use the fatty acid profile as a marker to determine the percentage of hybrids among citrus fruits due to the distinguishable difference in type lipid content of the various citrus fruits. Hopkirk and Wills (1981), indicated that a low linoleic acid content renders apple fruit more susceptible to soft scald.

For most fruits, their fatty acid composition profile is predominated by "major" fatty acids such as palmitic, oleic, linoleic and linolenic acids and are characterized by high degree of unsaturation (Kolesnik et al., 1987; Oboh and Oderinde, 1988; Aslanov and Mamedova, 1985; Takenaga et al., 1985; Couture et al., 1988; Blakesley et al., 1979). According to Takenaga et al. (1985), total lipid content is higher in seeds than that in endocarps (based on fresh weight) for bayberries.
Lipids in fruits during ripening. Ripening is a very complex process that consists of both synthesis and degradation of cell components (Whitaker, 1988). According to Blakesley et al. (1979), lipid content of mango, papaya and strawberry is highly dependent on the degree of maturity. Several lines of evidence indicate that free radical-induced lipid peroxidation plays a role in bringing about many of the deleterious changes associated with fruit ripening (Meir et al., 1991; Selvaraj, 1989; Galliard, 1968; Kimura et al., 1982). Both Gaydou et al. (1987) and Couture et al (1988), agreed that lipid content increased with increasing ripening of avocado and strawberry, respectively.

Donaire and Lopez-Gorge (1978) demonstrated that the lipid biosynthesis in olive fruit has been correlated with fruit development. In this study, the fatty acid composition of total lipids, and the incorporation of labeled carbon from [1-14C] acetate into the different lipid groups had been studied during the development of olive fruit. It was discovered that when [1-14C] acetate was applied to the fruit in the earlier stages of its development, there was a greater percentage of 14C incorporation into polar lipids (glycolipids and phospholipids). In the later stages, labeled carbon incorporating into neutral lipid increased, while 14C incorporation into glycolipids and phospholipids decreased.

Grosbois and Mazliak (1964), observed that during ripening the relative amounts of unsaturated fatty acids in the pulp lipid
of banana fruit decreased. This result coincided with the research done by Goldstein and Wick (1969); they concluded that the unsaturated fatty acids decreased in both pulp and peel during ripening. Wade and Bishop (1978) further indicated that the relative proportions of neutral lipid, glycolipid and phospholipid did not change in banana fruit pulp tissue during development. However, the fatty acid composition of the lipids did change during ripening. This change occurred mainly in the phospholipid fraction, in which, the proportion of linolenic acid increased, and the proportion of linoleic acid decreased. This results in an increase in total unsaturation of the fatty acids in the phospholipid fraction.

**Temperature effect on plant lipids.** Many researchers have suggested that low temperature induces an increase in the polyunsaturated fatty acid content of plant cell membranes, by which optimum membrane fluidity is maintained (Radwan et al., 1978; Breidenbach and Waring, 1977; Mazliak, 1981; Gawer et al., 1983). Willemot (1983) studied the effect of a lethal frost on the lipid composition of crown and root tissue of winter wheat; after thawing, rapid degradation of polar lipids occurred. This study further discovered that the polyunsaturated species of polar lipids were preferentially degraded. In 1989, Parkin and Kuo studied the chilling effect on lipids of the fruit of cucumber. When cucumber fruits were chilled at 4 °C in the dark, lipid
degradation occurred. Decreases in unsaturation (the ratio of unsaturated : saturated fatty acids) of both glycolipids and phospholipids were observed in fruits rewarmed after chilling for three and seven days, respectively.

Volatile Compounds of Strawberries

The qualitative and quantitative composition of the volatile constituents in strawberry fruits has been studied extensively. The number and components of volatiles of the fruits varies considerably between cultivars. In addition, the volatile profiles of strawberries were highly dependent on the degree of maturity (Blakesley et al., 1979). Pyysalo et al. (1979) identified 87 compounds in the wild strawberry, Fragaria vesca L., and 58 in the cultivated berries, Fragaria × ananassa, 'Senga Sengana'. The compound, 2,5-dimethyl-4-methoxy-3-(2H)-furanone was identified as the main volatile component in wild type strawberries and also as an abundant component in the cultivated varieties.

In 1983, Hirvi determined the concentrations of the most important aroma compounds, i.e., ethyl hexanoate, ethyl butanoate, trans-2-hexenal, 2,5-dimethyl-4-methoxy-3-(2H) furanone and linalool, among the cultivars 'Senga Sengana', 'Kristina' and 'Lihama × Senga Sengana'. Methyl and ethyl butanoate, methyl and ethyl hexanoate, trans-2-hexenyl acetate, trans-2-hexenal, trans-2-hexen-1-ol and 2,5-dimethyl-4-
methoxy-3(2H)-furanone were identified by Schreier (1980) as the main volatile components of the cultivated strawberries *Fragaria ananassa* 'Senga Sengana', 'Senga Litessa' and 'Senga Gourmella'. While Ueda and Iwata (1982) studied the aroma of fresh and frozen strawberries, they found that esters were the dominant compounds in fresh berries, but were hardly detected in frozen-thawed berries. Generally, many aliphatic alcohols and esters were found in the volatile compounds of strawberries and the importance of esters, acetals, alcohols and aldehydes to the strawberry aroma has been pointed out by Teranishi et al. (1963).

**Roles of Lipid in Flavors**

It might be thought that lipids are most important as sources of flavor compounds. The statement may be debatable because of many important flavor compounds are derived from proteins and carbohydrates. But generally, lipids are not only important as precursors of many volatile flavors, but also contribute to other flavors (Forss, 1969).

The formation of a number of flavor compounds can be traced back to lipid degradation. This is particularly true for the formation of aldehydes belonging to the *n*-alkanal, *n*-alk-2-enal, and *n*-alka-2,4-dienal group. These aldehydes can exist in a variety of foods both of high and low fat content (Eriksson, 1975). The release of short chain fatty acids by hydrolysis of ester bonds
in lipids (lipolysis) is responsible for the development of a rancid flavor in foods (Nawar, 1985). Lipid-derived aldehydes and other carbonyl compounds have in the past attracted the interest of researchers because these secondary autoxidation products are among the most important substances responsible for the flavor and off-flavor of many foodstuffs and beverages.

Autoxidation of Lipids

The major oxidative deterioration reaction involved in lipids is "autoxidation", which is the reaction with molecular oxygen (Nawar, 1985). In foods, lipids can be either oxidized by enzymatic or non enzymatic reactions. It is generally accepted that the autoxidation of lipids proceeds through a free radical chain reaction, and can be characterized by the following stages: (1) initiation, (2) propagation, and (3) termination. The process needs initiation through the formation of an initial radical. The production of the free radical in the initiation step occurs by decomposition of hydroperoxides, by metal catalysis of lipids, or by exposure to light (Nawar, 1985). In the propagation stage, hydrogen atoms are abstracted at the position $\alpha$ to double bonds ($\alpha$-methylene groups). Oxygen then is added to the positions where free radicals occurred, resulting in the formation of peroxy radicals, which in turn attack another $\alpha$-methylene group yielding a lipid hydroperoxide and a new lipid radical which propagates the chain reaction. The termination of autoxidation is
by the interaction of radicals to produce non-propagating and non-initiating species.

For the autoxidation of polyunsaturated fatty acid, the detachment of a hydrogen atom generally occurs at the central methylene group of a 1,4-pentadiene system. Because of resonance stabilization of the free radicals, the reaction sequence is usually accompanied with a shift in the position of double bonds, resulting in the formation of isomeric hydroperoxides which often contain conjugated double bonds (Nawar, 1985). Abstraction of a hydrogen atom at one of these methylene groups would yield a free radical that would be stabilized through resonance with the three contributing structures showed in FIG.1.

The abstraction of a hydrogen atom from the central methylene group of a 1,4-pentadiene should be easier than the detachment of a hydrogen atom from an ordinary α-methylene group by virtue of the greater resonance energy of the former radical. This appears to be the main reason for the higher rate of autoxidation of the fatty acids with 1,4-pentadiene structure.

Antioxidants shorten the length of the propagation sequence by enhancing the termination step. They are frequently phenolic compounds of natural or synthetic origin. The hydroperoxides, which are the first products of autoxidation, are rather unstable and easily decomposed into other products.
FIG. 1. Free radical formation from the methylene interrupted structure and its three contributing structures through resonance stabilization.
Flavors from Oxidation of Lipids by Enzymes. With few exceptions, enzymes manipulate the biosynthesis of natural flavors or tastes, many of which originate from lipid substrates. Flavors develop from lipids by a series of enzymatic reactions. Commonly, tissue damage by either crushing, freezing or macerating initiates enzymatic responses in reactions that transform the endogenous lipids of tissues. Normally, the lipolytic enzymes are inactive, and become active only when they are released from their association with specific subcellular organelles.

In plant tissue, free fatty acids are released mainly by lipolysis of galactolipid, phospholipid and triglyceride (Gardner, 1985). Once the free acids are released by lipolysis, lipoxygenase catalyzes the oxidation of polyunsaturated acids into hydroperoxides. Although free fatty acids are the usual substrates for lipoxygenase, certain isoenzymes also catalyze the oxidation of glyceride-bound fatty acid. Dillard et al. (1960), provided some evidence of the occurrence of two types of lipoxygenases, one attacking only free fatty acids and another attacking linoleic acid in triglycerides.

Lipoxygenase

Lipoxygenases (EC 1.13.11.12) are defined as enzymes that catalyze the oxidation of unsaturated fatty acids containing a 1,4-cis, cis-pentadiene structure. So far, known lipoxygenases
contain one non-heme iron center that is essential to proper catalytic functioning (Vliegenthart et al., 1982). Enzymes from plants produce cis, trans-conjugated monohydroperoxides as primary products. Moreover, Galliard and Chan (1980) indicated that lipoxygenases catalyze cooxidation reactions, resulting in the bleaching of natural pigments (chlorophylls, carotenoids, etc.) and modification of functional protein groups.

**Lipoxygenase activity in plants.** Much research has been done on activity of lipoxygenase in various plants. Vick and Zimmerman (1976) reported that the maximum activity of lipoxygenase of watermelon seedlings occurred on the sixth day after germination. For mango fruit, it was found that lipoxygenase activity increased from harvest maturity until the half-ripe stage and then declined (Selvaraj, 1989). In 1985, Zamora et al. indicated that lipoxygenase activity in grapes was higher in partially ripe than in fully ripe fruits. Hildebrand and Hymowitz (1983) discovered that the total activity of lipoxygenase-1 per seed increased to maturity and then decreased as germination proceeded. For cotton seedlings, the activity of lipoxygenase was very low in the seed but increased immediately after germination, reached a maximum after three to four days, and then declined (Vick and Zimmerman, 1981).

Lipoxygenase activity during storage was investigated in the core, flesh, and peel of apple (Feys et al., 1980). During
storage, activity increased in each part of the fruit. Increase in lipoxygenase preceded the browning of the core, and it was suggested that lipoxygenase may be responsible for the browning and may be involved in the induction of superficial scald (Feys et al., 1980). The activity of lipoxygenase increased when the plant tissue was ruptured, such as noted by Takeo and Tsushida (1980) when experimenting with plucking of tea shoots.

**Multiple forms of lipoxygenase in plants.**

Lipoxygenases are widely distributed in nature and have been found in both plants and animals. The highest lipoxygenase activity has been found in legume seeds, particularly, lipoxygenases of soybean are the mostly intensively studied (Christopher et al., 1972; Diel and Stan, 1978; Hildebrand and Hymowitz, 1983).

The possible existence of multiple forms of soybean lipoxygenase has been observed by several researchers (Christopher et al., 1972; Diel and Stan, 1978; Hildebrand and Hymowitz, 1983; Galliard and Phillips, 1971; Guss et al., 1967, 1968). At least two main isoenzymes of lipoxygenase have been identified in soybean seeds. Christopher et al. (1972) indicated that lipoxygenase-1 has a maximum activity at pH 9.5 and is most effective in the oxidation of free fatty acids containing a cis, cis-1,4-pentadiene structure. Lipoxygenase-2, which is optimally active at pH 6.5 (Galliard and Chan, 1980), is more
effective in the oxidation of esterified pentadiene-containing fatty acids. Additionally, lipoxygenase-1 has much greater heat stability than lipoxygenase-2 (Christopher et al., 1972; Diel and Stan, 1978).

Other evidences suggested that both types of lipoxygenases can be further separated into isoenzymatic forms (Yamamoto et al., 1970; Weber et al., 1974; Christopher et al., 1972). In 1972, Christopher et al. purified and characterized a third isoenzyme of lipoxygenase from soybeans. This lipoxygenase-3 possesses a broad pH activity profile throughout the pH range of 4.5 to 9.0. Most plants do not contain lipoxygenase-1 but contain lipoxygenase-2 instead (Chism, 1985).

**Distribution of lipoxygenase in plants.** Lipoxygenase activity has been demonstrated in many plant organs (Pinsky et al., 1971), including leaves (Grossman, 1969; Takeo and Tsushida, 1980), tubers (Berkeley and Galliard, 1976; Galliard, 1970; Pinsky et al., 1973; Wardale, 1980), seeds (Vick and Zimmerman, 1976; Hildebrand and Hymowitz, 1983; Christopher et al., 1972), cotyledon (Eriksson, 1967), roots (Wardale and Galliard, 1977) and fruits (Bonnet and Crouzet, 1977; Kim and Grosch, 1979; Zamora et al., 1985).

When studying lipoxygenase activity within the cell, variation of subcellular localization of lipoxygenase was greater than in whole plant. Evidences showed that the lipoxygenase of
potato tubers was located in fragile organelles separated from mitochondria, microbodies and plastids (Wardale and Galliard, 1975). In 1977, Wardale and Galliard further discovered that in pea roots, the activity of lipoxygenase was localized in the 'lysosomal' fraction, whereas with brassica florets (cauliflower and calabrese) it was present in a heavy body with a density similar to plastids. In cucumber fruits, Wardale and Lambert (1980) indicated that lipoxygenase activity was associated with the vacuoles.

**Optimum pH for lipoxygenase in plants.** The optimum pH for lipoxygenase activity varied between different plants. Kim and Grosch (1979) studied lipoxygenase activity of apples and found the enzyme had a pH optimum at 6.0. Wardale (1980) and Galliard (1970) suggested that at pH 5.5 - 5.6, high lipoxygenase activity was detected in potato tubers. In green peas, pH 7 was found to be the suitable condition for the activity of lipoxygenase. The pH optimum for most lipoxygenase ranges from 5.5 to 7.0 (Vick and Zimmerman, 1981,1982; Wardale and Lambert, 1980; Takeo and Tsushida, 1980; Grossman, 1969; Eskin and Henderson, 1974; Pinsky et al., 1973; Ganthavorn and Powers, 1989; Bonnet and Crouzet, 1977). Soybean lipoxygenase-1 is anomalous, having an optimum at about pH 9.
Aerobic and anaerobic reactions of lipoxygenases.

Under normal conditions, in the presence of molecular oxygen, lipoxygenase catalyzes the oxygenation of unsaturated fatty acids containing a cis,cis - 1,4-pentadiene structure and results in the production of cis, trans-conjugated monohydroperoxides as primary products (Vliegenthart et al., 1982). But, it has been suggested that besides the activity of oxygenation of unsaturated fatty acids, some lipoxygenases show catalytic ability under anaerobic conditions (Spaapen et al., 1977; Schieberle et al., 1981). For the anaerobic activity of soybean lipoxygenase-1, the primary product of the aerobic reaction of lipoxygenase, hydroperoxide, serves as the second substrate instead of oxygen.

Substrate specificity of lipoxygenase. As mentioned above, lipoxygenase catalyzes oxygenation of the unsaturated fatty acids contain cis, cis - 1,4-pentadiene structure. The efficiency of the reaction is likely to vary depending on the position of the 1,4-diene (Galliard and Chan, 1980). It was found that linoleic acid in the form of 9- cis, 12 -cis isomer was oxygenated at the highest rate. While Holman and Burr (1945) indicated that lipoxygenases in crude extracts attacked linoleic acid, linolenic acid, their esters, and methyl arachidonate. Furthermore, it was found that linoleate, linolenate, and arachidonate were oxidized at the same rate. Therefore, it was concluded that the position of the double bonds is not critical.
However, only the natural isomers (cis isomers) of linoleic and linolenic acids are attacked. The specificity of oxygen insertion in the substrate molecule depends on the oxygen concentration, pH and temperature (Christopher et al., 1972).

**Product specificity.** In aerobic reactions, lipoxygenases catalyze oxygenation of unsaturated fatty acids and result in the production of conjugated *cis, trans*-pentadiene hydroperoxides with the trans double bond next to the hydroperoxide. The product specificity of this reaction depends on the enzyme (source and purity), the type of fatty acid, and the incubation conditions (concentrations of the reactants and temperature). Generally, a mixture of 9- and 13-hydroperoxides is formed when linoleic acid is used as the substrate (Vliegenthart et al., 1982). Christopher et al., (1972) indicated that under optimal conditions (optimum pH, excess of oxygen), linoleic acid is converted predominantly into 13-hydroperoxide by lipoxygenase-1. While catalyzed by lipoxygenase-2, linoleic acid is converted into a mixture of 9- and 13-hydroperoxide, oxodienoic acids and other unidentified products.

Under anaerobic conditions, lipoxygenase-1 catalyze the reaction between 13-hydroperoxide and linoleic acid with the formation of 13-oxo-*cis (trans)*-9, *trans*-11-tridecadienoic acid, *n*-pentane and 13-oxo-9, 11- octadecadienoic acid (Garssen et al., 1971). Although the 13-hydroperoxide is the major product
from the aerobic reaction, the 9-hydroperoxide is a better substrate for the anaerobic reaction in the absence of linoleic acid (Chism, 1985).

Inhibitors. Many studies of the inhibition of lipoxygenase have been made, but, perhaps due to the crude enzyme preparations and the different conditions of incubation, there is little agreement in the reports.

A variety of inhibitors of lipoxygenase have been reported. Two lipoxygenase isoenzymes were separated from potato tubers (Pinsky, 1973). These two enzymes had an optimum pH of 5.5, and were not affected by calcium ions but were inhibited by cysteine. Research done by Belver et al. (1982), verified the inhibiting effect of boron on sunflower seed germination. Wardale and Lambert (1980) indicated that the enzyme activity of the crude extract of lipoxygenase of cucumber fruit was not inhibited by calcium, EDTA or cysteine. In 1970, Galliard also demonstrated that neither calcium nor EDTA had any effect on lipoxygenase activity. While Kim and Grosch (1979) showed that a membrane-bound lipoxygenase partially purified from apples, which had a pH optimum at 6.0, could be inhibited by EDTA, disodium salt, or cyanide. The lipoxygenase activity of asparagus was also inhibited by cyanide (Ganthavorn and Powers, 1989). In addition, detergent such as Triton X-100 or Tween-20 have been
reported to have an inhibitory effect on lipoxygenase activity in grapes (Zamora et al., 1985).

Activation of lipoxygenase by calcium ions was demonstrated by several researchers. Spaapen et al. (1977) observed that both the aerobic and anaerobic reactions at pH 9.0 by pea lipoxygenase were stimulated by calcium ions. Christopher et al. (1970) indicated that lipoxygenase-2 is stimulated by calcium. In 1972, Christopher et al. separated a third lipoxygenase (lipoxygenase-3) from soybean and further demonstrated that the activity of lipoxygenase-3 was inhibited by calcium ions.

In despite of little agreement on the inhibitors of lipoxygenase, polyphenols, which are antioxidants that act as free radical scavengers, are generally conceded to be inhibitors of lipoxygenase activity (Chism, 1985; Bonnet and Crouzet, 1977; Grossman, 1969).

**Lipoxygenase Assays**

Several methods have been used to measure lipoxygenase activity in plants. The reaction can be following by measuring (1) loss of fatty acid substrate, (2) oxygen consumption, (3) hydroperoxide formation, (4) appearance of a conjugated diene chromophore and, (5) cooxidation of a cosubstrate (Galliard and Chan, 1980).
The major concern of lipoxygenase assays is how well the fatty acid substrate dispersed into aqueous solution. The most commonly used substrate, linoleic acid, forms soluble soap at pH 9. It may be the optimal condition for lipoxygenase-1 (optimum pH at 9), but most lipoxygenases from plants possess an optimum pH of between 5.5 and 7.0, and in this pH range, fatty acid substrate dispersion is inadequate. Therefore, a variety of emulsifiers, such as Tween 80 (Schieberle et al., 1981) and Tween 20 (Chen and Whitaker, 1986; Eskin and Henderson, 1974) have been used to ensure that the fatty acid substrate becomes soluble.

**Methods based on loss of fatty acid substrate.** Measuring the amount of radioactively labeled fatty acid substrate left is a useful indicator of lipoxygenase activity in crude extracts, but it is neither specific nor rapid (Berkeley and Galliard, 1976).

**Methods based on oxygen consumption.** Two different methods were developed based on the fact that the oxidation of unsaturated fatty acids involves consumption of molecular oxygen by the substrate. The manometric method is one of the oldest techniques for lipoxygenase activity (Grossman and Zakut, 1979). This technique can be applied with crude or purified extracts, but the procedure is complicated and tedious.
Due to its extended assay time, it allows the secondary reactions to become more significant. Therefore, generally speaking, it is an obsolete method for measuring lipoxygenase activity.

Polarographic methods (oxygen electrode methods) which measure the same oxygen uptake as the manometric methods, are widely used and replace the manometric methods (Galliard and Chan, 1980). The polarographic method is rapid, sensitive, and specific and gives continuous recording, and it is normally the method of choice for crude extracts (Wardale and Galliard, 1975; Wardale, 1980; Ganthavorn and Powers, 1989; Belver et al., 1982; Kim and Grosch, 1979; Feys et al., 1980; Schieberle et al., 1981; Sekiya et al., 1978).

Methods based on the formation of hydroperoxides. Several techniques using colorimetric measurement of peroxides have been used for lipoxygenase assays. The ferric thiocyanate method has been used for measurements of the linoleate peroxide formed by lipoxygenase (Grossman and Zakut, 1979). The reaction is based on the conversion of Fe(CNS)₂ to the colored Fe(CNS)₃ by the hydroperoxide formed during the lipoxygenase reaction. Generally, peroxide determinations are limited to the easily extractable triglycerides. Furthermore, peroxides are unstable, and can be easily decomposed even when lipid oxidation is far advanced.
**Methods based on the formation of conjugated diene.** With the exception of polarographic methods, this method can be one of the most commonly used assays for lipoxygenase activity (Bonnet and Crouzet, 1977; Al-Obaidy and Siddiol, 1981; Vick and Zimmerman, 1981; Chen and Whitaker, 1986; Hildebrand and Hymowitz, 1983; Zamora et al., 1985; Andrawis et al., 1982; Wardale et al., 1978; Selvaraj, 1989). Formation of conjugated dienoic acids increases the absorbency at 234 nm. The method has the advantage of simplicity and short experimental time, and is particularly suitable for purified enzymes (Galliard and Chan, 1980). Optically clear solutions are required for this method.

**Methods based on cooxidation.** Cooxidation of carotenoids may act as an indicator for measuring lipoxygenase activity. The insolubility of carotenoids in aqueous medium and the pH dependent solubility of the fatty acid substrate bring obstacles to the coupled oxidation of carotene. Furthermore, not all lipoxygenase has the same ability to catalyze cooxidation of pigments to the same degree (Chism, 1985).

**Aroma Compounds Derived from Oxidized Lipids**

As mentioned previously, lipids are the precursors of certain flavor compounds (Eriksson, 1975; Zamora et al., 1985; Forss, 1969). The development of flavor compounds from lipids
involves the process of oxidation of lipids enzymatically or non-enzymatically. Although free unsaturated fatty acids are the normal substrates for peroxidation processes, some fatty acids in the form of triglycerides are also involved. The primary initial products of this peroxidation of lipids are hydroperoxides, and are relatively unstable (Nawar, 1985). Chain scission occurs to some degree during lipid oxidation, resulting in a variety of flavor compounds, the primary products being aliphatic aldehydes with some hydrocarbons and alcohols (Eriksson, 1975).

**Hydroperoxide formation of lipid autoxidation.**
Evidence has been shown that the autoxidation of lipids involves the free radical chain reaction (Gunstone, 1984; Nawar, 1985). This radical chain reaction involves initiation, propagation and termination (Fig. 2). The rate of reaction and the structure of the hydroperoxides depend on the structure of the resonance-stabilized allylic radical R produced from the unsaturated fatty acids RH and the type of isoenzyme of lipoxygenase when enzymatic oxidation is involved (Gunstone, 1984).

From the autoxidation point of view, a mono-unsaturated fatty acid (such as oleic acid), hydrogen was abstracted at C8 and C11, and due to the resonance of radicals, four radical intermediates were formed which resulted in the formation of 8-, 9-, 10-, and 11- allylic hydroperoxides (Nawar, 1985). For linoleic and linolenic acids, an equal mixture of conjugated 9- and
INITIATION \[ RH \rightarrow R^\cdot \]

PROPAGATION \[ R^\cdot + O_2 \rightarrow ROO^\cdot \]
\[ ROO^\cdot + RH \rightarrow ROOH + R^\cdot \]

TERMINATION interaction of radicals to produce nonradical products

FIG. 2. Scheme for radical chain reaction of lipid autoxidation.
13-diene hydroperoxides has been produced by oxidation of the former, and conjugated 9-, 12-, 13-, and 16-hydroperoxides from oxidation of the latter fatty acid (Nawar, 1985).

Lipoxygenases act only upon fatty acids containing methylene interrupted double bonds, such as linoleic, linolenic, and arachidonic acids and result in forming generally the same hydroperoxides as those formed by the autoxidation of these same fatty acids. The primary products of the aerobic reactions of this oxygenation of polyunsaturated fatty acids are conjugated cis, trans - pentadienyl hydroperoxides (Vliegenthart et al., 1982; Galliard and Chan, 1980).

Generally, a mixture of hydroperoxy fatty acids differ in the position of the hydroperoxide group, i.e., with linoleic acid as the substrate, a mixture of 9- and 13- hydroperoxides is formed. Most lipoxygenases catalyze the formation of one specific positional isomer. Kim and Grosch (1979) indicated that a membrane-bound lipoxygenase partially purified from apple, which had a pH optimum of 6.0, converted linoleic acid predominantly into the 13- hydroperoxide. Soybean lipoxygenase-1 was reported to be almost 100% specific for the formation of 13-hydroperoxide from linoleic acid at pH 9.0, while lipoxygenase-2 formed a mixture of equal amounts of the 9- and 13- hydroperoxides at pH 6.6 (Christopher and Axelrod, 1971). In addition, Chen and Whitaker (1986) showed that the
isoenzyme-1 of English pea formed 50.3% of 13-hydroperoxide and 49.7% of 9-hydroperoxide at pH 7.0.

It is believed that pH plays an important role in controlling the formation of particular hydroperoxide isomers. The positional specificity of partially purified corn germ lipoxygenase was investigated as a function of pH with linoleic acid as the substrate (Veldink et al., 1972). The 13-hydroperoxide was formed predominantly at pH 9.0 and major formation of 9-hydroperoxide at pH 6.6 respectively.

Galliard and Phillips (1971) demonstrated that at pH 5.5, soybean lipoxygenase catalyzed the oxidation of linoleic acid into the formation of 54% 9-hydroperoxide and of 46% 13-hydroperoxide. Another study was conducted to determine the pH effect on hydroperoxide specificity of soybean lipoxygenase-1 (Roza and Francke, 1973) and indicated that at pH 9, formation of the 13-isomer was predominant, while at pH 7 the same enzyme catalyzed the formation of almost equal amounts of the 9- and 13-isomers. However, the pH effects observed by the above researchers can be explained as more than one isoenzyme of lipoxygenases were involved, and certain isoenzymes are more active at specific pH (Christopher and Axelrod, 1971).

Decomposition of hydroperoxides. The monohydroperoxides, initial products of peroxidation of fatty acids, are rather unstable and easily decompose into a wide
variety of products. Generally speaking, lipid derived aldehydes are formed by the chain cleavage reactions on hydroperoxides. Different hydroperoxides form different sets of initial breakdown products which are based upon the position of the peroxide group located in the original hydroperoxides. These initial products can themselves undergo further decomposition and result in the formation of even larger secondary or tertiary products.

Although the complexity of the peroxidation reactions of lipids has not yet permitted clear identification of the pathways of biosynthesis of all aldehydes found in various peroxidation reactions of lipids, it is reliable to assume that most of the aldehydes are produced by one or more of the chain scission reactions shown in FIG. 3 (Nawar, 1985).

The first step in the decomposition of hydroperoxides is the scission reaction taking place at the oxygen-oxygen bond of the hydroperoxide group, resulting in the formation of an alkoxy radical and a hydroxy radical. On the second step, cleavage of the carbon-carbon bond on either side of the alkoxy radical occurred, thus contributing to the formation of an aldehyde and an acid or a hydrocarbon and an oxoacid, depending on whether the cleavage was taking place on the acid side or on the hydrocarbon side, respectively. However, if a vinylic radical was produced from such a scission, an aldehyde was formed (FIG. 4) (Nawar, 1985).
\[ R_1 - \text{CH} - R_2 \rightarrow R_1 - \text{CH} - R_2 + ^\cdot \text{OH} \]

- \text{alkoxy radical}

FIG. 3. Aldehyde formation by the scission reactions of hydroperoxides.
FIG. 4. Aldehyde formation from a vinylic radical.
Aldehydes belonging to the three groups, namely C1-10 n-alkanals, C3-12 n-2-alkenals, and the C5-12 n-2,4-alkadienals, are formed by the oxidation of unsaturated fatty acids (Eriksson, 1975; Forss, 1969). Therefore, decomposition of monohydroperoxides derived from linoleic acid results in the formation of 2,4-decadienal, 2-octenal, and n-hexanal (Forss, 1969).

Researchers have reported that C6 aldehydes and alcohols (n-hexanal, cis-3-hexenal, trans-2-hexenal, hexanol) are formed from linoleic and linolenic acids (Galliard et al., 1977; Takeo and Tsushida, 1980; Hatanaka et al., 1976; Zamora et al., 1985; Selvaraj, 1988). Arens and Grosch (1974) and Grosch et al., (1974) reported that the oxidation reaction between a purified pea lipoxygenase and linolenic acid substrate resulted in the formation of propanal, 2-pentenal and 2,4-heptadienal. In addition, a number of other carbonyl compounds such as cis-4-heptenal and trans-2, cis-6-nonadienal have been isolated from oxidized lipids, and it has been suggested that these flavor compounds resulted from the oxidation of other C18 unsaturated fatty acids (Forss, 1969).
Chapter III
MATERIALS AND METHODS

Materials

Fresh cultivated strawberries (*Fragaria x ananassa* cv. 'Tristar') were grown in the Ohio State University Horticultural greenhouse. 'Tristar' is a stele resistant, day-neutral cultivar. It bears fruit three times per season (Funt et al., 1985). Fruits were harvested at different stages of fruit maturity, i.e., green, white, pink, and red stages.

Treatments of Samples

*Temperature treatments.* Red strawberries (fruit with peduncle and calyx), were divided into five groups: fresh strawberry (no treatment), strawberry refrigerated at 5 °C for 4 days and 7 days respectively, strawberry heated at 40 °C for 2 hours, and strawberry frozen at -20 °C for 6 months.

*Chemical applications.* Each group described above for the temperature treatments was further divided into other subgroups: with butylatedhydroxytoluene (BHT), an antioxidant spray and control spray. BHT was dissolved in acetone (1% w/v)
with 0.1% Tween 20 and applied to strawberry as a spray. Strawberries sprayed with 0.1% Tween 20 in acetone served as the control.

**Lipid Analysis**

*Lipid extraction.* Since seed lipid is one of the more prominent features of the fatty acid composition of plant tissues, it was investigated in addition to the lipid content of whole strawberries. Duplicate samples (~50 g each for whole strawberry and ~0.5 g each for strawberry seed) were subjected to steam for 5 minutes to inactivate the LOX prior to lipid extraction.

Lipid from strawberry fruit (receptacle plus achenes) and seeds were extracted with Folch reagent (chloroform : methanol, 2 : 1, v/v) in 10 parts of reagent with one part of sample (v/w) (Folch et al., 1957) for 2 minutes by using the polytron homogenizer (Brinkmann Instruments, Westbury, NY) at room temperature and filtered with a Buchner funnel under reduced pressure. The residue was re-extracted with same portion of Folch reagent.

The combined extract was transferred quantitatively into a separatory funnel and washed with 0.25 volume of potassium chloride (0.88%) by shaking the mixture vigorously and allowing it to settle. The lower chloroform phase was then transferred to another separatory funnel and washed with 0.25 volume of
water-methanol (1:1, v/v). Again, the chloroform phase was filtered through anhydrous sodium sulfate to remove residual moisture and solvent was removed under vacuum by a rotary evaporator at reduced pressure at 45 °C. Extracted lipids were stored in a vacuum desiccator until a constant weight was obtained and stored in a freezer until analysis.

**Column chromatography.** Strawberry lipids were separated into three classes by silicic acid column chromatography (Rouser et al., 1967). A 1.1 cm diameter glass column was used with a 250 ml reservoir flask at a sample loading ratio of 2:100 (by weight) adsorbent. Neutral lipids were eluted by chloroform at an elution ratio of 25 ml/g adsorbent. Glycolipids and phospholipids were eluted by acetone and methanol at 40 and 25 ml/g adsorbent respectively. All three lipids were eluted at the same flow rate, 0.5 ml/minute.

Each fraction of lipid classes was collected in bulk and the solvent was removed by rotary vacuum. Each lipid class was monitored by thin layer chromatography (TLC) on silica gel GF plates, 5 x 20 cm, 250 μ thickness (Analtech, Newark, DE). The developing solvent for neutral lipid was chloroform, and chloroform: acetone: methanol: acetic acid: water (65:20:10:10:3, v/v) for glycolipids and phospholipids (Lepage, 1967). Diphenylamine solution was prepared and used for detecting lipid classes.
Gas chromatography (GC). Analyses of fatty acids were performed by a Hewlett Packard model 5890 A gas chromatograph equipped with a flame ionization detector (FID) and a Hewlett Packard model 3390 A electric integrator. The coiled stainless steel column (244 cm x 0.3 cm, i.d.) packed with 10% sp 2330 on 100/120 Chromosorb W AW (Supelco, Bellefonte, PA) was used for the analysis. The GC was operated under the following conditions: column temperature, 190 °C; detector temperature, 220 °C; injection port temperature, 220 °C; carrier gas, nitrogen; carrier gas flow rate, 20 ml/min. (Peng, 1974).

Fatty acids of each lipid class were converted into their methyl esters by boron-trifluoride methanol according to Metcalfe et al., (1966). Fatty acid composition was identified by comparing the retention time of known standards. The concentration of the fatty acids was expressed as the percent area of the total area of all methyl ester peaks.

Enzymatic Activity of Lipoxygenase

Substrate emulsion. Linoleic acid solution was prepared by dispersing 0.2 ml linoleic acid (NU - CHEK, > 99%) in 0.2 ml double distilled water with of an equal amount of Tween 20. The solution was neutralized with 1 N NaOH and diluted with water to 25 ml and emulsified in an ultra sonic bath for 1 min. Emulsions were prepared fresh.
Preparation of crude strawberry extract. Diced strawberries (~15 g) were homogenized with a polytron homogenizer for 2 minutes in sodium phosphate buffer (0.2 M, pH 9.1) in 100% suspension (15 g of strawberry per 15 ml of buffer), containing 0.005 M ethylene diaminetetracetic acid (EDTA) and 1% of Triton X-100. The homogenate was centrifuged at 15,000 g for 20 min. The pH of the supernatant was adjusted to pH 6.5 by dialyzing with 0.2 M sodium phosphate buffer (pH 6.5) in Spectra/Por molecular porous membrane tubing (m.w. cut off: 12,000 ~ 14,000, Fisher Scientific, Lexington, MA). The dialyzed supernatant was used as the source of enzyme extract.

Lipoxygenase (LOX) activity. Activity was determined at 25 °C with a Rank Electrode (Rank Brothers, Cambridge, England) connected to a Cole-Parmer dual channel flatbed recorder (Cole-Parmer Scientific Co., Chicago, IL). One ml of linoleic acid substrate and 0.5 ml of enzyme extract were incubated in the reaction vessel for 10 minutes to determine LOX activity. Linoleic acid solution was agitated in the reaction vessel for 3 minutes before adding the enzyme extract.

Enzyme activities were calculated from initial rates of O2 uptake. Initial dissolved O2 concentration was assumed relative to pure H2O. It was assumed that the O2 concentration was 5.776 ml/L (Seidell, 1919). All data were the mean of two runs.
and expressed as specific activity (oxygen consumption \( \mu l \) per mg protein per min.).

**Protein content of enzyme extract.** Protein content of the enzyme extract was measured by using a Coomassie Blue Binding method (Sedmak and Crossberg, 1977). To 0.1 ml of sample (in this case, 1 ml of sample was pre-diluted to 25 ml with sodium phosphate buffer), 1 ml of coomassie blue reagent was added, shaken, incubated for 10 minutes, and then measured at 595 nm. Bovine serum albumin (BSA, Sigma, St. Louis, MO) was used for the standard protein curve.

**Flavor Volatiles**

**Headspace volatile sampling by the purge and trap method.** Flavor compounds of mature strawberry (strawberry with temperature and chemical treatment included) were analyzed by using the purge and trap method developed by Ha et al. (1991). Duplicated strawberry (~10 g) samples were quartered and placed into a sealed glass serum bottle.

Volatile of strawberry were swept from their head space with a Tekmar model LSC 2000 purge-and-trap concentrator (Tekmar Co., Cincinnati, OH) with ultra high purity helium at a flow rate of 40 ml/minutes and adsorbed on a Tenax No. 1 trap which was baked 10 minutes at 225 °C immediately before analysis to avoid background artifacts. Adsorbed volatile
compounds were disorbed from the trap by heating at 180 °C for 5 minutes. The disrobed compounds were cryogenetically focused in the liquid nitrogen cooled capillary interface and then automatically injected into the gas chromatograph/mass spectrometer (GC/MS).

**Gas chromatography and mass spectrometry analysis.** Volatiles were identified by a Hewlett Packard 5890 series II gas chromatograph and model 5971 A Mass Selective Detector (MSD), equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm, 0.25 μm film thickness) from J & W Scientific Inc. (Rancho Cordova, CA). High purity helium served as the GC carrier gas at a flow rate of 1 ml/minute. Programmed oven temperature was at 35 °C for 5 minutes, to 180 °C at 5 °C/minute increment. The column was then heated to 200 °C at a rate of 15 °C/minute and held at the same temperature for 4 minutes to condition the column for the next injection.

Mass spectra analysis was programmed by the following conditions: transfer line temperature, 280 °C; mass analyzer temperature, 180 °C; ion source temperature, 180 °C. Tentative identification of flavor compounds was by computer matching of full or partial mass spectra in the NIST/EPA/MSDC 49K Mass Spectral Database (1988) at the Haas Chair Laboratory of Food Science Department at The Ohio State University.
Statistical Analysis

Analysis of variance and Tukey multiple comparison (Keppel, 1982) at 5% were used to compare means of controls and treatments. Statistical analysis were performed by using the MINITAB statistical package with a Macintosh personal computer.
Lipid Analysis

*Lipid content of strawberry.* The moisture contents (based on fresh weight) of strawberry fruits (receptacle plus achenes) are 84%, 91%, 89% and 82% for green, white, pink and red stages of maturity respectively. The oil contents of seeds and whole fruits (receptacle plus achenes) of the different stages are listed in Table 1. For the oil content of seeds (based on fresh weight) from the four stages of maturity, the green stage contains significantly less percent oil than the red and pink stages, but there are no differences between the green and white stages nor the red and pink stages. On the aspect of oil content of whole fruits (based on fresh weight) of the four stages of maturity, only the green stage contains a significantly higher percentage oil than the red stage, no difference is found among the other stages.
Table 1. Percentage lipid content\(^1\) of seeds and whole fruits of strawberry.

<table>
<thead>
<tr>
<th>STAGE</th>
<th>% LIPID OF SEEDS</th>
<th>% LIPID OF FRUITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>GREEN STAGE</td>
<td>3.22(_a)</td>
<td>0.36(_a)</td>
</tr>
<tr>
<td>WHITE STAGE</td>
<td>14.36(_{ab})</td>
<td>0.26(_{ab})</td>
</tr>
<tr>
<td>PINK STAGE</td>
<td>17.10(_b)</td>
<td>0.26(_{ab})</td>
</tr>
<tr>
<td>RED STAGE</td>
<td>17.28(_b)</td>
<td>0.22(_b)</td>
</tr>
</tbody>
</table>

1. % of lipid content in seeds and whole fruits is the mean of two measurements.

Means within the same column followed by the same letter are not significantly different (\(\alpha = 0.05\)).

Tukey's \(W\) for % oil of seeds = 13.07
Tukey's \(W\) for % oil of fruits = 0.11
Distribution of lipid classes. The weight percentage of three lipid classes; neutral lipids (NL), glycolipids (GL) and phospholipids (PL) separated from the crude oils of seeds and fruits from the four stages of maturity, are listed in Table 2. Table 2 clearly shows that NL of fruits and seeds increases as maturity increases. On the other hand, for strawberry fruits and seeds, PL decreases as maturity increases. GL of fruits decreases with the increase of maturity, but this trend is not observed in the GL of seeds.

When comparing the distribution of lipid classes within fresh, red fruits with no spray, control spray and with BHT spray (Figure 5); it is found that for NL of whole fruits, the berries with the control spray contain the highest NL content, with no difference between fruits with no spray and BHT spray. However, for GL of whole fruits, berries with the control spray have the lowest GL content among the three types of treatments, and again no difference is observed between fruits without spray and with BHT spray. For PL of whole fruits, only strawberries sprayed with BHT showed a significantly higher PL content than fruits with no spray.

As for the lipid class distributions in the seeds of strawberries with the same treatments described above (Figure 6), the seeds of fresh fruits with no spray contain the lowest NL among the three treatments, and no difference is observed
Table 2. Weight percentage\(^1\) of neutral lipids (NL), glycolipids (GL) and phospholipids (PL) in seeds and fruits of strawberry.

<table>
<thead>
<tr>
<th>STAGE</th>
<th>SEED NL%</th>
<th>SEED GL%</th>
<th>SEED PL%</th>
<th>FRUIT NL%</th>
<th>FRUIT GL%</th>
<th>FRUIT PL%</th>
</tr>
</thead>
<tbody>
<tr>
<td>GREEN</td>
<td>41.06a</td>
<td>22.76a</td>
<td>36.18a</td>
<td>29.54a</td>
<td>26.90a</td>
<td>43.55a</td>
</tr>
<tr>
<td>WHITE</td>
<td>63.13b</td>
<td>12.92b</td>
<td>23.95ab</td>
<td>54.06b</td>
<td>17.44b</td>
<td>28.49b</td>
</tr>
<tr>
<td>PINK</td>
<td>75.32b</td>
<td>12.60b</td>
<td>12.08b</td>
<td>67.74b</td>
<td>13.77b</td>
<td>18.50b</td>
</tr>
<tr>
<td>RED</td>
<td>69.09b</td>
<td>18.26ab</td>
<td>12.65b</td>
<td>64.05b</td>
<td>16.22b</td>
<td>19.74b</td>
</tr>
</tbody>
</table>

1 % of weight of NL, GL and PL in seeds and whole fruits is the mean of two measurements.

Means within the same column followed by the same letter are not significantly different (\(\alpha = 0.05\)).

Tukey's \(W\) for % NL of seeds = 13.20
Tukey's \(W\) for % GL of seeds = 6.69
Tukey's \(W\) for % PL of seeds = 13.45
Tukey's \(W\) for % NL of fruits = 18.58
Tukey's \(W\) for % GL of fruits = 7.09
Tukey's \(W\) for % PL of fruits = 11.95
Figure 5: Fresh weight percentage distribution of lipid classes in red fruits from no spray, control spray or BHT spray samples.

Tukey's $W$ for % NL of fruits = 8.02
Tukey's $W$ for % GL of fruits = 6.63
Tukey's $W$ for % PL of fruits = 2.69
Tukey's $W$ for % NL of seeds = 13.05  
Tukey's $W$ for % GL of seeds = 3.86  
Tukey's $W$ for % PL of seeds = 10.57

Figure 6:  Fresh weight percentage distribution of lipid classes in the seeds of red strawberry from no spray, control spray or BHT spray samples.
between the control and BHT spray treatments. For GL in seeds, no difference is found between the control and BHT spray treatments, and seeds in fresh fruits without any spray has the highest GL content. There is no significant difference within the three treatments when the PL of seeds is compared.

Comparisons of the effects of temperature treatments (Temp 1: no temperature treatment; Temp 2: 5 °C, 4 days; Temp 3: 5 °C, 7 days; Temp 4: 40 °C, 2 hours; Temp 5: -20 °C, 6 months) and BHT spray on the % of distribution of lipid classes of seeds and whole fruits of red strawberry are illustrated in Figures 7 to 12.

For the NL of whole red fruits, berries treated with the BHT spray show a significantly lower content than those with the control spray (Figure 7). From the temperature treatment point of view, only Temp 1 berries show a significantly higher NL content than the Temp 2 berries. From Figure 8, it is not possible to conclude whether the content of GL in whole fruits with BHT spray is significantly different than strawberries with the control spray. Only the Temp 1 treated samples show that fruits with BHT spray contain more GL than the control sprayed fruits. No significant difference is found between the BHT and control sprayed samples of berries subjected to the other temperature treatments. Temp 2 berries with the control spray show a higher GL content than the Temp 1 and Temp 4 berries with the control spray. As for the PL of whole fruits (Figure 9),
Tukey's $W = 7.03$

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 7: The effects of temperature treatments and BHT spray on the % of distribution of NL content of red whole fruits.
Tukey’s $W = 4.80$

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 8: The effects of temperature treatments and BHT spray on the % of distribution of GL content of red whole fruits.
Tukey's $W = 5.42$

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: 20 °C for 6 months.

Figure 9: The effects of temperature treatments and BHT spray on the % of distribution of PL content of red whole fruits.
berries with the BHT spray have higher PL contents than those with the control spray. For the temperature treatments, only Temp 3 fruits show higher PL contents than the Temp 5 fruits.

No significant difference is observed in the NL of seeds of red strawberries between the BHT and control spray (Figure 10). Seeds of Temp 1 berries show higher NL content than seeds of either Temp 2 or Temp 3 berries. Both seeds of Temp 4 and Temp 5 berries show no significant difference in NL content when compared to seeds of Temp 1 berries.

For GL of seeds of red strawberry (Figure 11), a difference occurs only in Temp 3 treated samples, seeds with the control spray have a higher GL content than the seeds with BHT spray. No other difference in GL content between the BHT and control sprays is observed. When sprayed with the control spray, seeds of both Temp 2 and Temp 3 berries have a higher GL content than the seeds of Temp 1 berries. For samples sprayed with BHT, only seeds of Temp 2 berries show a higher GL content than the seeds of Temp 1 berries.

Statistically, for PL in seeds of red strawberries (Figure 12), no significant difference is observed for the five temperature treatments between the two sprays.
Tukey’s $W = 9.89$

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 10: The effects of temperature treatments and BHT spray on the % of distribution of NL content of seeds of red strawberries.
Tukey's $W = 5.56$

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 11: The effects of temperature treatments and BHT spray on the % of distribution of GL content of seeds of red strawberries.
Tukey's $W = 13.57$

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 12: The effects of temperature treatments and BHT spray on the % of distribution of PL content of seeds of red strawberries.
The effect of different stages of maturity on fatty acid composition of the lipid classes of whole fruits (based on mg/100 g of sample). In this study, the fatty acids listed below are the ones commonly found in all lipid classes of strawberry: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. The results of different stages of maturity on fatty acid composition of lipid classes of whole strawberry are listed on Tables 3 to 5.

Neutral lipids  For lauric acid, the green stage contains significantly more than the red, pink and white stages (Table 3). There are no significant differences between the stages for myristic, stearic and linoleic acids. For palmitic acid, the green stage is greater than the red and pink stages. For oleic acid, only the white stage is greater than the green stage. As for linolenic acid, the green stage has a lower content than any of the red, pink and white stages. Finally, the pink stage has a higher linolenic acid content than that of the white stage.

Glycolipids  For lauric, and oleic acids, no difference is observed among the four stages of maturity (Table 4). Strawberries at the green stage have the highest content of myristic, palmitic and stearic acids. On the contrary, the green stage has the lowest content of linoleic and linolenic acids.

Phospholipid  No differences occurred for the lauric acid content of the four different stages of maturity. As shown in
Table 3. Fatty acid content (mg /100 g of sample) of neutral lipid (NL) of whole fruits from four stages of maturity (red, pink, white and green).

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>Fatty Acid</th>
<th>Red Stage</th>
<th>Pink Stage</th>
<th>White Stage</th>
<th>Green Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL 12 : 0</td>
<td>0.16a</td>
<td>0.14a</td>
<td>0.32a</td>
<td>1.68b</td>
<td></td>
</tr>
<tr>
<td>NL 14 : 0</td>
<td>0.14a</td>
<td>0.14a</td>
<td>0.34a</td>
<td>1.73a</td>
<td></td>
</tr>
<tr>
<td>NL 16 : 0</td>
<td>7.86a</td>
<td>9.23a</td>
<td>14.78ab</td>
<td>35.08b</td>
<td></td>
</tr>
<tr>
<td>NL 18 : 0</td>
<td>1.38a</td>
<td>1.51a</td>
<td>3.57a</td>
<td>7.76a</td>
<td></td>
</tr>
<tr>
<td>NL 18 : 1</td>
<td>16.85ab</td>
<td>16.28ab</td>
<td>28.15b</td>
<td>5.62a</td>
<td></td>
</tr>
<tr>
<td>NL 18 : 2</td>
<td>59.36a</td>
<td>71.20a</td>
<td>42.22a</td>
<td>17.91a</td>
<td></td>
</tr>
<tr>
<td>NL 18 : 3</td>
<td>54.80ab</td>
<td>70.67b</td>
<td>41.11a</td>
<td>17.97c</td>
<td></td>
</tr>
</tbody>
</table>

Fatty acid 12 : 0 - lauric acid; Fatty acid 14 : 0 - myristic acid; Fatty acid 16 : 0 - palmitic acid; Fatty acid 18 : 0 - stearic acid; Fatty acid 18 : 1 - oleic acid; Fatty acid 18 : 2 - linoleic acid; Fatty acid 18 : 3 - linolenic acid.

Tukey's W for lauric acid = 1.15
Tukey's W for myristic acid = 2.14
Tukey's W for palmitic acid = 22.60
Tukey's W for stearic acid = 7.66
Tukey's W for oleic acid = 20.36
Tukey's W for linoleic acid = 55.55
Tukey's W for linolenic acid = 19.70

Means in the same row followed by the same letter are not significantly different (α = 0.05).
Table 4. Fatty acid content (mg / 100 g of sample) of glycolipid (GL) of whole fruits from four stages of maturity.

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>Fatty Acid</th>
<th>Red Stage</th>
<th>Pink Stage</th>
<th>White Stage</th>
<th>Green Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL 12 : 0</td>
<td>0.09a</td>
<td>0.07a</td>
<td>0.00a</td>
<td>0.52a</td>
<td></td>
</tr>
<tr>
<td>GL 14 : 0</td>
<td>0.20a</td>
<td>0.15a</td>
<td>0.25a</td>
<td>0.99b</td>
<td></td>
</tr>
<tr>
<td>GL 16 : 0</td>
<td>8.42a</td>
<td>7.46a</td>
<td>12.60a</td>
<td>54.92b</td>
<td></td>
</tr>
<tr>
<td>GL 18 : 0</td>
<td>1.21a</td>
<td>1.07a</td>
<td>2.11a</td>
<td>14.06b</td>
<td></td>
</tr>
<tr>
<td>GL 18 : 1</td>
<td>3.23a</td>
<td>2.55a</td>
<td>3.38a</td>
<td>2.69a</td>
<td></td>
</tr>
<tr>
<td>GL 18 : 2</td>
<td>9.28a</td>
<td>9.44a</td>
<td>9.62a</td>
<td>2.59b</td>
<td></td>
</tr>
<tr>
<td>GL 18 : 3</td>
<td>10.42a</td>
<td>11.22a</td>
<td>13.29a</td>
<td>1.86b</td>
<td></td>
</tr>
</tbody>
</table>

Fatty acid 12 : 0 - lauric acid; Fatty acid 14 : 0 - myristic acid; Fatty acid 16 : 0 - palmitic acid; Fatty acid 18 : 0 - stearic acid; Fatty acid 18 : 1 - oleic acid; Fatty acid 18 : 2 - linoleic acid; Fatty acid 18 : 3 - linolenic acid.

Tukey's W for lauric acid = 0.53
Tukey's W for myristic acid = 0.13
Tukey's W for palmitic acid = 7.64
Tukey's W for stearic acid = 2.45
Tukey's W for oleic acid = 1.39
Tukey's W for linoleic acid = 2.14
Tukey's W for linolenic acid = 3.75

Means in the same row followed by the same letter are not significantly different (α = 0.05).
Table 5, for myristic acid, the white strawberries have a significantly higher content than the strawberries of the other three stages of maturity. For palmitic acid and stearic acid, no difference is observed between red and pink strawberries or between white and green strawberries. The green strawberries contain the highest amount of oleic and linoleic acids, but no difference is observed among the other three stages. For linolenic acid, the green strawberry still has the highest content, and the white strawberries have the least content among the four stages. No difference is observed between red and pink strawberries for the fatty acid content of linolenic acid.

The effect of different stages of maturity on fatty acid composition of the lipid classes of seeds (based on mg/g of sample of seeds). The seeds of strawberries carry the same type of fatty acids as the whole strawberry fruits do; lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids. The fatty acid contents of strawberry seeds are listed in Tables 6 to 8.

Neutral lipid For lauric acid, no difference occurs among the four stages of maturity (Table 6). Seeds of the green and pink stages have a higher myristic acid content than seeds of the red stage, but there are no differences between seeds of the red and white stages. For palmitic acid, seeds of the green stage have the lowest fatty acid content. Pink stage seeds have a higher
Table 5. Fatty acid content (mg / 100 g of sample) of phospholipid (PL) of whole fruits from four stages of maturity.

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>Fatty Acid</th>
<th>Red Stage</th>
<th>Pink Stage</th>
<th>White Stage</th>
<th>Green Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>12:0</td>
<td>0.01a</td>
<td>0.01a</td>
<td>0.03a</td>
<td>0.03a</td>
</tr>
<tr>
<td>PL</td>
<td>14:0</td>
<td>0.10a</td>
<td>0.09a</td>
<td>0.20b</td>
<td>0.13a</td>
</tr>
<tr>
<td>PL</td>
<td>16:0</td>
<td>14.95a</td>
<td>14.68a</td>
<td>46.52b</td>
<td>54.36b</td>
</tr>
<tr>
<td>PL</td>
<td>18:0</td>
<td>1.43a</td>
<td>1.51a</td>
<td>5.44b</td>
<td>6.78b</td>
</tr>
<tr>
<td>PL</td>
<td>18:1</td>
<td>2.09a</td>
<td>2.09a</td>
<td>2.34a</td>
<td>4.33b</td>
</tr>
<tr>
<td>PL</td>
<td>18:2</td>
<td>15.27a</td>
<td>17.12a</td>
<td>11.10a</td>
<td>56.53b</td>
</tr>
<tr>
<td>PL</td>
<td>18:3</td>
<td>7.65a</td>
<td>9.65a</td>
<td>4.58b</td>
<td>31.14c</td>
</tr>
</tbody>
</table>

Fatty acid 12:0 - lauric acid; Fatty acid 14:0 - myristic acid; Fatty acid 16:0 - palmitic acid; Fatty acid 18:0 - stearic acid; Fatty acid 18:1 - oleic acid; Fatty acid 18:2 - linoleic acid; Fatty acid 18:3 - linolenic acid.

Tukey's W for lauric acid = 0.02
Tukey's W for myristic acid = 0.04
Tukey's W for palmitic acid = 17.52
Tukey's W for stearic acid = 2.20
Tukey's W for oleic acid = 1.11
Tukey's W for linoleic acid = 11.08
Tukey's W for linolenic acid = 2.71

Means in the same row followed by the same letter are not significantly different (α = 0.05).
Table 6. Fatty acid content (mg / 1 g of sample) of neutral lipid (NL) of strawberry seeds from four stages of maturity.

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>Fatty Acid</th>
<th>Red Stage</th>
<th>Pink Stage</th>
<th>White Stage</th>
<th>Green Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL 12:0</td>
<td>0.03a</td>
<td>0.04a</td>
<td>0.04a</td>
<td>0.05a</td>
<td></td>
</tr>
<tr>
<td>NL 14:0</td>
<td>0.06a</td>
<td>0.08b</td>
<td>0.07ab</td>
<td>0.08b</td>
<td></td>
</tr>
<tr>
<td>NL 16:0</td>
<td>5.37a</td>
<td>7.34b</td>
<td>6.14ab</td>
<td>2.56c</td>
<td></td>
</tr>
<tr>
<td>NL 18:0</td>
<td>1.18ab</td>
<td>1.36a</td>
<td>1.10b</td>
<td>0.43c</td>
<td></td>
</tr>
<tr>
<td>NL 18:1</td>
<td>11.60a</td>
<td>20.52b</td>
<td>12.39a</td>
<td>1.22c</td>
<td></td>
</tr>
<tr>
<td>NL 18:2</td>
<td>41.63a</td>
<td>50.68b</td>
<td>33.82c</td>
<td>5.35d</td>
<td></td>
</tr>
<tr>
<td>NL 18:3</td>
<td>33.02a</td>
<td>43.96b</td>
<td>32.64a</td>
<td>2.29c</td>
<td></td>
</tr>
</tbody>
</table>

Fatty acid 12:0 - lauric acid; Fatty acid 14:0 - myristic acid; Fatty acid 16:0 - palmitic acid; Fatty acid 18:0 - stearic acid; Fatty acid 18:1 - oleic acid; Fatty acid 18:2 - linoleic acid; Fatty acid 18:3 - linolenic acid.

Tukey's $W$ for lauric acid = 0.05
Tukey's $W$ for myristic acid = 0.02
Tukey's $W$ for palmitic acid = 1.26
Tukey's $W$ for stearic acid = 0.22
Tukey's $W$ for oleic acid = 2.18
Tukey's $W$ for linoleic acid = 6.62
Tukey's $W$ for linolenic acid = 7.81

Means in the same row followed by the same letter are not significantly different ($\alpha = 0.05$).
palmitic acid content than red stage seeds, but no difference is found between the palmitic acid content of seeds of pink and white or red and white stages. For stearic acid, green stage seeds have the lowest content, while pink stage seeds have a higher fatty acid content than white stage seeds but not the red stage seeds. For oleic and linolenic acids, seeds from the green stage have the lowest fatty acid content, seeds from the pink stage have higher contents than those of either red or white stages, and there is no difference between the red and white stages. Seeds of the green stage contain the lowest amount of linoleic acid. Seeds of the pink stage have a higher linoleic acid content than either the red or white stages. In addition, the linoleic acid content of seeds of the red stage is higher than that of the white stage seeds.

Glycolipids For lauric acid, no difference is found in the seeds of the four stages of maturity (Table 7). For myristic acid, both seeds of red and pink stages have a higher fatty acid content than seeds of either the green or white stages. Seeds of the red stage have the highest palmitic and stearic acid content with no differences observed among the other three stages. For oleic acid, seeds of the red stage have a higher fatty acid content than those of either the white or green stages, but there is no difference between the red and pink stages. Seeds of the white stage seem to have the highest linoleic acid but no significant difference is found between the white and pink stages, nor is
Table 7. Fatty acid content (mg / 1 g of sample) of glycolipid (GL) of strawberry seeds from four stages of maturity.

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>Fatty Acid</th>
<th>Red Stage</th>
<th>Pink Stage</th>
<th>White Stage</th>
<th>Green Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL 12:0</td>
<td>0.03a</td>
<td>0.04a</td>
<td>0.01a</td>
<td>0.05a</td>
<td></td>
</tr>
<tr>
<td>GL 14:0</td>
<td>0.33a</td>
<td>0.23a</td>
<td>0.11b</td>
<td>0.09b</td>
<td></td>
</tr>
<tr>
<td>GL 16:0</td>
<td>5.19a</td>
<td>3.41b</td>
<td>2.61b</td>
<td>2.46b</td>
<td></td>
</tr>
<tr>
<td>GL 18:0</td>
<td>2.57a</td>
<td>1.09b</td>
<td>0.64b</td>
<td>0.62b</td>
<td></td>
</tr>
<tr>
<td>GL 18:1</td>
<td>7.24a</td>
<td>4.28ab</td>
<td>2.12b</td>
<td>1.04b</td>
<td></td>
</tr>
<tr>
<td>GL 18:2</td>
<td>2.55a</td>
<td>5.14b</td>
<td>6.98c</td>
<td>1.15a</td>
<td></td>
</tr>
<tr>
<td>GL 18:3</td>
<td>2.44ab</td>
<td>4.18a</td>
<td>5.30a</td>
<td>0.80b</td>
<td></td>
</tr>
</tbody>
</table>

Fatty acid 12:0 - lauric acid; Fatty acid 14:0 - myristic acid; Fatty acid 16:0 - palmitic acid; Fatty acid 18:0 - stearic acid; Fatty acid 18:1 - oleic acid; Fatty acid 18:2 - linoleic acid; Fatty acid 18:3 - linolenic acid.

Tukey's W for lauric acid = 0.13
Tukey's W for myristic acid = 0.11
Tukey's W for palmitic acid = 1.64
Tukey's W for stearic acid = 1.31
Tukey's W for oleic acid = 4.89
Tukey's W for linoleic acid = 1.46
Tukey's W for linolenic acid = 3.03

Means in the same row followed by the same letter are not significantly different (α = 0.05).
there a difference between the red and green stages. For linolenic acid, seeds of both the pink and white stages have higher fatty acid contents than seeds of the green stage, but there is no difference between seeds of the red and green stages.

Phospholipids For lauric acid, white stage seeds contain the highest amount of fatty acid, with no differences among the other stages (Table 8). For myristic and linoleic acids, only white stage seeds show a significantly higher fatty acid content than green stage seeds with no other differences observed. Seeds of the white stage contain more palmitic acid than seeds of the red stage. There are no differences between stages of maturity for stearic and oleic acids. For linolenic acid, the white stage contains more fatty acid than either the red or green stages, but no difference is found between the white and pink stages.

The effect of different temperature treatments and chemical sprays on fatty acid composition of the lipid classes of whole fruits (based on mg / 100 g of sample). Seven fatty acids are commonly found in the strawberry fruits of this study, they are, lauric acid (12 : 0), myristic acid (14 : 0), palmitic acid (16 : 0), stearic acid (18 : 0), oleic acid (18 : 0), linoleic acid (18 : 2) and linolenic acid (18 : 3).

Neutral lipids The effects of temperature treatments and chemical sprays (BHT spray and control spray) on fatty acid composition of NL of whole fruits is listed in Table 9 of the
Table 8. Fatty acid content (mg / 1 g of sample) of phospholipid (PL) of strawberry seeds from four stages of maturity.

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>Fatty Acid</th>
<th>Red Stage</th>
<th>Pink Stage</th>
<th>White Stage</th>
<th>Green Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL 12 : 0</td>
<td>0.01a</td>
<td>0.06a</td>
<td>0.15b</td>
<td>0.01a</td>
<td></td>
</tr>
<tr>
<td>PL 14 : 0</td>
<td>0.10ab</td>
<td>0.20ab</td>
<td>0.30a</td>
<td>0.04b</td>
<td></td>
</tr>
<tr>
<td>PL 16 : 0</td>
<td>2.96a</td>
<td>4.09ab</td>
<td>7.82b</td>
<td>3.55ab</td>
<td></td>
</tr>
<tr>
<td>PL 18 : 0</td>
<td>1.05a</td>
<td>0.96a</td>
<td>2.05a</td>
<td>0.72a</td>
<td></td>
</tr>
<tr>
<td>PL 18 : 1</td>
<td>3.22a</td>
<td>3.98a</td>
<td>4.73a</td>
<td>0.89a</td>
<td></td>
</tr>
<tr>
<td>PL 18 : 2</td>
<td>6.06ab</td>
<td>6.09ab</td>
<td>9.94a</td>
<td>3.77b</td>
<td></td>
</tr>
<tr>
<td>PL 18 : 3</td>
<td>3.33ab</td>
<td>3.70ab</td>
<td>6.63b</td>
<td>2.02a</td>
<td></td>
</tr>
</tbody>
</table>

Fatty acid 12 : 0 - lauric acid; Fatty acid 14 : 0 - myristic acid; Fatty acid 16 : 0 - palmitic acid; Fatty acid 18 : 0 - stearic acid; Fatty acid 18 : 1 - oleic acid; Fatty acid 18 : 2 - linoleic acid; Fatty acid 18 : 3 - linolenic acid.

Tukey's $W$ for lauric acid = 0.08
Tukey's $W$ for myristic acid = 0.22
Tukey's $W$ for palmitic acid = 4.70
Tukey's $W$ for stearic acid = 2.80
Tukey's $W$ for oleic acid = 4.54
Tukey's $W$ for linoleic acid = 5.80
Tukey's $W$ for linolenic acid = 2.96

Means in the same row followed by the same letter are not significantly different ($\alpha = 0.05$).
Appendix. Figure 13 clearly shows that, with the control spray, fruits from the Temp 1 berries have the lowest lauric acid content among the five temperature treatments. When sprayed with BHT, Temp 3 berries have a higher lauric acid content than Temp 1 berries, with no differences occurring between Temp 1 and the other temperature treatments. Fruits sprayed with BHT show a higher lauric acid content than fruits with the control spray only in Temp 1 and Temp 3 berries.

For myristic acid, Temp 1 fruits have the highest content among the five temperature treatments when berries are sprayed with the control spray (Figure 14). With the BHT spray, no significant differences in myristic acid content are observed among the five temperature treatments. Only in Temp 1 berries, do fruits with the control spray show a higher myristic acid content than the BHT sprayed fruits.

In Figure 15, both Temp 3 and Temp 4 berries have a higher palmitic acid content than Temp 1 berries. No differences are observed in the palmitic acid contents between Temp 1 and Temp 2 or Temp 1 and Temp 5 berries. Strawberries with the control spray show a higher palmitic acid content than berries with the BHT spray.
Tukey's $W$ for lauric acid = 0.11
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 13 : The effects of temperature treatments and chemical sprays (control and BHT spray) on lauric acid (12 : 0) of NL of whole fruits.
Tukey's $W$ for myristic acid = 5.80
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 14: The effects of temperature treatments and chemical sprays (control and BHT spray) on myristic acid (14:0) of NL of whole fruits.
Tukey's $W$ for palmitic acid = 2.06

Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 15 : The effects of temperature treatments and chemical sprays (control and BHT spray) on palmitic acid (16 : 0) of NL of whole fruits.
Figure 16 illustrates the stearic acid content of NL from whole fruits subjected to five temperature and two chemical treatments. Temp 4 berries have a higher stearic acid content than Temp 1 berries when sprayed with the control spray. There is no difference between Temp 1 and the other three temperature treatments. When berries are treated with BHT, no significant differences are observed between Temp 1 and the other four temperature treatments. Only Temp 4 berries show the control sprayed berries having more stearic acid than the BHT sprayed samples.

For oleic acid content (Figure 17), with the control spray, Temp 1 berries have a higher fatty acid content than the other four temperature treatments. When sprayed with the BHT spray, no differences in oleic acid contents are observed between Temp 1 and Temp 2 or Temp 1 and Temp 5 berries. Both Temp 3 and Temp 4 berries have more oleic acid than Temp 1 berries when the berries are treated with BHT. BHT treated berries have a higher oleic acid content than berries with the control spray for both Temp 3 and Temp 4 berries. On the contrary, Temp 1 berries show that control sprayed samples contain more oleic acid than BHT sprayed samples.

With the control spray, both Temp 3 and Temp 5 berries have significantly more linoleic acid than Temp 1 berries, but no differences are found between Temp 1 and Temp 2 or Temp 1 and Temp 4 berries (Figure 18). With the BHT spray, Temp 1
Tukey's $W$ for stearic acid = 1.58
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 16: The effects of temperature treatments and chemical sprays (control and BHT spray) on stearic acid (18:0) of NL of whole fruits.
Tukey's $W$ for oleic acid = 5.16
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 17 : The effects of temperature treatments and chemical sprays (control and BHT spray) on oleic acid (18 : 1) of NL of whole fruits.
Tukey's $W$ for linoleic acid = 12.89

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 18: The effects of temperature treatments and chemical sprays (control and BHT spray) on linoleic acid (18 : 2) of NL of whole fruits.
berries have more linoleic acid than the fruits from the other temperature treatments with the exception of Temp 5 berries. For Temp 1 berries, samples with the BHT spray show more linoleic acid than the samples with the control spray. On the other hand, Temp 3 berries show that the control sprayed samples have a higher linoleic acid content than the BHT sprayed samples.

Figure 19 shows the linolenic acid content of NL from whole fruits subjected to five different temperature and chemical spray treatments. With the control spray, both Temp 5 and Temp 3 berries have higher linolenic acid contents than Temp 1 berries, but there are no differences between Temp 1 and Temp 2 or Temp 1 and Temp 4 berries. There are no significant differences in linolenic acid between Temp 1 and the other four temperature treatments when berries are sprayed with BHT. Both Temp 3 and Temp 5 berries show that the control sprayed samples have higher linolenic acid contents than the BHT sprayed samples.

**Glycolipids**  The fatty acid compositions of GL of whole fruits subjected to five temperature treatments and chemical sprays are listed in the Table 10 of the Appendix. For lauric acid content (Figure 20), of the control sprayed berries, no significant differences are observed between Temp 1 and the other four temperature treatments. When sprayed with BHT, both Temp 3 and Temp 4 berries have more lauric acid than Temp 1 berries,
Tukey's $W$ for linolenic acid = 12.81

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 19: The effects of temperature treatments and chemical sprays (control and BHT spray) on linolenic acid (18:3) of NL of whole fruits.
Tukey's $W$ for lauric acid = 0.41
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 20: The effects of temperature treatments and chemical sprays (control and BHT spray) on lauric acid (12:0) of GL of whole fruits.
but no differences are found between Temp 1 and Temp 2 or Temp 1 and Temp 5 berries. For Temp 3 and Temp 4 berries, fruits with the BHT spray have more lauric acid than those with the control spray. There are no differences in lauric acid content between BHT and control sprayed samples for Temp 1, Temp 2 and Temp 5 berries.

Statistically, no differences are found in myristic acid content between the Temp 1 and the other four temperature treatments for either the control or BHT sprays (Figure 21). BHT sprayed samples show higher myristic acid than the control samples only in Temp 4 berries.

The palmitic acid content of GL of whole fruits subjected to five temperature treatments and chemical sprays are illustrated in Figure 22. With the control spray, Temp 2 and Temp 5 berries show more palmitic acid than Temp 1 berries, and no differences occur between either Temp 1 and Temp 3 or Temp 1 and Temp 4 berries. When strawberries are sprayed with BHT, no differences in palmitic acid content are observed between Temp 1 and the other four temperature treatments. BHT treated samples have higher palmitic acid than samples with the control spray in both Temp 1 and Temp 4 berries. No differences in palmitic acid content are found between the BHT and control sprayed samples in Temp 2, Temp 3 and Temp 5 berries.
Tukey's $W$ for myristic acid = 0.53
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 21: The effects of temperature treatments and chemical sprays (control and BHT spray) on myristic acid (14:0) of GL of whole fruits.
Tukey's $W$ for palmitic acid = 3.60

Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 22: The effects of temperature treatments and chemical sprays (control and BHT spray) on palmitic acid (16:0) of GL of whole fruits.
Neither temperature treatments nor chemical sprays have any effect on the stearic acid content of GL of whole fruits (Figure 23).

Figure 24 shows the oleic acid content of GL of whole fruits treated at five temperatures and two chemical sprays. With the control spray, Temp 1 berries have the lowest oleic acid content among the five temperature treatments. On the contrary, with the BHT spray, Temp 1 berries show the highest oleic acid among the five temperature treatments. For Temp 1, Temp 3 and Temp 4 berries, BHT sprayed samples show more oleic acid than control sprayed samples. For Temp 2 and Temp 5 berries, BHT treated samples have a lower oleic acid content than control sprayed samples.

Figure 25 shows the linoleic acid content of berries treated at five temperature treatments and with the two chemical sprays. Temp 1 berries with the control spray have the lowest linoleic acid content compared with the other four temperature treated control spray berries. But when berries are sprayed with BHT, no significant difference is observed between Temp 1 and the other four temperature treatments. BHT treated samples show more linoleic acid than samples treated with the control spray only in the Temp 1 berries. There are no differences in linoleic acid content between BHT and control spray berries in the other four temperature treatments.
Tukey's $W$ for stearic acid = 2.08
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 23: The effects of temperature treatments and chemical sprays (control and BHT spray) on stearic acid (18:0) of GL of whole fruits.
Tukey's $W$ for oleic acid = 1.30
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 24: The effects of temperature treatments and chemical sprays (control and BHT spray) on oleic acid (18:1) of GL of whole fruits.
Tukey's $W$ for linoleic acid = 2.58
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 25: The effects of temperature treatments and chemical sprays (control and BHT spray) on linoleic acid (18:2) of GL of whole fruits.
Figure 26 clearly shows that, for the control spray, Temp 1 berries have the lowest linolenic acid content compared with Temp 2, Temp 3 and Temp 5 berries. There is no difference in linolenic acid content between Temp 1 and Temp 4 berries when both are sprayed with the control spray. With the BHT spray, no differences in linolenic acid content are observed between Temp 1 and the other four temperature treatments. There are no differences found between the BHT and the control sprayed samples in Temp 3, Temp 4 and Temp 5 berries. For Temp 1 berries, BHT sprayed berries have a higher linolenic acid than control sprayed berries. On the other hand, for Temp 2 berries, control sprayed samples show a higher linolenic acid than BHT sprayed samples.

**Phospholipids** In Table 11 of the Appendix, the fatty acid compositions (mg /100 g of sample) of PL of whole fruits subjected to five temperature treatments and two chemical sprays are shown. For lauric acid content (Figure 27), when berries are sprayed with the control spray, Temp 1 berries have a lower fatty acid content than Temp 3 berries. However, no differences in lauric acid content are found between Temp 1 and the other three temperature treatments. With the BHT spray, Temp 1 berries have less lauric acid than either Temp 3 or Temp 4 berries. There are no differences observed in lauric acid contents between either Temp 1 and Temp 2 or Temp 1 and
Tukey's $W$ for linolenic acid = 4.00
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 26 : The effects of temperature treatments and chemical sprays (control and BHT spray) on linolenic acid (18 : 3) of GL of whole fruits.
Tukey's $W$ for lauric acid = 0.31
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 27: The effects of temperature treatments and chemical sprays (control and BHT spray) on lauric acid (12:0) of PL of whole fruits.
Temp 5 berries when the samples are sprayed with BHT. For Temp 1, Temp 2 and Temp 5 berries, there are no significant differences in lauric acid content between the BHT and control sprayed samples. For Temp 3 and Temp 4 berries, BHT treated samples show more lauric acid than samples with the control spray.

Figure 28 clearly shows that, for either the control or BHT spray, Temp 3 berries have more myristic acid than Temp 1 berries, but no differences are found between Temp 1 and the other three temperature treatments. Only for Temp 3 berries, do BHT treated samples show more myristic acid than samples with the control spray, no differences are found between the two sprays in the other temperature treatments.

For the palmitic acid content, Figure 29 shows that BHT sprayed berries have a higher fatty acid content than berries with the control spray. From the temperature treatment point of view, berries treated at either Temp 3 or Temp 4 temperature have more palmitic acid than Temp 1 berries. Additionally, no differences are observed between the palmitic acid content of Temp 1 and Temp 5 or Temp 1 and Temp 2 berries.

Statistically, no significant differences are found in the stearic acid content of berries subjected to the five temperature and two chemical treatments (Figure 30).
Tukey's $W$ for myristic acid = 0.46
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 28: The effects of temperature treatments and chemical sprays (control and BHT spray) on myristic acid (14 : 0) of PL of whole fruits.
Tukey's $W$ for palmitic acid = 3.99
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 29: The effects of temperature treatments and chemical sprays (control and BHT spray) on palmitic acid (16:0) of PL of whole fruits.
Tukey's $W$ for stearic acid = 0.75
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 30 : The effects of temperature treatments and chemical sprays (control and BHT spray) on stearic acid (18 : 0) of PL of whole fruits.
With the control spray, Temp 3 berries have more oleic acid than Temp 1 berries, and no differences are found between Temp 1 and the other three temperature treatments (Figure 31). For berries sprayed with the BHT spray, Temp 1 berries have more oleic acid than both Temp 2 and Temp 5 berries, with no differences observed between either Temp 1 and Temp 3 or Temp 1 and Temp 4 berries. Both Temp 1 and Temp 4 berries show that the BHT sprayed samples have more oleic acid than the control sprayed samples. There are no differences in oleic acid content between BHT and control sprayed samples for Temp 2, Temp 3 and Temp 5 berries.

For linoleic acid content, berries with the BHT spray have a significantly higher fatty acid content than berries with the control spray (Figure 32). From the temperature treatment point of view, Temp 3 berries have the highest linoleic acid content among the five temperature treatments.

There are no significant differences in linolenic acid content between Temp 1 and the other four temperature treatments for either the control or BHT sprays (Figure 33). In addition, the linolenic acid content shows no difference between the control and the BHT sprayed samples for all five temperature treatments.
Figure 31: The effects of temperature treatments and chemical sprays (control and BHT spray) on oleic acid (18 : 1) of PL of whole fruits.

Tukey's $W$ for oleic acid $= 1.96$

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.
Tukey's $W$ for linoleic acid = 6.94
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 32: The effects of temperature treatments and chemical sprays (control and BHT spray) on linoleic acid (18:2) of PL of whole fruits.
Tukey's $W$ for linolenic acid = 3.91
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 33: The effects of temperature treatments and chemical sprays (control and BHT spray) on linolenic acid (18:3) of PL of whole fruits.
The effect of different temperature treatments and chemical sprays on fatty acid composition of the lipid classes of strawberry seeds (based on mg / 1 g of sample). The fatty acid compositions of strawberry seeds are similar to the fatty acid compositions of whole fruits. They are composed of lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids.

Neutral lipids Table 12 of the Appendix lists the fatty acid compositions of NL of strawberry seeds subjected to five temperature and two chemical spray treatments. For lauric acid content (Figure 34), when samples are treated with the control spray, Temp 4 seeds have more lauric acid than Temp 1 seeds, but no differences are found between Temp 1 and the other three temperature treatments. With the BHT spray, only Temp 2 seeds show a higher lauric acid than Temp 1 seeds and no differences are found between Temp 1 and the other three temperature treatments. For Temp 2 seeds, samples with the BHT spray have more lauric acid than samples with the control spray, and no differences between the BHT and control samples are found in the other temperature treatments.

From the seeds treated with the control spray, no differences in myristic acid content are found between Temp 1 and the other four temperature treatments (Figure 35). With the BHT spray, Temp 1 seeds have the most myristic acid among the five temperature treatments. Only for Temp 1 seeds, do BHT
Tukey's $W$ for lauric acid = 0.04
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 34: The effects of temperature treatments and chemical sprays (control and BHT spray) on lauric acid (12 : 0) of NL of strawberry seeds.
Tukey's $W$ for myristic acid = 11.84
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 35: The effects of temperature treatments and chemical sprays (control and BHT spray) on myristic acid (14:0) of NL of strawberry seeds.
sprayed samples show higher amounts of myristic acid than the samples with the control spray. No differences in myristic acid content are found between the BHT and control sprayed samples for the other four temperature treatments.

With the control spray, Temp 1 seeds have more palmitic acid than either Temp 2 or Temp 3 seeds, but no differences are observed between either Temp 1 and Temp 4 or Temp 1 and Temp 5 seeds (Figure 36). When treated with the BHT spray, Temp 2 seeds have more palmitic acid than Temp 1 seeds with no differences found between Temp 1 and the other three temperature treatments. For Temp 1 seeds, samples with the control spray show more palmitic acid than samples with the BHT spray. On the contrary, for Temp 2 seeds, the BHT samples have more palmitic acid than the control samples. No differences in palmitic acid content are found between BHT and control samples of Temp 3, Temp 4 and Temp 5 seeds.

With the control spray, there are no differences in stearic acid content between Temp 1 and the other four temperature treatments (Figure 37). For seeds with the BHT spray, Temp 2 has more stearic acid than Temp 1, but no differences are found between Temp 1 and the other three temperature treatments. For the seeds of Temp 1, Temp 3, Temp 4 and Temp 5, no differences in stearic acid content are observed between BHT and control treated samples. For the seeds of Temp 2, BHT treated
Tukey's $W$ for palmitic acid = 4.13

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 36: The effects of temperature treatments and chemical sprays (control and BHT spray) on palmitic acid (16:0) of NL of strawberry seeds.
Tukey's $W$ for stearic acid = 1.99
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 37: The effects of temperature treatments and chemical sprays (control and BHT spray) on stearic acid (18 : 0) of NL of strawberry seeds.
samples show more stearic acid than samples with the control spray.

For the oleic acid content of NL of strawberry seeds (Figure 38), when treated with the control spray, Temp 1 seeds have a higher oleic acid content than Temp 2 seeds, but no differences are found between the seeds of Temp 1 and the other three temperature treatments. For the BHT treated seeds, Temp 2 samples have more oleic acid than Temp 1 samples, but no differences are observed between Temp 1 and the other three temperature treatments. For the seeds of Temp 3, Temp 4 and Temp 5, no differences in oleic acid content occur between the BHT and control sprayed samples. The BHT sprayed samples have more oleic acid than the control sprayed samples for Temp 2 seeds. For Temp 1 seeds, the control samples have more oleic acid than the samples sprayed with BHT.

Figure 39 shows the linoleic acid content of NL of strawberry seeds subjected to five temperature and two chemical sprays. When sprayed with the control spray, Temp 1 seeds have more linoleic acid than either Temp 2 and Temp 3 seeds, and there are no differences between either Temp 1 and Temp 4 or Temp 1 and Temp 5 seeds. With the BHT spray, only Temp 2 seeds show more linoleic acid than Temp 1 seeds with no differences found between Temp 1 and the other three temperature treatments. There are no differences in linoleic acid content between the BHT and control sprayed samples for Temp
Tukey's $W$ for oleic acid = 14.11
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 38: The effects of temperature treatments and chemical sprays (control and BHT spray) on oleic acid (18 : 1) of NL of strawberry seeds.
Tukey's $W$ for linoleic acid = 13.73
Temp 1: no temperature treatment.
Temp 2: 5°C for 4 days.
Temp 3: 5°C for 7 days.
Temp 4: 40°C for 2 hours.
Temp 5: -20°C for 6 months.

Figure 39: The effects of temperature treatments and chemical sprays (control and BHT spray) on linoleic acid (18:2) of NL of strawberry seeds.
3 and Temp 5 seeds. For Temp 1 and Temp 4 seeds, control sprayed samples have higher amounts of linoleic acid than samples with the BHT spray. For Temp 2 seeds, samples with the BHT spray have more linoleic acid than samples with the control spray.

There are no differences in linolenic acid content between Temp 1 and the other four temperature treatments for seeds with the control spray (Figure 40). When sprayed with BHT, Temp 2 seeds show more linolenic acid than Temp 1 seeds, but there are no differences between Temp 1 and the other three temperature treatments. Seeds treated with BHT show more linolenic acid than seeds with the control spray only for the Temp 2 seeds. For Temp 1, Temp 3, Temp 4 and Temp 5 seeds, no differences in linolenic acid content are found between the BHT and control sprayed samples.

**Glycolipids** Table 13 of the Appendix lists the major fatty acid compositions of GL of strawberry seeds subjected to five temperature treatments and two chemical sprays.

Figure 41 demonstrates the lauric acid content of GL of strawberry seeds subjected to five temperature treatments and two chemical sprays. With the control spray, both Temp 2 and Temp 5 seeds have higher lauric acid contents than Temp 1 seeds, but no differences are observed between either Temp 1 and Temp 3 or Temp 1 and Temp 4 seeds. With the BHT spray, Temp 2 seeds show more lauric acid than Temp 1 seeds, but
Tukey's $W$ for linolenic acid = 15.89
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 40 : The effects of temperature treatments and chemical sprays (control and BHT spray) on linolenic acid (18 : 3) of NL of strawberry seeds.
Tukey's $W$ for lauric acid = 0.12

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 41: The effects of temperature treatments and chemical sprays (control and BHT spray) on lauric acid (12 : 0) of GL of strawberry seeds.
there are no differences between Temp 1 and the other three temperature treatments. For Temp 1, Temp 3 and Temp 4 seeds, there are no differences in lauric acid content between the BHT and control samples. Seeds with the BHT spray have more lauric acid than seeds with the control spray for the Temp 2 samples. On the other hand, for seeds of Temp 5, the control samples show more lauric acid than the samples with BHT.

When sprayed with the control spray, myristic acid contents of Temp 2, Temp 3 and Temp 5 seeds are higher than Temp 1 seeds, but no difference is observed between Temp 1 and Temp 4 seeds (Figure 42). With the BHT spray, only Temp 2 seeds have more myristic acid than Temp 1 seeds. With no differences found between Temp 1 and the other three temperature treatments. There are no differences in myristic acid content between the BHT and control sprayed samples for Temp 1, Temp 3 and Temp 4 seeds. For Temp 2 seeds, samples with the BHT spray show more myristic acid than samples with the control spray. For Temp 5 seeds, samples with the control spray have more myristic acid than the samples with the BHT spray.

With the control spray, Temp 2, Temp 3 and Temp 5 seeds have more palmitic acid than Temp 1 seeds, but no difference is found between Temp 4 and Temp 1 seeds (Figure 43). When treated with the BHT spray, both Temp 2 and Temp 3 seeds show more palmitic acid than Temp 1 seeds. With no differences between Temp 1 and the other two temperature treatments. For
Tukey's $W$ for myristic acid = 0.23
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 42: The effects of temperature treatments and chemical sprays (control and BHT spray) on myristic acid (14:0) of GL of strawberry seeds.
Tukey's $W$ for palmitic acid = 1.80
Temp 1 : no temperature treatment.
Temp 2 : 5 ºC for 4 days.
Temp 3 : 5 ºC for 7 days.
Temp 4 : 40 ºC for 2 hours.
Temp 5 : -20 ºC for 6 months.

Figure 43: The effects of temperature treatments and chemical sprays (control and BHT spray) on palmitic acid (16:0) of GL of strawberry seeds.
Temp 3 and Temp 5, seeds with the control spray show higher amounts of palmitic acid than seeds with the BHT spray. In the case of Temp 2 treatments, seeds sprayed with BHT have a higher palmitic acid content than seeds with the control spray. There are no differences in palmitic acid content between samples with the BHT and control sprays for Temp 1 and Temp 4 seeds.

When the samples are treated with the control spray, both Temp 2 and Temp 3 seeds show more stearic acid than Temp 1 seeds, but no differences are found between Temp 1 and the other two temperature treatments (Figure 44). With the BHT spray, only Temp 2 seeds have more stearic acid than Temp 1 seeds. With no differences observed between Temp 1 and the other three temperature treatments. For Temp 2 seeds, samples with the BHT spray have more stearic acid than samples with the control spray. There are no significant differences between the BHT and control sprayed samples for the other temperature treatments.

Figure 45 demonstrates the oleic acid content of GL of seeds subjected to five temperature treatments and two chemical sprays. With the control spray, seeds from Temp 2 and Temp 3 treatments have higher amounts of oleic acid than the seeds of Temp 1, with no differences found between Temp 1 and the other two temperature treatments. For the BHT spray, Temp 2 seeds show more oleic acid than Temp 1 seeds, with no differences between Temp 1 and the other three temperature treatments.
Tukey's $W$ for stearic acid = 1.30

Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 44: The effects of temperature treatments and chemical sprays (control and BHT spray) on stearic acid (18:0) of GL of strawberry seeds.
Figure 45: The effects of temperature treatments and chemical sprays (control and BHT spray) on oleic acid (18 : 1) of GL of strawberry seeds.

Tukey's $W$ for oleic acid = 2.06
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.
There are no differences between the oleic acid contents of BHT and control sprayed samples for Temp 1, Temp 4 and Temp 5 seeds. For Temp 2 seeds, BHT sprayed samples show more oleic acid than the control sprayed samples. On the other hand, the control sprayed samples have more oleic acid than the BHT sprayed samples for Temp 3 seeds.

When sprayed with the control spray, Temp 2 and Temp 3 seeds have more linoleic acid than Temp 1 seeds while there are no differences between Temp 1 and the other two temperature treatments (Figure 46). With the BHT spray, Temp 2 seeds have more linoleic acid than Temp 1 seeds while there are no differences between Temp 1 and the other three temperature treatments. There are no differences between the linoleic acid contents of BHT and control sprayed samples for Temp 1, Temp 4 and Temp 5 seeds. For Temp 2 seeds, BHT sprayed samples show more linoleic acid than the samples with the control spray. On the contrary, samples with the control spray have more linoleic acid than the samples with the BHT spray for Temp 3 seeds.

For linolenic acid content, the same results as those of linoleic acid content are observed except that there is no difference between the linolenic acid content of the BHT and the control sprayed samples (Figure 47).

**Phospholipids** In Table 14, the major fatty acid compositions of PL of strawberry seeds subjected to five temperature treatments and two chemical sprays are listed. For
Tukey's $W$ for linoleic acid = 3.37
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 46: The effects of temperature treatments and chemical sprays (control and BHT spray) on linoleic acid (18:2) of GL of strawberry seeds.
Tukey's $W$ for linolenic acid = 2.27
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 47: The effects of temperature treatments and chemical sprays (control and BHT spray) on linolenic acid (18:3) of GL of strawberry seeds.
lauric acid content (Figure 48), when the control spray is applied, seeds of Temp 5 berries have more lauric acid than the seeds of Temp 1 berries. With no differences between Temp 1 and the other three temperature treatments. For seeds with the BHT spray, Temp 2 samples show more lauric acid than Temp 1 samples, but no differences are found between Temp 1 and the other three temperature treatments. Only Temp 2 seeds show the BHT sprayed samples having higher lauric acid than the samples with the control spray. No differences are found between the BHT and control sprayed samples for the other four temperature treatments.

For the control spray, there are no differences in myristic acid contents of seeds between Temp 1 and the other four temperature treatments (Figure 49). For the BHT spray, Temp 2 seeds have higher myristic acids than Temp 1 seeds but no differences are found between Temp 1 and the other three temperature treatments. There are no differences in myristic acid contents between the BHT and control sprayed samples with the exception of Temp 2 treatment for which the BHT sprayed samples have more myristic acid than the control sprayed samples.

Figures 50, 51 and 52, clearly show that the palmitic, stearic and oleic acid contents of PL of seeds have the same results as the myristic acid content with respect to the temperature and chemical spray treatments.
Tukey's $W$ for lauric acid = 0.08

Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 48: The effects of temperature treatments and chemical sprays (control and BHT spray) on lauric acid (12:0) of PL of strawberry seeds.
Tukey's $W$ for myristic acid = 0.18
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 49: The effects of temperature treatments and chemical sprays (control and BHT spray) on myristic acid (14:0) of PL of strawberry seeds.
Tukey's $W$ for palmitic acid = 4.96
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 50: The effects of temperature treatments and chemical sprays (control and BHT spray) on palmitic acid (16:0) of PL of strawberry seeds.
Tukey's $W$ for stearic acid = 1.43
Temp 1 : no temperature treatment.
Temp 2 : 5 °C for 4 days.
Temp 3 : 5 °C for 7 days.
Temp 4 : 40 °C for 2 hours.
Temp 5 : -20 °C for 6 months.

Figure 51: The effects of temperature treatments and chemical sprays (control and BHT spray) on stearic acid (18:0) of PL of strawberry seeds.
Tukey's $W$ for oleic acid = 3.88
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 52: The effects of temperature treatments and chemical sprays (control and BHT spray) on oleic acid (18:1) of PL of strawberry seeds.
For linoleic acid content, Temp 4 control spray seeds have more linoleic acid than Temp 1 seeds but no differences are found between Temp 1 and the other three temperature treatments (Figure 53). With the BHT spray, Temp 2 seeds show more linoleic acid than Temp 1 seeds, while there are no differences between Temp 1 and the other three temperature treatments. For Temp 1, Temp 3 and Temp 5 seeds, no differences are found between the linoleic acid contents of the BHT and the control sprayed samples. Seeds with the BHT spray show more linoleic acid than seeds with the control spray only occurred in Temp 2 samples. On the contrary, BHT sprayed seeds have less linoleic acid than seeds with the control spray for Temp 4 samples.

When sprayed with the control spray, Temp 4 seeds have more linolenic acid than Temp 1 seeds, but no differences are found between Temp 1 and the other three temperature treatments (Figure 54). With the BHT spray, there are no differences in linolenic acid contents between Temp 1 and the other four temperature treatments. For Temp 1, Temp 2, Temp 3 and Temp 5 seeds, no differences in linolenic acid are observed between the BHT and the control sprayed samples. For Temp 4 seeds, the control sprayed samples have more linolenic acid than samples with the BHT spray.
Figure 53: The effects of temperature treatments and chemical sprays (control and BHT spray) on linoleic acid (18:2) of PL of strawberry seeds.

Tukey's $W$ for linoleic acid = 8.14

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.
Tukey's $W$ for linolenic acid = 5.01
Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.

Figure 54: The effects of temperature treatments and chemical sprays (control and BHT spray) on linolenic acid (18:3) of PL of strawberry seeds.
Lipoxygenase (LOX) Activity of Strawberry

LOX activity of strawberry for four stages of maturity. Table 15 lists LOX activity (expressed as specific activity: oxygen consumption µl per mg protein per min.) of strawberries at different stages of maturity. White strawberries seem to have the highest LOX activity of the four maturity stages, although they are not significantly higher than the pink and red berries. There is no difference in LOX activity between pink and red berries. For green strawberries, no LOX activity is detected.

LOX activity of red strawberries subjected to temperature and chemical spray treatments. Table 16, clearly shows that the sprays have no effect on LOX activity of strawberry as no differences in LOX activity are found between the BHT and the control sprayed samples. With either the control or the BHT spray, only Temp 5 treatment shows lower LOX activity than Temp 1 treatment. No differences are found between Temp 1 and the other temperature treatments.

C₆ Aldehydes Derived from Oxidized Lipids

C₆ aldehydes (n - hexanal, cis -3- hexenal, trans -2- hexenal) have been reported as aroma compounds derived from lipid oxidation (linoleic and linolenic acids) by various studies(Galliard et al., 1977; Takeo and Tsushida, 1980; Hatanaka
Table 15. LOX activity of strawberry of four stages of maturity¹.

<table>
<thead>
<tr>
<th>Strawberry</th>
<th>LOX activity ($\mu l$ O₂/mg/min.)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green strawberry</td>
<td>0.00a</td>
</tr>
<tr>
<td>White strawberry</td>
<td>4.00b</td>
</tr>
<tr>
<td>Pink strawberry</td>
<td>0.84ab</td>
</tr>
<tr>
<td>Red strawberry</td>
<td>0.66ab</td>
</tr>
</tbody>
</table>

1. Results are the mean of two determinations of each sample.
2. Means with the same superscripts in the same column are not significantly different at $\alpha = 0.05$.

Tukey's $W = 3.54$. 
Table 16. LOX activity of red strawberries subjected to temperature and chemical spray treatments\(^1\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LOX activity (µl O(_2)/mg/min.)(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp 1, control spray</td>
<td>0.63abc</td>
</tr>
<tr>
<td>Temp 1, BHT spray</td>
<td>0.68abc</td>
</tr>
<tr>
<td>Temp 2, control spray</td>
<td>0.29cd</td>
</tr>
<tr>
<td>Temp 2, BHT spray</td>
<td>0.37bcd</td>
</tr>
<tr>
<td>Temp 3, control spray</td>
<td>0.36bcd</td>
</tr>
<tr>
<td>Temp 3, BHT spray</td>
<td>0.56abc</td>
</tr>
<tr>
<td>Temp 4, control spray</td>
<td>0.72a</td>
</tr>
<tr>
<td>Temp 4, BHT spray</td>
<td>0.82a</td>
</tr>
<tr>
<td>Temp 5, control spray</td>
<td>0.00d</td>
</tr>
<tr>
<td>Temp 5, BHT spray</td>
<td>0.00d</td>
</tr>
</tbody>
</table>

1. Results are the mean of two determinations of each sample.
2. Means with the same superscripts in the same column are not significantly different at \(\alpha = 0.05\).

Tukey's \(W = 0.40\)

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.
et al., 1976; Zamora et al., 1985; Selvaraj, 1988). In this study, detection of hexanal is used to demonstrate the oxidation of lipids. Amounts (average of two measurements) of hexanal detected for the strawberries at different stages of maturity are 25.70%, 26.95%, 19.06% and 20.14% for green, white, pink and red strawberries respectively. The percentages of hexanal released from strawberries at the different stages of maturity have no significant differences.

Comparisons are made for hexanal production of red strawberries subjected to five temperature and two chemical spray treatments (Table 17). From the effect of BHT spray point of view, only Temp 1 treatment shows that the BHT sprayed berries release less hexanal than the control sprayed berries. Temperature wise, when sprayed with the control spray, Temp 1 berries produce more hexanal than either strawberries subjected to Temp 3 or Temp 5 treatments. With the BHT spray, only strawberries treated at Temp 4 show a higher hexanal content than Temp 1 berries.
Table 17. % of hexanal released from red strawberries subjected to five temperature and two chemical spray treatments\(^1\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hexanal production(% of area)(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp 1, control spray</td>
<td>16.12(^a)</td>
</tr>
<tr>
<td>Temp 1, BHT spray</td>
<td>4.22(^c)</td>
</tr>
<tr>
<td>Temp 2, control spray</td>
<td>11.48(^{ab})</td>
</tr>
<tr>
<td>Temp 2, BHT spray</td>
<td>5.14(^{bc})</td>
</tr>
<tr>
<td>Temp 3, control spray</td>
<td>3.76(^c)</td>
</tr>
<tr>
<td>Temp 3, BHT spray</td>
<td>3.52(^c)</td>
</tr>
<tr>
<td>Temp 4, control spray</td>
<td>10.73(^{ab})</td>
</tr>
<tr>
<td>Temp 4, BHT spray</td>
<td>10.68(^{ab})</td>
</tr>
<tr>
<td>Temp 5, control spray</td>
<td>0.00(^c)</td>
</tr>
<tr>
<td>Temp 5, BHT spray</td>
<td>0.00(^c)</td>
</tr>
</tbody>
</table>

1. Results are the mean of two determinations of each sample.
2. Means with the same superscripts in the same column are not significantly different at \(\alpha = 0.05\).

Tukey's \(W = 6.41\).

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.
Chapter V
DISCUSSION

Lipid Analysis

*Lipid content.* The moisture content of whole strawberry fruit decreased gradually from 91% to 82% (White stage to red stage). This change has also been observed in the fruit of Persen Americana during fruit development (Gaydou et al., 1987). In this study, the percentage of lipid in the green fruits is significantly higher than that of red fruits (based on fresh weight). No significant difference in lipid content occurred for the whole fruits of the red, pink and white stages, although Couture et al. (1988), did suggest that ripe fruit contained slightly more lipid than unripe fruit. On the other hand, lipid content of seeds (based on fresh weight) does increase with increasing maturity. In addition, from Table 1, it is clear that the seeds have a higher lipid content than the whole fruits. Takenaga et al. (1985) observed that the total lipid content is higher in seeds than in endocarps (based on fresh weight) for bayberries.
Distribution of lipid classes. For both strawberry seeds and whole fruits, NL is the predominant lipid of the three lipid classes for the red, pink and white stages. Table 2 clearly shows that the % PL of whole fruits and seeds decreases as the maturity increases. On the contrary, the content of NL increased as the maturity increased. In addition, the content of GL of whole fruits decreases with the increase of maturity, but no such trend is observed in the GL content of strawberry seeds. These changes also occurred in the study of lipid biosynthesis of olive fruit during fruit development (Donaire and Lopez-Gorge, 1978) which demonstrated greater incorporation of labeled carbon into polar lipids (glycolipids and phospholipids) in the early stages of fruit development, while in later stages, the amount of labeled carbon incorporated into neutral lipid increased and labeled carbon incorporation into polar lipids decreased.

When the effect of antioxidant on distribution of lipid classes was observed on the red fresh fruits with no spray, control spray and BHT spray (Figure 5 and Figure 6), no conclusive results were obtained as to the NL and GL contents of either whole fruits and seeds. The BHT spray also showed no effect on the distribution of PL in either whole fruits or seeds.

When the combined effects of temperature treatments and the antioxidant spray were taken into consideration on the distribution of lipid classes, in the whole fruits, only PL exhibited any differences. The BHT sprayed sample possessed a higher % of
PL than the control sprayed sample did (Figure 9). This phenomena is probably due to the oxidation of lipids in PL of control sprayed sample which does not have BHT to prohibit the lipid oxidation. Pun et al. (1980), reported that off-flavors are often related to the deterioration of the functional lipids (such as PL) in foods.

Looking at the effect of temperature treatment on distribution of lipid classes (Figure 7 to Figure 12), fruits with cold temperature (Temp 2 and Temp 3), hot temperature (Temp 4) and freezing temperature (Temp 5) were compared with fruits without temperature treatment (Temp 1). Only the cold temperature treatments (Temp 2 and Temp 3) consistently showed different results from those of Temp 1 treatment. More precisely, for both fruits and seeds, cold temperature treatment seems to decrease NL and tends to increase GL content in both fruits and seeds. Gawer et al. (1983), revealed that low temperatures induce an increase in the polyunsaturated fatty acid content of plant cells in order to maintain the optimum membrane fluidity. Both glycolipids and phospholipids have been equated with membrane lipids (Wade and Bishop, 1978). On the other hand, Parkin and Kuo (1989) observed decreases in unsaturation of peel tissue glycolipids in cucumber fruits rewarmed after 3 days of chilling. However, they found no consistent changes in the glycolipid content.
The effect of different stages of maturity on fatty acid composition of lipid classes of whole fruits (based on mg/100 g of sample). In this study, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were determined. Both lauric acid and myristic acids were generally presented in insignificant quantities (less than 1 mg/100 g of sample). Whereas Couture et al. (1988), observed two less fatty acid composition from lipid extraction of strawberry, i.e., lauric and myristic acids were not detected.

Generally speaking, for NL of whole strawberry, unsaturated fatty acids such as oleic, linoleic and linolenic acids seem to increase with the increase of maturity (Table 3). Though the differences were not statistically significant, oleic acid content seems to reach the highest level at the white stage then starts to decrease toward more mature stages. This phenomena to a certain degree confirms the observation of Couture et al. (1988), that lipids of ripe fruit were richer in oleic acid. Although it is not statistically significant, linoleic and linolenic acid content seem to decrease from pink to red stage. Couture et al. (1988), did indicate that the ripe fruit contained less linoleic acid than unripe fruit. As for saturated fatty acids, i.e., palmitic and stearic acids (lauric and myristic acids are in such a small quantity that they are negligible), fatty acid content decrease with the increase of maturity.
For GL of whole strawberry, saturated fatty acid (palmitic and stearic) content decreased as the maturity of fruit increased. Basically, for the unsaturated fatty acids (especially linoleic and linolenic acids), the fatty acid content increased with the increase of maturity only when the green and white stages were considered. Table 4 shows that ripe fruit seems to have less polyunsaturated fatty acids (linoleic and linolenic) than less mature fruit (not statistically different though).

In Table 5, it is clear that the saturated fatty acids (palmitic and stearic) of PL of whole strawberry showed the same pattern as those of GL of whole strawberry. As for the unsaturated fatty acids, the green stage possess the highest fatty acid content. Viewing the fatty acid content of linoleic and linolenic acids of pink and red stages from Table 3 to Table 5, shows the trend of decreasing fatty acid content with increasing maturity. This observation coincides with the study of Grosbois and Mazliak (1964), that during ripening the relative amounts of unsaturated fatty acids in the pulp lipid of banana fruit decreased.

The effect of different stages of maturity on fatty acid composition of lipid classes of seeds (based on mg/g of sample of seeds). Strawberry seeds contained the same type of fatty acids as those of the whole fruits. At the beginning of this research, lipid of the flesh (fruits without seeds) was extracted. Same type of fatty acids as those in the seeds and
whole fruits were detected. This differs from comparisons of lipid contents of fruits and seeds of other species. Asilbekova et al. (1985) found that the lipids of the pericarp of fruit of Acanthopanax sessiliflorus contain a more complex set of components than the seeds. Takenaga et al. (1985), also indicated that although the main fatty acid in seeds and endocarps of bayberries were palmitic, oleic and linoleic acids, there was a little difference between two parts.

For the NL, seeds of the pink stage seem to contain the highest fatty acid content of both saturated (palmitic and stearic acids) and unsaturated fatty acids (oleic, linoleic and linolenic acids). Generally, fatty acids increased with the increase of maturity (from the green to pink stage). Between the red and pink stages, the fatty acid content (for saturated and unsaturated fatty acids) decreased with the increase of maturity. In Table 7 and Table 8, for the linoleic and linolenic acids in both GL and PL portions, seeds of the white stage contained the highest fatty acid content. This may be due to the greater incorporation of carbon into polar lipids in the early stages of fruit development (Donaire and Lopez-Gorge, 1978). Linoleic acid predominated in every lipid class of seeds during the maturation of fruit. The same result was obtained by Ichihara and Noda (1980) on the study of fatty acid composition in developing safflower seeds. Palmitic, stearic and oleic acids in the GL portion of seeds increased with increasing maturity of strawberries. As in the PL portion of
strawberry seeds, palmitic, stearic and oleic acids seem to reach the highest level at the white stage. Therefore, generally speaking, lipids of seeds changed during maturation of fruit with respect to the fatty acid composition of three lipid classes. On the other hand, Blenko and Datunashvili (1984) disclosed that the total lipids of seeds of grape remained virtually unchanged in the course of ripening with respect to the fatty acid composition.

The effect of different temperature treatments and chemical sprays on fatty acid composition of lipid classes of whole fruits (based on mg/100 g of sample). Of the seven fatty acids, lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids detected in the lipids of whole fruits, the unsaturated fatty acids (especially polyunsaturated fatty acids) were used to monitor the effects of temperature treatments and chemical sprays due to their higher susceptibility to lipid oxidation.

For linoleic acid content of NL, control sprayed samples showed higher fatty acid content than BHT sprayed samples only when both samples were treated at Temp 3. On the other hand, Temp 1 treated samples showed that BHT sprayed samples contain higher linoleic acid content than of control sprayed samples. With BHT spray, Temp 1 samples contained higher linoleic acid content than either Temp 2, Temp 3 or Temp 4 samples, but no difference was observed between Temp 1 and
Temp 5 treatments. With the control spray, both Temp 3 and Temp 5 samples showed higher linoleic acid content than of Temp 1 samples with no difference observed either between Temp 1 and Temp 2 or between Temp 1 and Temp 4 samples.

For GL, only Temp 1 treated samples showed BHT sprayed samples containing higher linoleic acid content than of control sprayed samples. With BHT spray, no difference in linoleic acid content was observed for comparisons between Temp 1 samples and other temperature treatments. For the control spray, Temp 2, Temp 3, Temp 4 and Temp 5 treated samples all contained a higher linoleic acid content than the Temp 1 samples.

For linoleic acid content of PL, all five different temperature treatments showed a definite effect in that BHT sprayed samples had a higher fatty acid content than that of control sprayed samples. Temp 3 berries have more linoleic acid than Temp 1 berries.

As for the linolenic acid content of NL, control sprayed samples showed a higher fatty acid content than the BHT sprayed samples occurred in fruits treated at Temp 3 and Temp 5. With the BHT spray, all five temperature treatments had no effect on the linolenic acid content. When sprayed with the control spray, both Temp 3 and Temp 5 samples showed higher linolenic acid contents than Temp 1 samples.

For the linolenic acid content of GL, BHT sprayed samples showed a higher fatty acid content than the control sprayed
sample when both samples were treated at Temp 1. With the BHT spray, temperature treatments showed no effect on linolenic acid content. When sprayed with the control spray, strawberries treated at Temp 2, Temp 3 or Temp 5 had a higher linolenic acid content than the Temp 1 treated berries. However, no difference in linolenic acid content was observed between Temp 1 and Temp 4 samples.

In PL, no difference in linolenic acid content was observed between BHT sprayed and control sprayed samples for all temperature treatments. In addition, there was no difference in linolenic acid content observed when the temperature treatments were compared with Temp 1 samples.

The effect of different temperature treatments and chemical sprays on fatty acid composition of lipid classes of strawberry seeds (based on mg/g of sample). For the linoleic acid content of NL, the BHT sprayed samples had a higher fatty acid content than the control sprayed samples when both samples were treated at Temp 2. With the BHT spray, only Temp 2 samples showed a higher linoleic acid content than the Temp 1 samples. But when sprayed with the control spray, Temp 1 samples had a higher linoleic acid content than either Temp 2 or Temp 3 samples.

In GL, Temp 2 samples again showed that BHT sprayed samples had a higher linoleic acid content than the control
sprayed samples. With the BHT spray, only Temp 2 samples showed a higher linoleic acid content than that of Temp 1 samples. With control spray, both Temp 2 and Temp 3 samples had a higher linoleic acid content than the Temp 1 samples.

For the linoleic acid content of PL, once again, the BHT sprayed samples showed a higher fatty acid content than the control sprayed samples when both samples were at Temp 2. On the other hand, when samples were at Temp 4, the control sprayed samples showed a higher linoleic acid content than the BHT sprayed samples. With the BHT spray, only Temp 2 had a higher linoleic acid content than the Temp 1 treated samples. With the control spray, only the Temp 4 samples had a higher linoleic acid content than the Temp 1 samples.

For the linolenic acid content of NL, Temp 2 samples showed that BHT sprayed samples had a higher fatty acid content than the control sprayed samples. With the BHT spray, only Temp 2 samples showed a higher linolenic acid content than the Temp 1 samples. With control spray, no difference was observed in the linolenic acid content of seeds when comparisons were made between Temp 1 samples and samples subjected to either cold temperatures (Temp 2, Temp 3 and Temp 5) or hot temperature (Temp 4).

For GL of the Temp 3 samples, samples from the control spray had a higher linolenic acid content than the BHT sprayed samples. With the BHT spray, only Temp 2 samples showed a
higher linolenic acid content than the Temp 1 samples. With the control spray, only Temp 2 and Temp 3 samples showed higher linolenic acid contents than the Temp 1 samples.

As for the linolenic acid content of PL, Temp 4 samples sprayed with the control spray had a higher fatty acid content than the BHT sprayed samples. With the BHT spray, no difference in linolenic acid content between Temp 1 samples and the other temperature treatment was observed. Only Temp 4 samples showed a higher linolenic acid content than the Temp 1 samples when sprayed with the control spray.

Theoretically, a BHT spray stops lipid degradation as a chain stopper for the free radical chain mechanism of lipid oxidation (Stucky, 1977) while strawberries are subjected to cold or hot temperature treatments which will induce a loss of linoleic and linolenic acids (Willemot, 1983; Ichihara and Noda, 1980; Parkin and Kuo, 1989). Therefore, the BHT sprayed samples were supposed to have higher linoleic and linolenic acid contents than the control sprayed samples. This only occurred in certain cases in this study. On the other hand, the reverse effect (the control sprayed samples containing higher linoleic or linolenic acids than the BHT sprayed samples) was also observed in some cases. Therefore, it was impossible to conclude a definite effect of the antioxidant spray on polyunsaturated fatty acid content. This is probably due to the fact that the spray only covers the surface of
the fruits thus making limited contact with the lipids that the chemical sprays were supposed to have an effect on.

Observing the linoleic and linolenic acid contents of whole fruits and seeds in three lipid classes, regularly showed that cold storage treatments (Temp 2, Temp 3 and Temp 5) seem to cause an increase. This is especially true with the control sprayed samples. This can be explained by the theory that low temperatures induce an increase in the polyunsaturated fatty acid content of plant lipids (Hopkirk and Wills, 1981; Gawer et al., 1983; ). In addition, in certain rare occasions, the cold temperature treatments (especially Temp 2 and Temp 3) showed a tendency to decrease the linoleic acid content. As mentioned previously, other researchers have found a decrease in unsaturated fatty acid when plants were subjected to cold temperature treatments (Willemot, 1983; Parkin and Kuo, 1989). Therefore, it is likely that during cold storage (especially Temp 2 and Temp 3 treatments), there is both an increase of unsaturation in order to maintain the membrane fluidity and the degradation of polyunsaturated fatty acid occurred but these occur at different degrees. Which of these is the predominant effect during cold storage may vary with the temperature of cold storage and the time that the fruits are kept in the cold storage. As for the effect of a hot temperature treatment (Temp 4 treatment) on polyunsaturated fatty acid content, no decisive conclusion can be drawn.
LOX Activity of Strawberry

Extraction of LOX enzyme. A preliminary study indicated that the LOX of strawberry was active at pH 5.5 to pH 7.0 with the maximum activity of LOX at pH 6.5. After extracting the crude enzyme extract with sodium phosphate buffer at pH 9.0, no LOX activity was detected. LOX-1 catalyzes the oxidation of substrates optimally at pH 9.0 while LOX-2 shows maximum activity at pH 6.5 provided that Ca\(^{+2}\) is absent (Schieberle et al., 1981). Since the optimum pH of strawberry LOX is 6.5, strawberry LOX is probably LOX-2. Generally speaking, strawberry is a very high acid fruit. In this study, pHs of 3.4 to 3.9 were observed in strawberry slurries. Homogenizing strawberries in sodium phosphate buffer at pH 7 resulted in a homogenate with pH 4.0 to pH 4.5 therefore deactivating LOX. It was discovered that when a high pH buffer (at pH 8 to pH 9) was used, better activity of LOX was detected. Blending strawberries with a high pH sodium phosphate buffer resulted in a more neutral homogenate. Gelation was observed in the enzyme extract, this was probably caused by the high concentration of pectin in strawberries. Therefore, it was necessary to incorporate a chelating agent (such as EDTA) to prevent the gelation of enzyme extract. The crude extract of enzyme lost its activity rapidly even when it was kept below 5 °C. Dialysis of the enzyme extract improves the stability and activity of LOX.
**LOX activity of strawberry in four stages of maturity.** Table 15 shows that no LOX activity was detected in green fruits. This results may be questionable due to the smaller size (~ 5 g) of samples were used because of a low number of green fruits available. The red, pink and white stages had sample sizes of ~ 15 g. However, it was assumed that if the green stage had contained LOX, it had to be in very small amounts. The white stage showed the highest LOX activity among the four stages of maturity. Although no significant difference in LOX activity was observed between the red and pink stages, the LOX activity seems to decline in the more mature stages (from 0.84 to 0.67). Selvaraj (1988) reported that LOX activity of mango fruit increased from harvest maturity until the 1/2 ripe stage and then declined. In addition, in 1985, Zamora et al., also observed that LOX activity in grapes was higher in partially ripe than in fully ripe grapes.

**LOX activity of red strawberries subjected to temperature and chemical spray treatments.** Table 16 clearly shows that no LOX activity was detected in the red strawberries stored at -20 °C for 6 months (both of control and BHT spray). Chen and Whitaker (1986) indicated that 80% of the LOX activity of the frozen concentrate of immature English peas was lost in 2 weeks at -18 °C. Within the same temperature treatment, no difference in LOX activity was observed between
BHT sprayed samples and control sprayed samples. This is not to be expected, since BHT prohibits lipid oxidation by the termination of the free radical chain reaction of lipid oxidation and BHT itself does have inhibiting effect on LOX activity which is measured by the oxygen uptake of lipid oxidation. This effect of BHT spray on LOX activity may depended upon how well the BHT dispersed in the enzyme extract.

When comparing the LOX activity of red strawberries subjected to hot temperature (Temp 4) and cold temperature (Temp 2 and Temp 3) treatments with that of Temp 1 berries, no significant difference was observed. But Temp 5 (sub-zero temperature) treatment showed the effect of decreasing LOX activity. Although not statistically different, the hot temperature treatment seems to increase the LOX activity for the red stage.

Al-Obaidy and Siddiol (1981) reported that the LOX activity of broad bean increased with the rise of temperature from 1 °C to 30 °C. In addition, they indicated that the LOX showed no loss of activity up to 55 °C, but between 55 °C and 65 °C, about 40% activity was lost.

C₆ Aldehydes Derived from Oxidized Lipids

It is thought that a number of flavor compounds can be related to lipid oxidation. This is particularly true for the formation of aldehydes, especially C₆ aldehyde. Lipid oxidation generally involves free radical chain reactions (either enzymatic
or non-enzymatic). Polyunsaturated fatty acids (linoleic and linolenic acids) are more susceptible to oxidation due to their 1,4-pentadiene structure which LOX catalyzes the oxygenation of. Both linoleic and linolenic acids produce hexanal by an autoxidation process (Nawar, 1985). As mentioned previously, C₆ aldehydes (n-hexanal, cis-3-hexenal, trans-2-hexenal) have been well proven as aromas derived from lipid oxidation of linoleic and linolenic acids (Galliard et al., 1977; Takeo and Tsushida, 1980; Hatanake et al., 1976; Zamora et al., 1985; Selvaraj, 1988). Therefore, detection of hexanal was used as an indicator of lipid oxidation.

In this study, fruits at all four stages of maturity released hexanal, i.e., 25.70%, 26.95%, 19.06% and 20.14% for green, white, pink and red strawberries respectively. This indicated that green fruits may contain LOX. Additionally, another aroma compound derived from lipid oxidation, 2-hexenal, was also detected in the red, pink, and white stage. The stages contained 35.74%, 34.56% and 48.42% for red, pink and white strawberries respectively. No 2-hexenal was detected in the green stage. These results for flavor confirmed the LOX activity of the four stages, i.e., the green fruits may contain a small amount of LOX, since hexanal but no 2-hexenal was observed; the white fruits released the highest amount of both hexanal and 2-hexenal while also showing the highest LOX activity among the four stages of maturity.
From the temperature point of view, neither hexanal nor 2-hexenal were detected for Temp 5 (-20 °C for 6 months). This observation agreed well with the lack of LOX activity of Temp 5. Also, Table 17 shows that Temp 4 (40 °C for 2 hours) did increase the hexanal production which also agrees with the fact that Temp 4 contains higher (not statistically) LOX activity than the Temp 1. Again, the antioxidant spray did not show consistent results of reducing the release of hexanal.
Chapter VI
SUMMARY AND CONCLUSION

Cultivated strawberries were grown in the green house of the Department of Horticulture of the Ohio State University. Strawberry fruits were harvested at green, white, pink and red stages. The lipid composition of the lipid classes of whole fruits and seeds, LOX activity and lipid derived flavor compounds, hexanal were studied. In addition, mature red fruits were subjected to different temperature treatments (5 °C for 4 days, 5 °C for 7 days, 40 °C for 2 hours and -20 °C for 6 months) and chemical sprays (BHT spray and control spray). Again, the change of lipid composition of lipid classes of fruits and seeds, LOX activity and production of hexanal were studied. Generally it is believed that lipids contribute to certain flavor compounds by means of oxidation during fruit development and storage. LOX was believed to play an important role in lipid oxidation of fruits.

Lipids were extracted from whole fruits and seeds by Folch reagent (chloroform : methanol, 2 : 1, v/v) (Folch et al., 1957). Extracted lipids were further separated into three lipid classes, NL, GL and PL by silicic acid column chromatography (Rouser et
The fatty acid compositions of each lipid class were analyzed by GC. For the LOX enzyme assay, LOX activity was determined by the oxygen probe method. Crude enzyme extracts were prepared using sodium phosphate buffer with EDTA added to prevent coagulation. Dialysis was applied to the enzyme extract to stabilize its activity. Pure linoleic acid solution was the substrate for the enzyme assay. Volatile compounds were detected by GC-MS.

The results of this study are summarized as follows:

1. Seeds contained a higher % lipid content than the whole fruits (on the fresh weight basis).

2. For the whole fruits of different stages of maturity, green fruits contained the highest % lipid content (on the fresh weight basis), On the contrary, seeds from the green fruits contained the lowest % of lipid content (on the fresh weight basis).

3. PL of whole fruits and seeds decreased as the maturity increased, and NL increased as the maturity increased.

4. Cold temperature (Temp 2 and Temp 3) seems to decrease NL but increase GL content in both fruits and seeds. No definite effect of BHT spray on lipid classes of strawberry was observed.
5. Seven fatty acids were detected in three lipid classes of whole fruits and seeds; lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid.

6. In the three lipid classes of whole fruits and seeds, lauric and myristic acids were mostly present in insignificant quantities (less than 1 mg/100 g of sample and 1 mg/g of sample for whole fruits and seeds respectively).

7. Oleic, linoleic and linolenic acids were the predominant fatty acids present in three lipid classes of fruits and seeds.

8. Generally speaking, linoleic and linolenic acids decreased as the maturity increased (pink to red stages) for both fruits and seeds.

9. In this study, no definite effect of BHT spray on polyunsaturated fatty acids was observed. Since the spray was a surface application, this result may be affected by limited contact between BHT and polyunsaturated fatty acids inside the fruits.

10. Cold temperature treatments (Temp 2, Temp 3 and Temp 5) showed both increasing and decreasing polyunsaturated fatty acids.

11. A hot temperature treatment (Temp 4) showed no conclusive effect on polyunsaturated fatty acids.

12. The optimum pH of strawberry LOX is pH 6.5 indicating that the strawberry LOX may be LOX-2.
13. White fruits contained the highest LOX activity among four stages of maturity.

14. LOX activity tends to decrease as maturity increases.

15. The BHT spray showed no effect on LOX activity.

16. The Hot temperature treatment tends to increase the LOX activity.

17. LOX was sensitive to freezing. No LOX activity was detected in the fruits subjected to -20 °C for 6 months.

18. LOX may be at least partially responsible for the production of hexanal, since the LOX activity correlated well with the quantity of hexanal released.
Table 9. Fatty acid composition (mg / 100 g of sample) of NL of whole fruits with five temperature treatments and chemical sprays.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Spray</th>
<th>Temp 1</th>
<th>Temp 2</th>
<th>Temp 3</th>
<th>Temp 4</th>
<th>Temp 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 : 0</td>
<td>Control</td>
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<td>0.14</td>
<td>0.23</td>
<td>0.26</td>
<td>0.16</td>
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<tr>
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<td>0.13</td>
<td>0.66</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>14 : 0</td>
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<td>0.30</td>
<td>0.28</td>
<td>0.20</td>
</tr>
<tr>
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<td>3.09</td>
<td>1.42</td>
<td>1.31</td>
<td>0.94</td>
</tr>
<tr>
<td>16 : 0</td>
<td>Control</td>
<td>7.74</td>
<td>9.12</td>
<td>10.61</td>
<td>12.12</td>
<td>9.65</td>
</tr>
<tr>
<td>16 : 0</td>
<td>BHT</td>
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<td>10.14</td>
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<td>8.34</td>
</tr>
<tr>
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<td>54.59</td>
<td>53.82</td>
<td>62.53</td>
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</table>

1. Chemical sprays: Control and BHT sprays.
   Temp 1 : no temperature treatment.
   Temp 2 : 5 °C for 4 days.
   Temp 3 : 5 °C for 7 days.
   Temp 4 : 40 °C for 2 hours.
   Temp 5 : -20 °C for 6 months.
Table 10. Fatty acid composition (mg / 100 g of sample) of GL of whole fruits with five temperature treatments and chemical sprays.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Spray</th>
<th>Temp 1</th>
<th>Temp 2</th>
<th>Temp 3</th>
<th>Temp 4</th>
<th>Temp 5</th>
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<tr>
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<td>0.50</td>
<td>0.36</td>
<td>0.20</td>
<td>0.43</td>
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<tr>
<td>12 : 0</td>
<td>BHT</td>
<td>0.20</td>
<td>0.29</td>
<td>0.96</td>
<td>1.01</td>
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<td>0.45</td>
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<td>0.94</td>
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<td>9.49</td>
<td>11.29</td>
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</tr>
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<td>1.61</td>
<td>1.43</td>
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</table>

1. Chemical sprays: control and BHT sprays.

   Temp 1: no temperature treatment.
   Temp 2: 5 °C for 4 days.
   Temp 3: 5 °C for 7 days.
   Temp 4: 40 °C for 2 hours.
   Temp 5: -20 °C for 6 months.
Table 11. Fatty acid composition (mg / 100 g of sample) of PL of whole fruits with five temperature treatments and chemical sprays.$^1$

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Spray</th>
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<th>Temp 2</th>
<th>Temp 3</th>
<th>Temp 4</th>
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<td>2.18</td>
<td>2.24</td>
<td>1.24</td>
</tr>
<tr>
<td>18 : 0</td>
<td>BHT</td>
<td>1.53</td>
<td>1.20</td>
<td>1.53</td>
<td>1.48</td>
<td>1.27</td>
</tr>
<tr>
<td>18 : 1</td>
<td>Control</td>
<td>2.53</td>
<td>3.16</td>
<td>4.69</td>
<td>3.03</td>
<td>3.80</td>
</tr>
<tr>
<td>18 : 1</td>
<td>BHT</td>
<td>9.68</td>
<td>3.20</td>
<td>5.38</td>
<td>6.25</td>
<td>2.60</td>
</tr>
<tr>
<td>18 : 2</td>
<td>Control</td>
<td>19.31</td>
<td>22.08</td>
<td>28.58</td>
<td>14.77</td>
<td>18.34</td>
</tr>
<tr>
<td>18 : 2</td>
<td>BHT</td>
<td>22.78</td>
<td>21.99</td>
<td>34.70</td>
<td>24.78</td>
<td>22.22</td>
</tr>
<tr>
<td>18 : 3</td>
<td>Control</td>
<td>10.81</td>
<td>10.20</td>
<td>12.72</td>
<td>6.99</td>
<td>9.51</td>
</tr>
<tr>
<td>18 : 3</td>
<td>BHT</td>
<td>9.75</td>
<td>11.07</td>
<td>10.98</td>
<td>10.15</td>
<td>11.62</td>
</tr>
</tbody>
</table>

1. Chemical sprays: control and BHT sprays.

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.
Table 12. Fatty acid composition (mg / 1 g of sample) of NL of seeds with different temperature treatments and chemical sprays.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Spray</th>
<th>Temp 1</th>
<th>Temp 2</th>
<th>Temp 3</th>
<th>Temp 4</th>
<th>Temp 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>Control</td>
<td>0.00</td>
<td>0.02</td>
<td>0.02</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>12:0</td>
<td>BHT</td>
<td>0.01</td>
<td>0.07</td>
<td>0.04</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>14:0</td>
<td>Control</td>
<td>4.30</td>
<td>4.04</td>
<td>2.09</td>
<td>10.63</td>
<td>0.09</td>
</tr>
<tr>
<td>14:0</td>
<td>BHT</td>
<td>39.31</td>
<td>0.25</td>
<td>0.07</td>
<td>10.44</td>
<td>0.07</td>
</tr>
<tr>
<td>16:0</td>
<td>Control</td>
<td>10.65</td>
<td>5.13</td>
<td>6.39</td>
<td>7.69</td>
<td>8.42</td>
</tr>
<tr>
<td>16:0</td>
<td>BHT</td>
<td>6.32</td>
<td>15.25</td>
<td>6.05</td>
<td>5.89</td>
<td>7.20</td>
</tr>
<tr>
<td>18:0</td>
<td>Control</td>
<td>2.53</td>
<td>1.06</td>
<td>1.19</td>
<td>1.36</td>
<td>1.99</td>
</tr>
<tr>
<td>18:0</td>
<td>BHT</td>
<td>1.29</td>
<td>3.50</td>
<td>1.39</td>
<td>1.12</td>
<td>1.54</td>
</tr>
<tr>
<td>18:1</td>
<td>Control</td>
<td>30.92</td>
<td>15.04</td>
<td>19.87</td>
<td>27.51</td>
<td>18.00</td>
</tr>
<tr>
<td>18:1</td>
<td>BHT</td>
<td>13.26</td>
<td>53.79</td>
<td>12.69</td>
<td>18.51</td>
<td>14.91</td>
</tr>
<tr>
<td>18:2</td>
<td>Control</td>
<td>72.10</td>
<td>44.17</td>
<td>54.19</td>
<td>70.51</td>
<td>64.51</td>
</tr>
<tr>
<td>18:2</td>
<td>BHT</td>
<td>56.29</td>
<td>95.04</td>
<td>50.91</td>
<td>56.60</td>
<td>55.10</td>
</tr>
<tr>
<td>18:3</td>
<td>Control</td>
<td>55.37</td>
<td>39.96</td>
<td>47.13</td>
<td>58.11</td>
<td>51.27</td>
</tr>
<tr>
<td>18:3</td>
<td>BHT</td>
<td>47.62</td>
<td>66.09</td>
<td>43.02</td>
<td>48.94</td>
<td>41.32</td>
</tr>
</tbody>
</table>

1. Chemical sprays: Control and BHT sprays.

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.
Table 13. Fatty acid composition (mg / 1 g of sample) of GL of seeds with different temperature treatments and chemical sprays.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Spray</th>
<th>Temp 1</th>
<th>Temp 2</th>
<th>Temp 3</th>
<th>Temp 4</th>
<th>Temp 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 : 0</td>
<td>Control</td>
<td>0.00</td>
<td>0.17</td>
<td>0.08</td>
<td>0.01</td>
<td>0.19</td>
</tr>
<tr>
<td>12 : 0</td>
<td>BHT</td>
<td>0.00</td>
<td>0.41</td>
<td>0.08</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>14 : 0</td>
<td>Control</td>
<td>0.01</td>
<td>0.32</td>
<td>0.35</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>14 : 0</td>
<td>BHT</td>
<td>0.17</td>
<td>0.79</td>
<td>0.21</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>16 : 0</td>
<td>Control</td>
<td>0.94</td>
<td>5.44</td>
<td>4.82</td>
<td>0.61</td>
<td>2.78</td>
</tr>
<tr>
<td>16 : 0</td>
<td>BHT</td>
<td>0.91</td>
<td>10.86</td>
<td>3.02</td>
<td>0.29</td>
<td>0.65</td>
</tr>
<tr>
<td>18 : 0</td>
<td>Control</td>
<td>0.51</td>
<td>2.16</td>
<td>2.19</td>
<td>0.25</td>
<td>1.46</td>
</tr>
<tr>
<td>18 : 0</td>
<td>BHT</td>
<td>0.52</td>
<td>3.80</td>
<td>1.69</td>
<td>0.24</td>
<td>0.28</td>
</tr>
<tr>
<td>18 : 1</td>
<td>Control</td>
<td>0.10</td>
<td>6.59</td>
<td>6.40</td>
<td>0.34</td>
<td>1.81</td>
</tr>
<tr>
<td>18 : 1</td>
<td>BHT</td>
<td>0.62</td>
<td>11.52</td>
<td>1.98</td>
<td>0.13</td>
<td>0.35</td>
</tr>
<tr>
<td>18 : 2</td>
<td>Control</td>
<td>0.18</td>
<td>5.86</td>
<td>5.27</td>
<td>0.68</td>
<td>0.96</td>
</tr>
<tr>
<td>18 : 2</td>
<td>BHT</td>
<td>1.85</td>
<td>11.51</td>
<td>1.28</td>
<td>0.32</td>
<td>0.26</td>
</tr>
<tr>
<td>18 : 3</td>
<td>Control</td>
<td>0.27</td>
<td>3.88</td>
<td>3.86</td>
<td>0.36</td>
<td>0.70</td>
</tr>
<tr>
<td>18 : 3</td>
<td>BHT</td>
<td>1.09</td>
<td>5.64</td>
<td>0.69</td>
<td>0.18</td>
<td>0.17</td>
</tr>
</tbody>
</table>

1. Chemical sprays: Control and BHT sprays.

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.
Table 14. Fatty acid composition (mg / 1 g of sample) of PL of seeds with five temperature treatments and chemical sprays\textsuperscript{1}.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Spray</th>
<th>Temp 1</th>
<th>Temp 2</th>
<th>Temp 3</th>
<th>Temp 4</th>
<th>Temp 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>Control</td>
<td>0.00</td>
<td>0.04</td>
<td>0.03</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>12:0</td>
<td>BHT</td>
<td>0.05</td>
<td>0.33</td>
<td>0.10</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>14:0</td>
<td>Control</td>
<td>0.05</td>
<td>0.10</td>
<td>0.13</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>14:0</td>
<td>BHT</td>
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<td>0.21</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
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<td>2.43</td>
<td>3.17</td>
<td>5.35</td>
<td>4.84</td>
</tr>
<tr>
<td>16:0</td>
<td>BHT</td>
<td>3.78</td>
<td>9.42</td>
<td>5.62</td>
<td>1.00</td>
<td>3.54</td>
</tr>
<tr>
<td>18:0</td>
<td>Control</td>
<td>0.92</td>
<td>0.84</td>
<td>1.39</td>
<td>1.36</td>
<td>1.52</td>
</tr>
<tr>
<td>18:0</td>
<td>BHT</td>
<td>1.10</td>
<td>2.92</td>
<td>1.96</td>
<td>0.32</td>
<td>1.00</td>
</tr>
<tr>
<td>18:1</td>
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<td>2.53</td>
<td>3.65</td>
<td>3.79</td>
<td>3.38</td>
</tr>
<tr>
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<td>1.58</td>
<td>10.97</td>
<td>1.59</td>
<td>0.54</td>
<td>1.82</td>
</tr>
<tr>
<td>18:2</td>
<td>Control</td>
<td>2.98</td>
<td>5.42</td>
<td>6.59</td>
<td>15.90</td>
<td>8.55</td>
</tr>
<tr>
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<td>4.95</td>
<td>14.48</td>
<td>11.09</td>
<td>2.04</td>
<td>4.84</td>
</tr>
<tr>
<td>18:3</td>
<td>Control</td>
<td>1.52</td>
<td>2.16</td>
<td>2.45</td>
<td>6.80</td>
<td>4.05</td>
</tr>
<tr>
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<td>5.88</td>
<td>4.74</td>
<td>0.83</td>
<td>2.97</td>
</tr>
</tbody>
</table>

1. Chemical sprays: Control and BHT sprays.

Temp 1: no temperature treatment.
Temp 2: 5 °C for 4 days.
Temp 3: 5 °C for 7 days.
Temp 4: 40 °C for 2 hours.
Temp 5: -20 °C for 6 months.
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Ha, E. Y. W., Morr, C. V. and Seo, A. 1991. Effect of three different soaking conditions on the production of isoflavone aglucones and headspace volatile organic compounds in soybeans. Laboratory report. Department of Food Science, The Ohio State University, OH.


