AN ABSTRACT OF THE THESIS OF

Chockchai Theerakulkait for the degree of Doctor of Philosophy in Food Science and Technology presented on February 22, 1994.

Title: Sweet Corn Germ Lipoxygenase: Isolation, Purification, Characterization and Involvement in Off-Aroma Formation

Abstract approved: _____
Diane M. Barrett

Lipoxygenase activity in the germ fraction of sweet corn (var. Jubilee) was determined and compared with that in the degermed fraction. Lipoxygenase activity per gram of germ was found to be approximately three times as great as that of the degermed fraction. Optimized procedures for isolation of lipoxygenase from the germs were developed. Lipoxygenase was isolated by preparation as an acetone powder, extraction with 0.2 M Tris-HCl, pH 8.0 (4°C), fractionation with 40-60% saturated ammonium sulfate and dialysis. The optimum pH and temperature for activity of lipoxygenase were 6-7 and 50°C, respectively. The enzyme appeared to be stable in the range of pH 5-8 and approximately 90% of original activity was inactivated after heating in pH 7 buffer at 70°C for 3 min.

Lipoxygenase in a 40-60% ammonium sulfate preparation was purified by conventional column chromatography on Sephacryl S-300 HR and fast protein liquid chromatography (FPLC) on a Mono Q

column. The purification was 124 fold with 26.3 percent recovery. Further purification was achieved by FPLC on Mono P and Superose-12. The estimated molecular weight and isoelectric point were 90,500 and 5.06, respectively. Lipoxygenase was inhibited by butylated hydroxyanisole, butylated hydroxytoluene, quercetin, and chlorogenic acid (at 0.2 mM) with 57.2, 16.3, 61.4 and 32.3 percent inhibition, respectively. The activation energy for thermal inactivation of the enzyme was 56.3 kcal/mol.

The mean overall intensity, and most off-aroma descriptors of a homogenate of frozen stored unblanched corn on the cob were significantly higher than that of a blanched sample. To investigate the involvement of sweet corn germ enzymes, particularly lipoxygenase, in off-aroma formation, crude enzyme extract, purified lipoxygenase, and purified peroxidase were prepared from the germs and added back to blanched corn homogenate. The samples, including blanched and unblanched samples were incubated, heated, and evaluated by a trained descriptive panel. Adding lipoxygenase significantly increased "painty" and "stale/oxidized" descriptors and significantly lowered "sweet" and "corn". Other enzymes in the germs may be involved in off-aroma formation. There was evidence suggesting that peroxidase in the germs is not as important. Lipoxygenase in the germs is important in off-aroma formation and it might be more appropriate than peroxidase for use as a blanching index.

Sweet Corn Germ Lipoxygenase: Isolation, Purification, Characterization and Involvement in Off-Aroma Formation

by

Chockchai Theerakulkait

A THESIS

Submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed February 22, 1994

Commencement June 1994

APPROVED:

Assistant Cooperative Extension Specialist of Food Science and Technology in charge of major

Head of Department of Food Science and Technology

Dean of Graduate School

Date thesis is presented: February 22, 1994

Typed by the author: Chockchai Theerakulkait

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to Dr. Diane M. Barrett, my major professor, for support, guidance, encouragement, and friendship throughout my study. I deeply appreciate the support, encouragement and friendship of my immediate advisor and thesis committee member, Dr. Mina R. McDaniel. My sincere appreciation is extended to Dr. Michael H. Penner, Dr. Wilbert Gamble, my thesis committee; Dr. Lorraine T. Miller, my graduate council representative; and Dr. Leonard M. Libbey, my former thesis committee member for their support, understanding and encouragement.

I am very grateful to the International Rotary Foundation for providing me with a three year "Freedom From Hunger Scholarship" for my Ph.D. study. My gratitude is extended to Dr. Harold W. Schultz, my Rotarian Counselor, and his wife for their support, encouragement, guidance and friendship through out my Ph.D. program and my time in U.S.A. I wish to express my appreciation to Dr. & Mrs. Horace Cheney for their support and friendship. I also would like to thank the committee on Grants-in-Aid of Research, Sigma Xi, The Scientific Research Society for partial support the research. Special thanks to Mr. Dennis Spink of the Agri-Pac Co., Woodburn, Oregon, and the National Frozen Food Co., Albany, Oregon, in providing the sweet corn samples for the research.

Special appreciation is extended to Dr. Dan Farkas for his genuine concern, support, advice and encouragement throughout my

Ph.D. program. I also wish to express my appreciation to Dr. Ron Wroldstad, for serving as my academic advisor in the first year and for his concern, support and encouragement; to Dr. Alan Bakalinsky for advising and, training me in genetic engineering research for about a year, and for his understanding, concern and friendship; and to all faculty in the Department of Food Science and Technology in providing me the knowledge, training, and support.

Special thanks to Brian Yorgey, Bob Durst, all staff in the main office: Cheryl Houk, Anne Adams, Alice True, Anita Brown, Tracy Mitzel, and Rosanna Leeson, and stock room officer, Boyd Wilcox who have always been very concerned, helpful and friendly to me.

Special acknowledgment for the contribution, suggestions and/or support from the following persons: Dr. Reg McParland, Central Service Laboratory for Gene Research and Biotechnology, Dr. Dean Malencik at Dr. Sonia Anderson's laboratory, Dept. of Biochem. & Biophys., Dr. David Thomas, Dept. of Statistics, MS. Gail Nickerson, Dept. of Agricultural Chemistry, Dr. Sonia Rubico, Dept. of Food Sci. & Tech., Dr. John Whitaker, Dept. of Food Sci. & Tech., U. of California at Davis, Davis, CA. Very special thanks to all sweet corn panelists for contributing their valuable time and efforts.

Thanks to my good friends: Blaine Mooers, Michell Wise, Gayle Orner, Dwyane Meadows, Aaron Cady and all of my friends for their friendship, support and/or editorial/technical assistance throughout my studies. Sincere appreciation is also extended to Mr. & Mrs. Melvin Cady for their concern and friendships.

My greatest gratitude and love to my parents, sisters, and brothers for their concern, support, understanding and encouragement. Finally, I would like to dedicate this thesis to my parents for their endless love, sacrifice, patience, understanding, support and encouragement.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

page

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF TABLES

LIST OF APPENDIX TABLES

LIST OF APPENDIX TABLES (continued)

SWEET CORN GERM LIPOXYGENASE: ISOLATION, PURIFICATION, CHARACTERIZATION AND INVOLVEMENT IN OFF-AROMA FORMATION

CHAPTER 1: INTRODUCTION

Sweet corn (*Zea mays* L.) is one of the most popular vegetables in the United States. It ranks second in farm value for processing and fourth for fresh-market among vegetable crops (Marshall, 1987) and it is an important crop for processing in Oregon (USDA, 1988). Quality deterioration, especially off-flavor and off aroma, is one of the most important problems of sweet corn quality after harvesting (Smittle et al., 1972). In order to maintain desirable quality and to make it available year around, sweet corn is processed or preserved by various techniques. Freezing is one of the most acceptable and successful techniques that is used to preserve sweet corn and other vegetables. Blanching is a major step prior to freezing vegetables. An important purpose of blanching is to inactivate endogenous enzymes that are still active under freezing conditions and that could cause undesirable quality changes in frozen vegetables during storage.

Off-flavor and off-aroma development are considered major problems of unblanched (raw) and underblanched frozen sweet corn (Wagenknecht, 1959; Lee, 1981; Velasco et al., 1989). For maximum quality retention of frozen sweet corn or other vegetables, sufficient

heat treatment is needed to inactivate the enzymes that cause quality deterioration, but at the same time, it is desirable to minimize quality loss due to heating. This observation led to the use of an endogenous enzyme as an indicator of blanching (Williams et al., 1986; Lim et al., 1989; Velasco et al., 1989; Reid, 1990). Peroxidase has been used as the index of adequate blanching for sweet corn and other vegetables prior to freezing since peroxidase is ubiquitously distributed, is generally considered to be the most heat stable of the endogenous enzymes in vegetables, and a simple colorimetric test is available for activity testing. However, use of peroxidase is not without problems since it could be regenerated after being heated and there is no evidence that peroxidase causes or is directly associated with off-flavor and other types of quality deterioration in most plant materials including sweet corn. Complete inactivation of peroxidase prior to freezing is suggested to be unnecessary for quality preservation during frozen storage. It might also result in overblanching which leads to unnecessary loss of color, flavor, texture, nutrient and economic loss due to excessive use of water and energy for blanching (Schwimmer, 1981; Williams et al., 1986; Lim et al., 1989; Velasco et al., 1989; Reid, 1990).

Lipoxygenase (Linoleate:oxygen oxidoreductase, EC 1.13.11.12, previously known as lipoxidase) is an important endogenous enzyme that is still active in vegetables under freezing conditions which has been suggested as the cause of off-flavor development in various unblanched and underblanched frozen vegetables including sweet corn (Wagenknecht and Lee, 1958; Wagenknecht, 1959; Lee, 1981;

Ganthavorn, 1989; Velasco et al., 1989; Sheu and Chen, 1991). Lipoxygenase may, therefore, be more important and more appropriate than peroxidase for use as the blanching index in sweet corn freezing. One criterion for choosing the enzyme as the blanching index is that it should have a major responsibility for the flavor or other type of quality deterioration (Williams et al., 1986; Lim et al., 1989; Velasco et al., 1989; Reid, 1990).

Previous studies suggested that in unblanched frozen sweet corn lipoxygenase activity induced off-flavors in the fraction containing the germ (Wagenknecht, 1959; Lee, 1981). The germ fraction also contains high amount of lipid with high polyunsaturated fatty acids (Gardner and Inglett, 1971; Flora and Wiley, 1972; Pascual and Wiley, 1974; Puangnak, 1976; Weber, 1978a and 1978b). Lipoxygenase in the germs might catalyze the oxidation of the *cis,cis*-1,4-pentadiene portion of an unsaturated fatty acid producing a *cis-trans* conjugated fatty acid hydroperoxide which may bring about a secondary deteriorative reaction that can lead to off-flavor and off-aroma development. Therefore, lipoxygenase in the germ fraction might play an important role in aroma deterioration of sweet corn. However, lipoxygenase in sweet corn germs has not been thoroughly investigated.

Prior to addressing whether lipoxygenase is more appropriate than peroxidase to use as the blanching indicator for sweet corn, the involvement of lipoxygenase in off-aroma formation in sweet corn needs to be investigated. Therefore, there is a need to first isolate and purify the lipoxygenase from sweet corn germs as the first step

and then characterize the purified lipoxygenase for better understanding of this enzyme. Finally, the purified lipoxygenase from the germs will be used for studying its involvement in off-aroma formation in sweet corn. Understanding the properties of corn germ lipoxygenase could be useful in improving the isolation and purification methods, in explaining the possible role of lipoxygenase in quality deterioration of sweet corn; and in controlling undesirable changes from the enzyme activity.

The results of this research will aid in the identification of the proper enzyme to be used as a blanching indicator of sweet corn. If lipoxygenase is appropriate for use as blanching indicator, and is less heat stable, the amount of heat used for blanching could be reduced. As a result, tremendous benefits would be realized in terms of improved flavor, texture, aroma and nutritional quality. A higher quality sweet corn product could be produced with substantial energy and cost savings.

The goals of this research were to develop and optimize the methods for extraction, isolation and purification of lipoxygenase from sweet corn germs; to study its characteristics; and to investigate the involvement of sweet corn germ enzymes, particularly lipoxygenase, in off-aroma formation. Three phases of the research were performed with the following objectives:

Part one:

To determine the lipoxygenase activity in the germ fraction of sweet corn compared to that in the degermed fraction.

To develop a procedure for rapid isolation of sweet corn germs.

To optimize the appropriate procedures and conditions for extraction and isolation of lipoxygenase from the sweet corn germ fraction.

To investigate some physicochemical properties of lipoxygenase from sweet corn germs including pH activity and stability, temperature activity and stability.

Part two:

To develop a procedure to further purify lipoxygenase from sweet corn germs by a combination of conventional chromatography with fast protein liquid chromatography (FPLC).

To determine characteristics of sweet corn germ lipoxygenase including molecular weight (MW), isoelectric point (pI), inhibition by synthetic and natural phenolic antioxidants, and thermal inactivation.

Part three:

To study the aroma profile of homogenate prepared from unblanched frozen stored corn on the cob.

To investigate the involvement of enzymes from isolated germs of sweet corn, especially lipoxygenase and peroxidase, in off-aroma formation using descriptive analysis.

CHAPTER 2:
LITERATURE REVIEW

SWEET CORN

Sweet corn (*Zea mays* L.) is a member of the Gramineae or grass family. It is an important crop in the United States and is primarily grown in North America (Marshall, 1987; Wiley et al. 1989). Oregon is one of the leading states for sweet corn production and processing (USDA, 1988). Sweet corn differs from field corn by a mutation at the sugary (*Su*) locus on chromosome 4. Sweet corn has the gene *su* at this locus. The *su* mutation affects the endosperm composition by causing it to accumulate more sugar and water soluble polysaccharides than normal field corn has at the immature state when it is normally consumed (Creech, 1968). The sugary (*su*) gene blocks the conversion of sugar (mostly sucrose) to starch. Because the sugar molecules are smaller than starch molecules and pack more tightly when dried, sweet corn kernels are wrinkled on appearance. Almost all commercially grown sweet corn is a Sugary (normal) Hybrids (*su*). Common examples of standard sweet corn cultivars are Golden Cross Bantam, Gold Cup, and Jubilee. The standard sweet corns are also those listed in seed catalogs as "normal", "traditional", "standard", "conventional", and "sugar". The predominant sugar in sweet corn is sucrose (Cobb and Hannah, 1981; Marshall, 1987).

Structure and composition

Sweet corn structure is composed primarily of edible kernels, husk and cob. However, most food industry interest and research attention has focused on the immature edible corn kernels. The sweet corn kernel is composed of the following major parts: pedicel (tip cap), germ (embryo), pericarp (epicarp and mesocarp), and endosperm (Fig. 2.1).

The germ fraction of the sweet corn kernel is composed of coleptile, plumule, primary root, and coleorhiza. The germ of sweet corn is approximately 5 percent of the whole kernel weight (Puangnak, 1976) and it is high in lipid content and polyunsaturated fatty acids, especially linoleic acid (Wagenknecht, 1959; Gardner and Inglett, 1971; Flora and Wiley, 1972; Pascual and Wiley, 1974; Puangnak, 1976; Weber, 1978a and 1978b). Lipid content and composition, however, also depends on genotype. In contrast, the endosperm which includes more than 80 percent of the kernel contains the bulk of sugars, starches and the water soluble polysaccharides (Wiley et al., 1989).

Aroma quality deterioration

Wiley (1985) defined the optimum or best quality of sweet corn for market quality corn as "a corn with a large well-filled ear with plump kernels wrapped in a tight dark green husk". The kernels themselves should hold quality for a long period, have a sweet taste, tender pericarp, and a *corn-like aroma*". For processing:

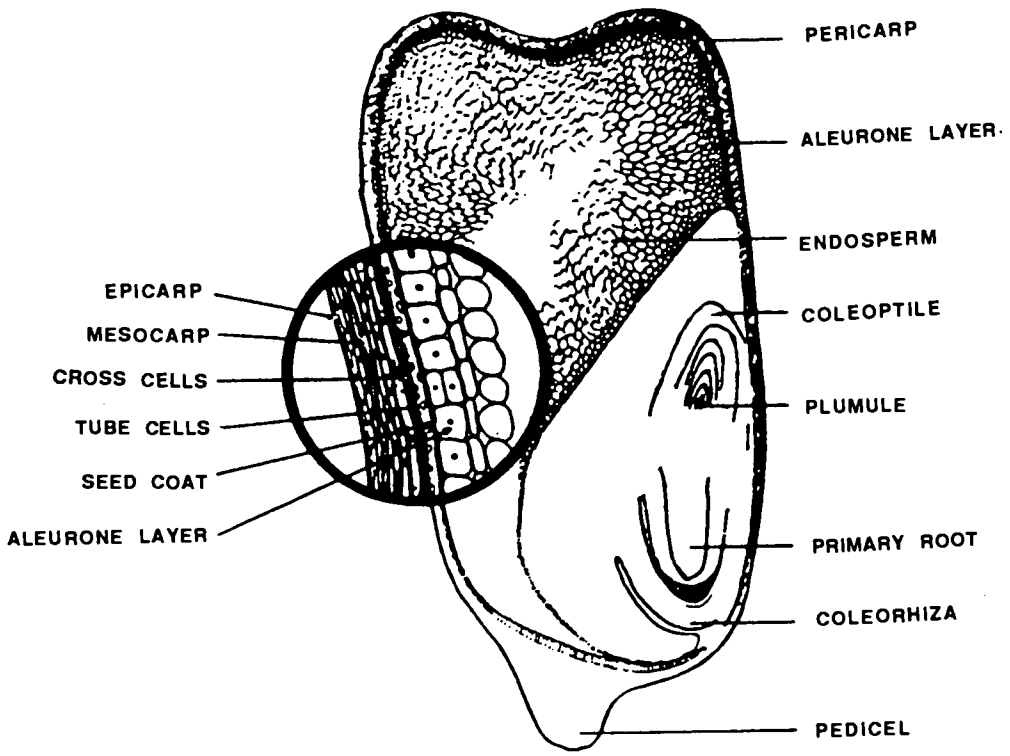

Fig. 2.1- Schematic diagram of a longitudinal section of a corn kernel (Khalil and Kramer, 1971).

sweet corn kernels should have a sweet fresh flavor (not sugary), bright deep yellow color, tender pericarp (not starchy), and a well-proportioned kernel shape with *no off-flavor or odor*". In general raw corn kernels give off very little aroma, and it is believed that the typical cooked corn aroma is heat activated. Dimethyl sulfide (DMS) is one of the key low-boiling volatiles that contributes to the cooked corn aroma (Bills and Keenan, 1968; Williams and Nelson, 1973; Flora and Wiley, 1974; Dignan and Wiley, 1976; Wiley, 1985).

Off-flavor and off-aroma development are considered major reasons for quality deterioration of sweet corn after harvesting, and during frozen storage for an extensive period (Wagenknecht, 1959; Smittle et al., 1972; Lee, 1981; Velasco et al, 1989). The cause of off-aroma formation in frozen stored raw or underblanched sweet corn is believed to be the result of enzymatic action during frozen storage. Of the enzymes in sweet corn, lipoxygenase has frequently been suggested as a causative agent of off-flavor development in unblanched frozen sweet corn (Wagenknecht, 1959; Lee, 1981; Velasco et al., 1989), especially in the fraction containing the germs (Wagenknecht, 1959; Lee, 1981).

Blanching and blanching index

The term "blanching" or "scalding" was originally used to designate those heat treatment operations in the processing of frozen foods which prevent deteriorative changes in food not treated during subsequent storage (Schwimmer, 1981). Blanching is an important step prior to freezing sweet corn and other vegetables. One of the

critical purposes of blanching is to inactivate the endogenous enzymes that are still active under freezing conditions which could cause undesirable quality changes in frozen vegetables. Overblanching can lead to loss in texture, color, flavor, and nutritional quality, formation of cooked flavor, loss in soluble solids and economic losses due to large amounts of water and energy requirements. Underblanching results in quality deterioration such as off-flavor and off-aroma occurring during frozen storage and distribution. To optimize the blanching process, the amount of heat should be sufficient to inactivate only enzyme(s) responsible for deteriorious changes during freezing and frozen storage, but at the same time also minimize quality loss due to heating. Therefore, selection of an appropriate indicator enzyme to use as a blanching index is necessary (Williams et al., 1986; Lim et al., 1989; Velasco et al., 1989; Reid, 1990).

Peroxidase is used as the index of adequate blanching for sweet corn and other vegetables prior to freezing since peroxidase is ubiquitously distributed, easy to test for and is generally considered the most heat stable of the endogenous enzymes in vegetables (Joslyn, 1949; Morris, 1958; Schwimmer, 1981; Lim et al., 1989; Velasco et al., 1989). Moreover, blanching to a negative peroxidase activity is often used to assure inactivation of other enzymes, and thus product stability. However, use of peroxidase is not without problems since it can be regenerated under certain conditions and there is no evidence that peroxidase causes or is directly associated with off-flavor and other types of quality deterioration in most plant

materials including sweet corn. It has been suggested that complete inactivation of peroxidase prior to freezing is unnecessary for quality preservation during frozen storage of some vegetables such as peas, green beans, cauliflower, and Brussels sprouts (Winter, 1969; Lu and Whitaker, 1974; Bottcher, 1975; Burnette, 1977; Williams et al., 1986).

Lipoxygenase is one of the endogenous enzymes that has been suggested as a causative agent of the off-aroma development in some vegetables such as soybeans, green beans, peas, and sweet corn (Lee and Wagenknecht, 1958; Wagenknecht, 1959; Eskin et al., 1977; Lee, 1981; Schwimmer, 1981, Williams et al., 1986; Velasco et al., 1989; Sheu and Chen, 1991). One criterion for choosing the enzyme as the blanching index is that it should have a major role in the flavor or other type of quality deterioration (Williams et al., 1986; Lim et al., 1989; Velasco et al., 1989; Reid, 1990). Therefore, lipoxygenase might be more important and appropriate than peroxidase for use as an indicator of blanching adequacy for particular vegetables including sweet corn.

LIPOXYGENASE

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12), was formerly known as "lipoxidase", "carotene oxidase" and "fat oxidase". It catalyzes the oxidation, by molecular oxygen, of fatty acids containing a *cis,cis*-1,4-pentadiene system to produce a *cis-trans* conjugated fatty acid and their subsequent degradation form volatile and nonvolatile constituents responsible for flavors in food plants such as soybeans. Lipoxygenase is of particular interest to food scientists and technologists primarily due to its role in quality changes of various plant products both undesirable and desirable approaches, especially, its role in production of flavor and aroma compounds (Axelrod, 1974; Eskin et al., 1977).

Occurrence in nature

Lipoxygenase was originally thought to be restricted to certain grains and leguminous seeds (Tappel, 1963). However, it is clear that lipoxygenases are widely distributed in both plants and animals (Hildebrand, 1989). In plants, lipoxygenase is found not only in seeds but also in the other plant organs including leaves, flowers, roots and fruits. Extensive lists of plants in which lipoxygenase has been found have been published in several papers (Rhee and Watts, 1966a; Pinsky et al., 1971; Axelrod, 1974; Eskin et al., 1977; Galliard and Chan, 1980).

Lipoxygenase is generally high concentration in the legume seeds such as soybeans, peas, and beans. Chang and McCurdy (1985)

investigated lipoxygenase activity in 14 different legumes and found that the activity was highest in soybeans, followed by lentil and cowpea, respectively. Lipoxygenase activity has been found in cereal grains such as wheat, barley, rice, oats; with the activity localized mainly in the germ (embryo) tissue (Auerman et al., 1971; Lulai and Baker, 1976; Yamamoto et al., 1980; Lulai et al., 1981; Von Ceumern and Hartfiel, 1984). In field corn, activity of lipoxygenase has also been found and generally is higher in the germ (embryo) than in the endosperm tissues (Gardner, 1970; Belefant and Fong, 1991). Lipoxygenase activity in sweet corn was also highest in the section of kernel that contained germ tissue (Wagenknecht, 1959; Lee, 1981; Lee et al., 1989).

Subcellular localization

The literature regarding subcellular localization of lipoxygenase within plant cells is not very extensive. Research suggests that the location of lipoxygenase from different sources was variable. For example, Wardale and Galliard (1975) and Wardale and Lambert (1980) found that lipoxygenase was located in the lysosomal fraction of pea roots, potato shoots and cucumber fruits. Song et al. (1990) showed that lipoxygenase-1 and -2 in the cotyledons of germinating soybean seeds and seedlings were localized in the cytoplasm. Garder (1988) reported that lipoxygenase was located in the chloroplasts of some plants such as soybean and pea leaves. The actual subcellular

location of lipoxygenase which is primarily found in germ tissue of the cereal grains, is unknown (Gardner, 1988).

Isozyme forms

Soybean seed lipoxygenase was generally regarded as a single entity until Christopher et al. (1970 and 1972) separated cotyledon soybean lipoxygenase into four distinct isozymes which were originally designated lipoxygenase-1, -2, -3a, and -3b. Lipoxygenase-3a and -3b are very similar, however, and most of researchers associate only three isozymes (lipoxygenase-1, -2, and-3) with soybean cotyledon (Hildebrand et al., 1988). All three isozymes are globular, water-soluble proteins and consist of a single polypeptide. Lipoxygenase-2 and -3 are sometimes referred to as type 2 lipoxygenase while lipoxygenase-1 is refered to as type 1 lipoxygenase. The classical lipoxygenase (type 1) is now know to be somewhat unusual compared with lipoxygenase from other plants. The soybean lipoxygenase type 1 has an alkaline pH optimum (pH 8.0-9.5) and has relatively low bleaching activity, while type 2 enzyme has optimal activity near neutral pH and is more effective in bleaching reaction (Diel and Stan, 1978; Galliard and Chan, 1980; Galliard, 1983; Siedow, 1991).

More than one isozymes of lipoxygenase has also been found in field corn seeds. Belefant and Fong (1991) found that there were at least three lipoxygenase isozymes with distinct pH activity optimums in the dent corn whole kernel during kernel development. Poca et al.

(1990) reported that mature maize seeds after 5 days of germination contained at least two lipoxygenase isozymes, L1 and L2 with different substrate specificity. L1 has more affinity for linolenic acid, while L2 has more for linoleic acid.

Lipoxygenase isozymes are also found in other plants, such as two lipoxygenase isozymes in cowpeas (Den and Mendoza, 1982); three major lipoxygenase isozymes in wheat germs (Shiiba et al., 1991); and two lipoxygenase isozymes in chickpeas (Sanz et al., 1992a).

Lipoxygenase catalyzed reaction mechanism

Aerobic Reaction

Lipoxygenase is a dioxygenase which catalyzes the oxidation of fatty acids containing a *cis,cis*-1,4-pentadiene system to produce a *cis-trans* conjugated fatty acid hydroperoxide (Fig. 2.2). Soybean lipoxygenase was the first to be crystallized successfully (Theorell et al., 1947a and b), and it is the most well known, studied and characterized among lipoxygenases (Gardner, 1988 and 1991; Siedow, 1991).

Substrate specificity: Substrates for lipoxygenase are always considered as polyunsaturated fatty acids and derivatives that contain a *cis,cis*-1,4-pentadiene system. Among the substrates, the most common substrates in plants are the essential fatty acids such

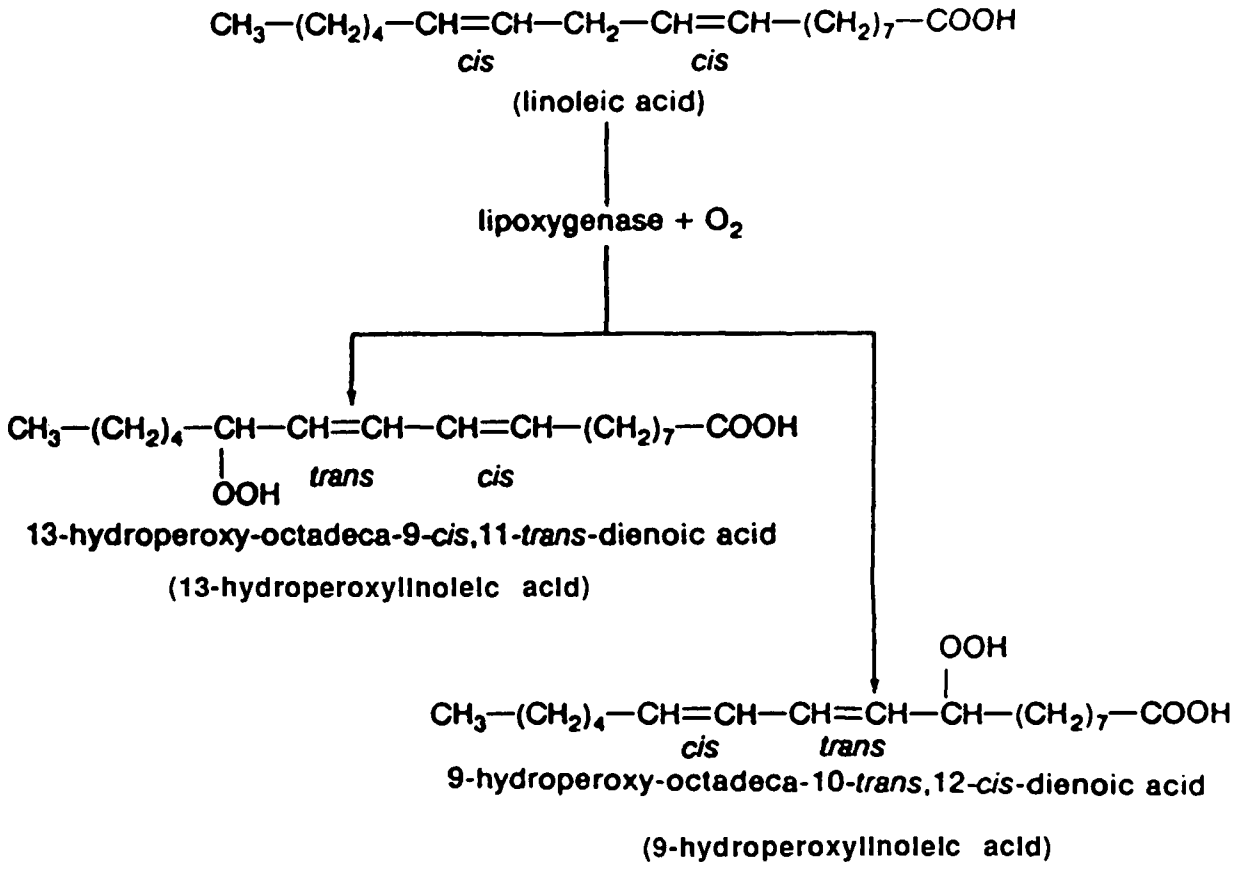

Fig. 2.2- The primary reaction catalyzed by lipoxygenase using linoleic acid (*cis, cis*-9, 12-octadecadienoic acid) as a substrate (Gaillard, 1983).

as linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid) and linolenic acid (*cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid). However, substrates for lipoxygenase are not only free fatty acids, but also fatty acid derivatives such as fatty acid esters, and alcohols with a *cis,cis*-1,4-pentadiene system. A *cis,cis* configuration of the two double bonds of a fatty acid is believed to be required for lipoxygenase activity, while the *cis,trans* and *trans,trans* forms are not catalyzed by the enzyme (Tappel, 1963; Axelrod, 1974; Eskin et al., 1977). However, Funk et al. (1987) demonstrated that both the *cis,trans* and the *trans,cis* isomers of the naturally occurring linoleic acid can serve as substrate for soybean cotyledon lipoxygenase-1.

Acylglycerols such as mono, di and tri-linolein can also be substrates for certain lipoxygenases (Veldink et al., 1977; Gardner, 1980). Efficiency of lipoxygenase catalysis is also affected by the position of the two *cis,cis* methylene interrupted double bonds of the substrates. The highest activity of soybean lipoxygenase-1 was obtained with 9, 12-isomer as found naturally in linoleic acid (Holman et al., 1969).

Reaction mechanism: The mechanism of lipoxygenase catalyzed reaction is discussed in detail in various papers (Veldink et al., 1977; Galliard and Chan, 1980; Vliegenthart et al., 1983; Hildebrand, 1989; Gardner, 1980, 1988 and 1991; Siedow, 1991). Most information was obtained from the studies of soybean lipoxygenase-1 since extensive data is not available on lipoxygenases from other plant sources.

One recognized mechanism for the conjugated *cis,trans*-diene hydroperoxide formation from the oxidation of a fatty acid substrate by lipoxygenase consists of the following steps (Fig. 2.3). Lipoxygenase binds to the fatty acid substrate, then a hydrogen radical is removed from the carbon atom of a methylene group (C_{11} of linoleic acid) between the double bonds to form a fatty acid radical. The removal of hydrogen is stereospecific and the specificity is dependent on the sources of lipoxygenase (Hamberg and Samuelsson, 1967; Egmond et al., 1972; Kuhn et al., 1982). The resulting radical is stabilized by resonance delocalization over the pentadiene system. The conjugation of the double bonds with a *trans* isomerization of the shifted double bond is accompanied by a rearrangement of the radical electron. Then, an oxygen insertion occurs which is position specific depending on the source of lipoxygenases. For example, the oxygen insertion can occur at C_9 or C_{13} of linoleic acid resulting in 9-hydroperoxy-*trans*-10,*cis*-12-octadecadienoic acid or 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid. The 13-hydroperoxy product is the predominant product of soybeans lipoxygenase-1 (Siedow, 1991) while 9-hydroperoxy products are formed predominantly from lipoxygenases from corn germs and potato tubers (Gardner and Weisleder, 1970; Galliard and Chan, 1980). However, reaction conditions such as pH, oxygen concentration, and temperature also influenced the positional specificity of insertion of the oxygen molecule (Galliard and Chan, 1980; Gardner, 1988). Some investigators have proposed other alternative reaction mechanisms,

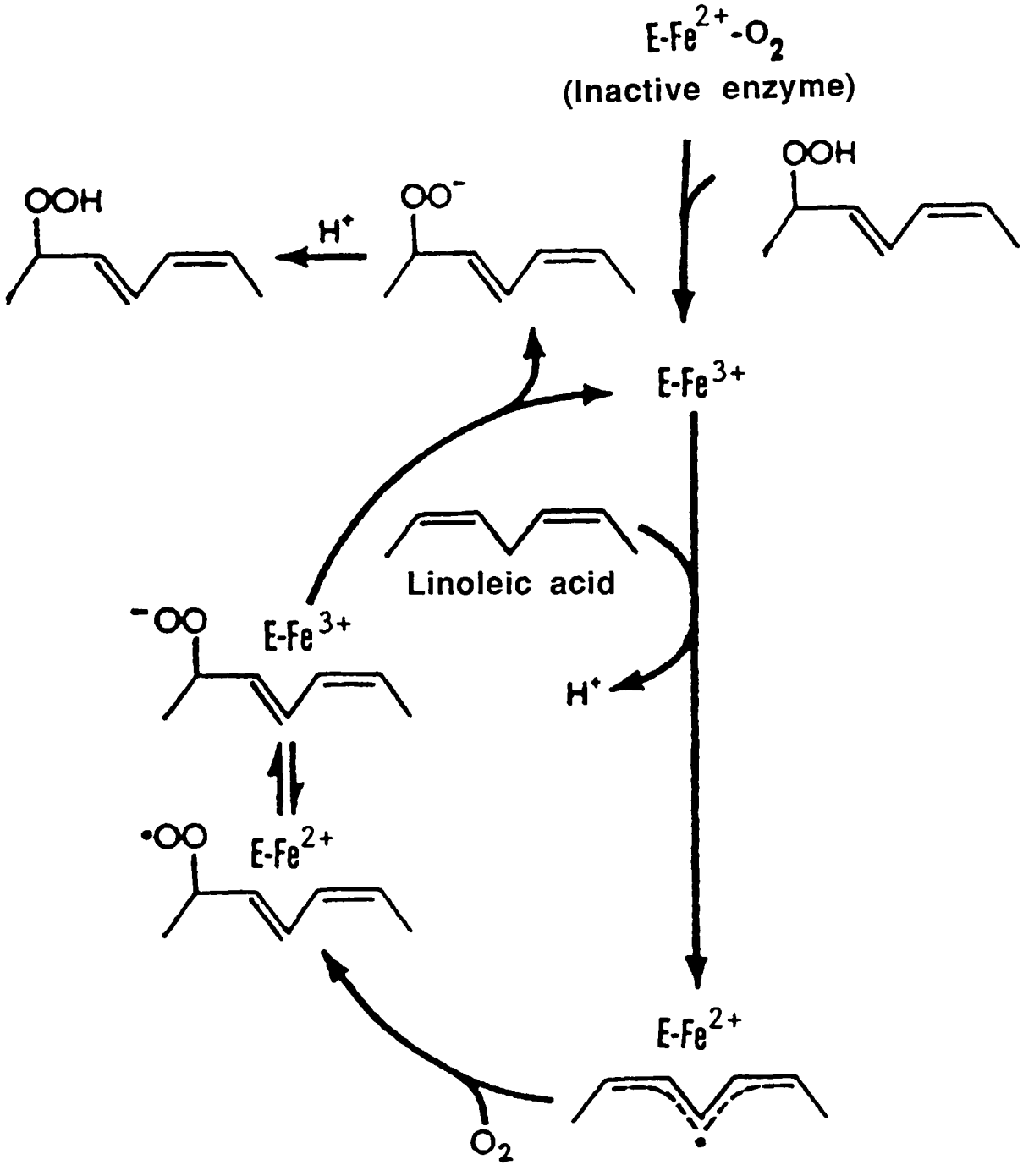

Fig. 2.3- Proposed mechanism for lipoxygenase oxidation of linoleic acid under aerobic condition. Adapted from Gardner (1991) and Veldink et al.(1977).

however, there is no consensus about those mechanisms (Siedow, 1991).

Role of iron in reaction: Soybean lipoxygenase-1 has been the most thoroughly characterized of the lipoxygenases. Each molecule of soybean lipoxygenase-1 contains one atom of iron that is believed to be present at the active site and essential to the enzyme activity (Chan, 1973; Roza and Francke, 1973; Pistorius and Axelrod, 1974). Iron appears to be involved in electron transfer during the incorporation of molecular oxygen into the substrate containing *cis,cis*-1,4-pentadiene system. Iron alternates between the Fe(II) and Fe(III) states during catalysis and it must be in the oxidized (FeIII) form for an oxidation reaction to proceed as shown in Fig. 2.3 (Hildebrand, 1989; Nelson et al., 1991). One of the characteristics of the lipoxygenase reaction is the initial lag period which can be overcome by addition of product hydroperoxides. The hydroperoxides react with the Fe(II) enzyme and oxidize it to the "active" Fe(III) enzyme (Vick and Zimmerman, 1987; Gardner, 1988). However, the initial lag period also depends on other factors such as concentration of the enzyme (Schilstra et al., 1993).

Anaerobic reaction

Under limited oxygen or anaerobic conditions, soybean lipoxygenase-1 is also able to catalyze the reaction. However, the reaction requires the presence of hydroperoxide products. The

complex mixture of products formed in the anaerobic reaction include both dimers and decomposition products of hydroperoxides such as pentane, oxo acid (Galliard and Chan, 1980; Gardner, 1988).

Cooxidation reaction

Lipoxygenases also show oxidizing activity toward other co-substrates such as carotenoids and chlorophyll. This ability of the enzyme has been used as the basis for lipoxygenase assay and in some commercial applications e.g. in flour bleaching. However, lipoxygenases from different sources differ in their cooxidation activity. For example, lipoxygenases from peas and beans (*Phaseolus*) have a high cooxidation potential; potato lipoxygenase is intermediate, whereas wheat, flax, and soybean lipoxygenase-1 have a poor cooxidation activity (Grosch et al., 1976; Eskin et al., 1977; Galliard and Chan, 1980).

Methods of activity assay

Several methods have been used to determine lipoxygenase activity (Holman, 1955; Eskin et al., 1977; Grossman and Zakut, 1979; Galliard and Chan, 1980). The enzymatic activity can be determined by relying on one of the following basis.

a. Oxygen consumption: One of the oldest techniques for lipoxygenase activity determination is the manometric technique based on the

measurement of oxygen consumption by the substrate in the presence of the enzyme (Holman, 1955). The technique has been developed by direct measurement of oxygen consumption using an oxygen electrode (polarographic technique). The polarographic technique is rapid, sensitive and does not require clear solution. Therefore, it is generally the method of choice for determining lipoxygenase activity in crude extract (Galliard and Chan, 1980). This technique was also adapted for measurement of lipoxygenase activity in homogenized vegetable tissue (Zhang et al., 1991). However, the oxygen consumption is not specific for only lipoxygenase activity in the crude extract and a purified lipoxygenase preparation may be subject to denaturation due to the continuous agitation required during measurement (Axelrod, 1974; Grossman and Zakut, 1979).

b. Conjugated diene formation: The spectrophotometric method is based on monitoring the increase in absorbance at 234 nm which results from the enzymatic formation of a conjugated diene. This method is widely used, simple, rapid, specific and accurate (Holman, 1955; Grossman and Zakut, 1979). It is recommended as one of the best choices for routine assays of lipoxygenase activity, and it is useful for pure lipoxygenase preparations (Eskin et al., 1977; Galliard, 1983). This method poses a difficulty in assaying the enzyme activity in the turbid solutions, however, an addition of emulsifying agents such as Tween 20 are useful in clarifying the substrate solution (Surrey, 1964; Ben Aziz et al., 1970).

c. Hydroperoxide formation: The hydroperoxide products from lipoxygenase activity can be measured colorimetrically by reacting with specific agents. For example, Koch et al. (1958) used procedures involving the oxidation of $Fe(CNS)_2$ to the colored $Fe(CNS)_3$ which can be measured spectrophotometrically at 480 nm. This method, however, is not very precise and is rarely used for quantitative determination. Williams et al. (1986) developed a KI-starch method for rapid semiquantitative determination of lipoxygenase activity of fruit and vegetable homogenate. The KI-starch method appears to work well, but only for some fruits and vegetables. For example, in the case of vegetables containing carotenes, such as corn and carrot, the carotenes may become oxidized before iodide, and therefore no activity would be detected. Various other colorimetric assays of lipoxygenase have been reported; however, they have not been very useful for quantitative activity determination due to their generally non-quantitative nature. They are mostly used to locate lipoxygenase isozymes in gel electrophoretograms (Guss et al., 1967; De Lumen and Kazeniac, 1976; Grossman and Zakut, 1979).

d. Co-substrate cooxidation: Lipoxygenase was first generally known by the term "carotene oxidase" since the coupled autoxidation of carotenoids was the first noted consequence of the enzyme's actions. Therefore, lipoxygenase activity can be determined by monitoring the bleaching rate of carotene in a coupled reaction with oxidized unsaturated fatty acids or esters. However, the coupled procedures

are not recommended for routine assays due to poor stoichiometry, instability of the substrate mixtures, and poor cooxidation activity of some lipoxygenases (Axelrod, 1974; Grossman and Zakut, 1979; Galliard and Chan, 1980).

e. Loss of fatty acid substrate: Lipoxygenase activity in crude systems can be determined by quantification of radioactive reaction products produced after incubation of lipoxygenase with radioactively labeled fatty acid substrates. This method is cumbersome and it is often the only approach to study animal lipoxygenases (Galliard and Chan, 1980; Schewe et al., 1986).

f. Other procedures: Several other procedures for determination of lipoxygenase activity have been developed. For example, Lilius and Laasko (1982) developed a sensitive assay for lipoxygenase using the ability of lipoxygenase to co-oxidize luminol, a chemiluminescent probe. However, the presence of any light quenching substance will interfere with the assay. Yabuuchi et al. (1982) used an enzyme-linked immunosorbent assay (ELISA) to determine lipoxygenase isozyme activity in soybeans. This method is sensitive, but it requires a highly purified lipoxygenase to produce specific immunoglobulins.

Isolation and purification of lipoxygenase

Lipoxygenase was first isolated and purified from soybeans by Theorell et at. (1947a and 1947b). Since then, various investigators have worked on the isolation and purification of lipoxygenase, primarily from soybeans (Mitsuda et al., 1967; Allen, 1968; Catsimpoolas, 1969; Steven et al., 1970; Grossman et al., 1972b; Finazzi-Agro et al., 1973; Alen et al., 1977; Kato et al., 1992; Shkarina et al., 1992). Lipoxygenase has also been isolated and purified from other plant sources including peas (Haydar and Hadziyev, 1973; Reynolds and Klein, 1982), broad beans (Al-obaidy and Siddiqi, 1981a and 1981b), egg plants (Grossman et al., 1972a), germinating sunflower seeds (Leoni et al., 1985), pumpkin (Hidaka et al., 1986), asparagus (Ganthavorn and Power, 1989), immature English peas (Chen and Whitaker, 1986), avocado (Marcus et al., 1988), germinating barley (Doderer et al., 1992), chickpeas (Sanz et al., 1992a), green beans (Adams and Ongley, 1989), cow peas (Den and Mendoza, 1982), flax seeds (Rabinovitch-Chable et al., 1992), and kidney beans (Sanz et al., 1993). Moreover, lipoxygenases from the germs (embryos) of cereal grains such as wheat (Wallace and Wheeler, 1979; Nicholas et al., 1982; Shiiba, 1991), barley (Lulai and Baker, 1976; Yabuuchi, 1976), and rice (Yamamoto et al., 1980; Ida et al., 1983) have also been isolated and purified.

Previously, most investigators have extracted and further isolated and purified lipoxygenase using conventional techniques such as ammonium sulfate fractionation, gel filtration, and ion-

exchange chromatography (Gardner and Weisleder, 1970; Steven et al., 1970; Veldink et al., 1972; Klein, 1976; Wallace and Wheeler, 1979; Al-obaidy and Siddiqi, 1981a and 1981b; Nicholas et al., 1982; Reynolds and Klein, 1982; Leoni et al., 1985; Ganthavorn and Power, 1989). These conventional techniques are considered to be reliable; however, there are some disadvantages in that they are time consuming and tedious (Eskin et al., 1977).

Other techniques have also been included in the schemes for isolation and purification of lipoxygenase; for example: isoelectric focusing (Catsimpoolas, 1969; Adams and Ongley, 1989; Poca et al., 1990), affinity chromatography (Grossman et al., 1972b; Vernooy-Gerritsen et al., 1982; Marcus et al., 1988), chromatofocusing (Funk et al., 1985; Chen and Whitaker, 1986), and high performance liquid chromatography (Ramadoss et al., 1982). More recently, in an attempt to increase the yield, purity and to decrease the time requirement, fast protein liquid chromatography (FPLC) has been incorporated into the procedures of isolation and purification of lipoxygenases from some plants such as soybeans (Kato et al., 1992; Shkarina et al., 1992), germinated barley (Doderer et al., 1992), and kidney beans (Sanz et al., 1993).

Isolation and purification of corn lipoxygenase

The isolation and purification of corn lipoxygenase has focused primarily on field corn seeds which include the germs (Gardner and Weisleder, 1970; Veldink et al., 1972; Gardner, 1988), and whole

kernels (Poca et al., 1990). Gardner and Weisleder (1970) partially purified lipoxygenase from mature corn germs by using 42-53% ammonium sulfate fractionation; however, the purification fold and percent recovery were not given. Veldink et al. (1972) partially purified lipoxygenase from mature maize germs with 30-60% ammonium sulfate fractional precipitation, followed by conventional size exclusion chromatography on a Sephadex-G 100 (Superfine) column. Purification fold and percent recovery of lipoxygenase activity were not reported by the investigators. Gardner (1988) indicated that maize germ lipoxygenase was isolated and purified by water extraction, pH 4.5 adjustment, 40-50% ammonium sulfate fractionation, adsorption on $Ca_3(PO_4)_2$ gel, and DEAE Sephadex A-50.

Poca et al. (1990) purified lipoxygenase from the whole mature maize seeds by preparation as an acetone powder, extraction with 0.1 M acetate buffer (pH 4.5), containing 2 mM sodium bisulfite, 0.1 M diethylenetriamine pentaacetic acid, and 0.1% Brij 99 (a non-ionic detergent), precipitation with 40-80% ammonium sulfate fractionation, anion exchange on DEAE-Tris-acryl column, and isoelectric focusing. The purification of lipoxygenase L1 and L2 after ion exchange column were 33.7 and 70 fold with percent recovery of 4.6 and 20.3 %, respectively.

Previous research on isolation and purification of lipoxygenase from sweet corn, particularly sweet corn germs, has been very limited. The only previous work on sweet corn germ lipoxygenase was carried out by Belefant and Fong (1991), who partially purified lipoxygenase from sweet corn germs by extraction with 0.1 M.

phosphate buffer (pH 7.4), precipitation with 20-50% ammonium sulfate and dialysis. However, the degree of purification, percent recovery of the enzyme, and sweet corn variety were not given. Velasco et al. (1989) partially purified lipoxygenase from whole sweet corn kernels by an acetone powder preparation, 0.2 M sodium phosphate buffer (pH 7.0) extraction, 10-50% ammonium sulfate fractional precipitation, and dialysis, followed by conventional size exclusion chromatography on an Ultrogel AcA34 column. The purification and percent recovery were 10.3 fold and 59%, respectively.

Characteristics of lipoxygenase

pH optimum for activity and pH stability

Optimum pH for activity of most plant lipoxygenases is near neutral pH, in the range of 5.5 to 7.5. This conclusion is based on studies of broad beans (Al-obaidy and Siddiqi, 1981b), egg plant (Grossman et al., 1972a), navy beans (Koch et al., 1971), soybean lipoxygenase-2 (Diel and Stan, 1978; Dreesen et al., 1982), pea isozyme-1 (Chen and Whitaker, 1986) and kiwifruit (Boyes et al., 1992). The optimum pH for activity of lipoxygenases from the germs of cereal grains, such as wheat germs (Nicholas et al., 1982), rice germs (Yamamoto et al., 1980), mature maize germs (Gardner, 1988; Belefant and Fong, 1991) and germinated barley germs (Doderer et al., 1992), were also in this pH range. However, lipoxygenase activity

of soybean lipoxygenase type-1 was found to have an optimum of pH 8.0-9.5 (Diel and Stan, 1978) and dry English pea seeds (var. Little Marvel) contained a small amount of a lipoxygenase type-1 with an optimum pH of 9.0-10.0 (Reynolds and Klein, 1982).

Lipoxygenase appeared to be most stable in the range of pH 5-8, for example in the cases of asparagus (Ganthavorn and Powers, 1989), broad bean (Al-obaidy and Siddiqi, 1981b) and pea (Chen and Whitaker, 1986).

Temperature optimum for activity

The optimum temperature for lipoxygenase activity of some plants, such as sunflower seeds, soybeans, broad beans, and cowpeas has been reported to be in the range of 30-35°C (Tappel et al., 1953; Al-Obaidy and Siddiqi, 1981; and Den and Mendoza, 1982; Leoni et al., 1985). However, the optimum for lipoxygenases from some plants were higher, for example, wheat germs and barleys were approximately 45 and 47°C, respectively (Lulai and Baker, 1976; Shiiba et al., 1991).

Thermal inactivation

Thermal inactivation of plant lipoxygenases has been studied by various investigators using enzyme preparations of different degrees of purification (Farkas and Goldblith, 1962; Svensson and Eriksson, 1972; Svensson and Eriksson, 1974; Park et al., 1988;

Ganthavorn et al., 1991). Factors such as type and concentration of impurities may influence the thermal stability of lipoxygenase. For example, Farkas and Goldblith (1962) found that pea solids had a protective effect for soybean lipoxygenase against thermal inactivation. Svensson and Eriksson (1974) also reported that pea lipoxygenase was more heat stable in pea press juice than in phosphate buffer at the same pH. The activation energy for thermal inactivation of pea lipoxygenase was 584 to 602 kJ/mol in the buffer pH range of 6.0-7.0, while in the presence of pea press juice (pH 6.8) was only 479 kJ/mol. Moreover, Alsoe and Adler-Nissen (1988) reported that the rate of denaturation of lipoxygenase at 72°C was considerably reduced when increasing concentrations of soybean solids (ranging from 0.024 to 0.249 g of dry soya beans/ g suspension) were present in the assay system. They suggested that the stabilizing effect is probably due to the binding of the enzymes to its natural substrates, linoleic and linolenic acids. However, in the presence of products of a lipoxygenase-catalyzed reaction (linoleate hydroperoxides), the stability of pea lipoxygenase to heat was decreased (Svensson and Eriksson, 1972).

Enzymes in their natural environment, where substrates are present, are generally better protected against thermal denaturation than the same enzymes in the purified state (Alsoe and Adler-Nissen, 1988). Heat stability of different lipoxygenase isozymes from the same source was also different, for instance, soybean lipoxygenase-1 is more heat stable than soybean lipoxygenase-2 (Christopher et al., 1970).

The activation energy for thermal inactivation of lipoxygenase from some plants were reported, such as 40.8 and 46.5 kcal/mol for two potato isozymes (Park et al., 1988), 48.9 kcal/mol for asparagus lipoxygenase in 50% ammonium sulfate (Ganthavorn et al., 1991), 24.6 kcal/mol for immature English peas (Chen and Whitaker, 1986), 100 and 140 kcal/mol for soybean and peas, respectively (Schwimmer, 1981; Svensson and Eriksson, 1974). Whitaker (1972) suggested that the activation energies required for denaturation of enzymes was in the range of 50-150 kcal/mol.

Molecular weight

In most plants, lipoxygenases appear to have molecular weights close to 100,000 daltons (in the range of 89,000 to 102,000 daltons). This range is true for lipoxygenases from germinating maize seeds (Poca et al., 1990), soybeans (Veldink et al., 1977; Shibata et al., 1987; Hildebrand et al., 1988; Shibata et al., 1988; Yenofsky et al., 1988); chickpeas (Sanz et al., 1992b), cucumber cotyledon (Matsui et al., 1993); rice embryo (Ohta et al., 1986), ungerminated and germinating barley (van Aarle et al., 1991; Doderer et al., 1992), immature English pea lipoxygenase (Chen and Whitaker, 1986); and wheat germ (Nicholas et al., 1982). However, the molecular weight of flaxseed lipoxygenase was found to be 130,000 daltons (Rabinovitch-Chable et al., 1992). The molecular weights of lipoxygenases from peanuts, pea seeds, cowpeas, and avocado were 73,000, 74,000, 68,000 and 74,000 (L-1 and L-2), and

74,000 daltons, respectively (Haydar and Hadziyev, 1973; Sanders et al., 1975; Den and Mendoza, 1982; Marcus et al., 1988).

Isoelectric point

The isoelectric points (pI) of lipoxygenase isozymes have been reported by various investigators. The pIs of lipoxygenase from various plants were in the range of 4.0 to 6.6. This includes data collected on the pIs of lipoxygenase isozymes from chickpea (Sanz et al., 1992b); germinating barley embryos (Yabuuchi, 1976), wheat germ (Nicholas et al., 1982), germinating maize seed (Poca et al., 1990), avocado (Marcus et al., 1988); soybeans (Hildebrand et al., 1988); wheat germs (Nicholas et al., 1982); horse beans (Nicholas and Drapron, 1977); and dry English pea seeds (Reynolds and Klein, 1982). Different isozymes of lipoxygenase from the same source also had different pIs. For example, the pIs of two lipoxygenase isozymes from wheat germ, L-1 and L-2, were 6.25 and 5.4, respectively (Nicholas et al., 1982). Sanz et al. (1992b) found that lipoxygenase L-1 and L-2 from chick peas had pIs of 4.92 and 4.74, respectively.

Inhibition by antioxidants

The enzyme-catalyzed reaction by lipoxygenase has been reported to be inhibited by various inhibitors (Eskin et al., 1977; Vliegenthart and Veldink, 1982). One of the most important group of inhibitors of lipoxygenase are antioxidants, especially phenolic

antioxidants. Synthetic phenolic antioxidants that are commonly used in food such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary-butylhydroquinone (TBHQ), and propyl gallate (PG) have been shown to inhibit an oxidation catalyzed by lipoxygenase (Eskin et al., 1977; Yasumato et al., 1970; Rhee and Watts, 1966; King and Klein, 1987). Yasumoto et al. (1970) reported the concentrations of various phenolic antioxidants for 50% inhibition of soybean lipoxygenase. Rhee and Watts (1966) reported the inhibitory effect of BHA on soybean and pea lipoxygenase.

Moreover, naturally occurring phenolic substances with antioxidant activity, such as flavonoids, have been reported to inhibit lipoxygenase activity (King and Klein, 1987; Dohi et al., 1991). Flavonoid compounds that inhibit lipoxygenase activity include quercetin, chlorogenic acid, and caffeic acid (King and Klein, 1987; Rhee and Watts, 1966; Takahama, 1985; Letan, 1966; Naim et al., 1976). The mechanism of inhibition utilized by flavonoid compounds such as quercetin has been proposed (Letan, 1966 and Takahama, 1985). Takahama (1985) proposed that quercetin inhibited soybean lipoxygenase-1-dependent linoleic acid peroxidation by reduction of the linoleic acid radical formed during lipoxygenation.

The inhibitory effects of each antioxidant on lipoxygenase activity are different. Yasumoto et al. (1970) reported that quercetin and BHA were more effective at inhibition of soybean lipoxygenase activity than BHT. King and Klein (1987) found that BHA and quercetin inhibited the conjugated diene formation by soybean

lipoxygenase more effectively than chlorogenic acid and BHT. Quercetin has also been reported to have a greater inhibitory effect than chlorogenic acid on the carotene bleaching activity of lipoxygenase (Oszmianski and Lee, 1990).

ENZYMATIC OFF-FLAVOR AND OFF-AROMA FORMATION IN PLANTS

Enzymes are responsible for most of the reactions that occur endogenously in plants. After harvest, many enzymes continue to act on remaining substrates and many of these reactions could affect the quality of food. Off-flavor (mostly off-aroma) is one of the detriments to quality that is initiated via the reaction of endogenous enzymes in plants, even under freezing conditions (Josyln, 1949; Schwimmer, 1981; Lim et al., 1989; Velasco et al., 1989; Williams et al., 1986). Lipoxygenase, lipase and protease are enzymes that have been suggested as being responsible for off-aroma development in vegetables (Williams et al., 1986). However, other enzymes such as cystine lyase have also been suggested as important enzymes in off-aroma development in broccoli (Lim et al., 1989).

The following literature review will emphasize off-flavor (especially off-aroma) in frozen vegetables, with particular reference to sweet corn, the involvement of lipoxygenase in off-aroma formation and previous investigations of endogenous enzyme(s) in off-aroma formation in food plants, especially vegetables.

Off-flavor and off-aroma in frozen vegetables

It has long been known that raw or underblanched vegetables deteriorate in quality when stored frozen for extended periods of time. Off-flavor (primarily off-aroma) formation is the main form of

quality deterioration in various vegetables, including sweet corn, during frozen storage. One important factor leading to this quality deterioration is due to the action of naturally endogenous enzymes that remain active under the freezing condition (Joslyn, 1949; Lee and Wagenknecht, 1951; Lee et al., 1955; Wagenknecht, 1959; Lee, 1981; Williams et al., 1986; Velasco et al., 1989).

Lee (1954) found that peroxide and acid values increased in frozen raw vegetables after prolonged storage and suggested that the off-flavor was a consequence of lipid oxidation by enzymes. Wagenknecht and Lee (1956) demonstrated that lipoxygenase was present in frozen raw peas and suggested that its reactions may contribute to off-flavor formation and other changes in quality of frozen raw peas. Wagenknecht and Lee (1958) found that objectionable off-flavors developed in blanched pea samples with added commercially available lipoxygenase or lipase after frozen storage at -17.8°C for 17 months. Flavor quality scores for underblanched frozen green beans and green peas appeared to be lower than those of commercially blanched samples during frozen storage (Halpin and Lee, 1987; Lee et al., 1988).

Off-flavor and off-aroma development have also been reported in frozen sweet corn. Wagenknecht (1959) suggested that off-flavors of frozen stored underblanched corn-on-the-cob were due in part to the action of residual lipoxygenase. Lee (1981) also demonstrated that off-flavor developed in unblanched frozen sweet corn, and off-flavored sweet corn was significantly higher in hexanal. The high hexanal peak was observed in the sterilized sweet corn homogenate

with added commercial lipoxygenase alone and in combination with other enzymes after frozen storage for 6 months. McDaniel et al. (1988) reported that total off-flavor and off-aroma were significantly higher in underblanched cut frozen sweet corn than the commercially blanched sweet corn. The underblanched sample was also significantly higher in aroma and flavor described as "stale/oxidized", "fishy", and "cobby/husk".

Lipoxygenase involvement in off-flavor and off-aroma formation

Off-flavor and off-aroma formation are widely known to be the main problems of legumes such as soybeans (Arai et al., 1970; Mattick and Hand, 1969; Rackis et al., 1972, 1979; Sessa, 1979; Kinsella and Damodaran, 1980), peas (Lee and Wagenknecht, 1958; Haydar and Hadziyev, 1973), and lentils (Kon et al., 1970). There is evidence indicating that lipoxygenase is involved in off-flavor and off-aroma formation, not only in legumes but also in other food plants which contain lipids (Eskin et al., 1977). Sessa (1979) and Rackis et al. (1979) indicated that lipoxygenase-mediated conversion of lipids to hydroperoxides and their subsequent degradation form volatile and nonvolatile constituents responsible for off-flavors in soybeans described as grassy-beany, green, oxidized, painty, and rancid flavors. Ashraf and Synder (1981) reported that soy milk with high residual lipoxygenase activity was high in "painty" off-flavor.

The "rancid" off-flavor development in legumes is believed to be the result of lipoxygenase activity (Eskin et al., 1977).

Johnsen et al. (1988) and Civille and Dus (1992) reported that off-flavor in peanuts and vegetable oil were described as "painty", "stale/oxidized" and related terms such as "cardboard", and "rancid." Mistry and Min (1992) indicated that one of the mechanisms involved in their off-flavor production was enzymatic lipid oxidation by lipoxygenase. In raw or underblanched frozen vegetables such as peas and sweet corn, lipoxygenase has also been suggested to cause off-flavor development (Wagenknecht and Lee, 1958; Lee and wagenknecht, 1958; Wagenknecht, 1959; Lee, 1981; Velasco et al., 1989). Lipoxygenase was demonstrated to be the key enzyme in the development of off-aroma in other plants such as English green peas and green beans (Williams et al., 1986).

Lipoxygenase catalyzed the formation of fatty acid hydroperoxides as primary products. However, hydroperoxides are very reactive and can undergo rapid degradation by either enzymatic or non enzymatic processes to form a variety of secondary products including several aldehydes, ketones, alcohols, acids (Gardner, 1975; Kinsella and Damodaran, 1980). Further reactions of hydroperoxides by enzymatic processes have been described in several papers (Gardner, 1975; Galliard and Chan, 1980; Veldink et al., 1977; Gardner, 1985; Vick and Zimmerman, 1987). Enzymes involved in enzymatic conversion of hydroperoxides include hydroperoxide lyase, and hydroperoxide isomerase. Flavor production via the lipoxygenase pathway is generally quiescent

unless it is triggered by cell damage such as freezing, crushing (Gardner, 1985).

The products from lipoxygenase catalyzed reactions have been reported to contribute to off-flavor and off-aroma development. (Eskin et al., 1977; MacLeod and Ames, 1988). Kalbrener et al. (1974) reported that hydroperoxide products of linoleic acid or their breakdown products prepared using soybean lipoxygenase were described as predominantly grassy/beany, musty/stale, and bitter. Linolenic hydroperoxide was described with a variety of terms with the most predominant description being grassy/beany followed by bitter and astringent. Some degradation products of polyunsaturated fatty acids, such as aldehydes and ketones, have been reported to be associated with grassy-beany and rancid off-flavors in soybeans (Mattick and Hand, 1969; Wolf, 1975; Rackis et al., 1979). Arai et al. (1970) reported that the major volatile compounds that affect the quality of soybean products are n-hexanal, n-hexanol, n-pentanol and n-heptanol. Rackis et al. (1979) reported that one of the major contributors to grassy-beany and green flavors in soybeans was n-hexanal.

Matoba et al. (1985) indicated that lipoxygenase-2 is the isozyme primarily responsible for the generation of hexanal in soybeans. Higher alk-2,4-dienals that have also been generated by enzymatic oxidation were described as oxidized, cardboard like, oily, and painty (Sessa, 1979). Moreover, non-volatile compounds produced by lipoxygenase may contribute to off-flavor in soybean oil; for example, bitterness in soybean oil may be due in part to fatty

acid dimers formed from lipoxygenase-generated hydroperoxides (Evan et al., 1960). Adding short chain aldehydes, n-hexanal and *cis*-3-hexanal, above a certain level has been found to produce green and rancid off-flavors in tomato juice (Kazeniac and Hall, 1970).

Investigations of enzyme(s) in off-aroma formation in vegetables

Various approaches has been used to investigate possible role of particular enzyme(s) in off-flavor and off-aroma formation. One approach has been based on the correlation of the flavor quality deterioration during storage with the residual activity of a particular enzyme of interest. This approach generally involves a storage study in which the particular enzyme is inactivated to various degrees. However, this approach provides useful information only for the empirical relationship of flavor quality and residual enzyme activity since other endogenous enzymes may still retain some activities even with complete inactivation of the enzyme of interest. This approach also requires prolonged storage time.

Another approach in evaluating the possible responsibility of the enzyme(s) of interest is performed as follows: Enzyme(s) thought to be important for flavor quality deterioration are obtained commercially or isolated and purified from the original plants of interest. The enzyme(s), singly and/or in combinations, are added to the homogenate of the plants in which all enzymes were previously inactivated by heat treatment. After incubation, the samples were

heated to inactivate the enzyme(s), and evaluated by sensory panel or objective analysis such as chemical analysis, gas chromatography analysis.

For example, Wagenknecht and Lee (1958) showed by panel evaluation that samples of blanched peas with added commercially available catalase (liver), peroxidase (horse radish), lipoxidase (soybean), and lipase (pancrease) developed off-flavors of varying intensity after frozen storage for 17 months at -17.8°C. Only mild off-flavors were observed in blanched peas with added catalase or peroxidase. Moderate off-flavors developed in samples with added lipoxygenase. Samples with added lipase had disagreeable off-flavors, which must be attributed in part to the flavor imparted by the lipase preparation itself. In general the effect of addition of mixtures of two or more enzymes was the same as the effects produced by the enzymes when added individually.

Lee and Wagenknecht (1958) repeated their study at a later date using the four enzymes extracted from their natural source, peas. The enzymes were partially purified and added back to blanched pea slurry as before. The results indicated that catalase produced the most offensive off-flavor; and lipoxidase and lipase also produced pronounced off-flavor. Peroxidase produced only a mild change in flavor. The results of the above studies also suggests us that sources of the enzymes should be considered since enzymes from other sources might not effect the flavor changes the same as those produced by their native sources. Moreover, other factors such

as degree of purity of enzymes used for the study are also important to be considered.

Lee (1981) also demonstrated the possible role of enzymes by adding commercial enzymes (lipoxygenase, catalase, peroxidase and lipase), singly and in combination, to the sterilized sweet corn homogenate, followed by frozen storage for 6 months. The samples were subjected to GC analysis. The results showed that hexanal peak was high in samples with added commercial lipoxygenase, either alone or in combination with other enzymes.

Williams et al. (1986) used a similar approach with some modifications to assess the important enzyme(s) in off-aroma formation in English green peas and green beans. Catalase, peroxidase, lipoxygenase and lipase purified from English green peas were added to blanched English green pea puree to give the same activity as that originally present in the raw sample. Samples were incubated at 30°C for 3 hours, heated at 97°C for 20 min, and then evaluated by a sensory descriptive trained panel. Green pea puree with added lipase, or lipase and peroxidase in combination, had flavor profiles similar to the blanched control. In the samples to which catalase was added along with lipase and peroxidase, three aroma descriptors, cooked, popcorn and putrid, became significantly higher than those of the control. The flavor profile of the sample with lipase, peroxidase, catalase and lipoxygenase added most closely resembled the profile of unblanched green pea puree. Similar experiments were performed with green beans. The sample with added lipase or peroxidase had aroma similar to the blanched

control. In the samples with added lipoxygenase, the aroma descriptors overall intensity, cooked corn, unriped banana, grassy, straw, sour and ammonia were significantly different from the control. The aroma profile of lipoxygenase-treated sample closely resembled the profile of unblanched green beans. Their findings indicate that lipoxygenase plays a major role in off-aroma development in green peas and green beans.

Lim et al. (1989) performed similar experiments with broccoli. Peroxidase, lipase and cystine lyase were purified from broccoli and used for the study. The sample with added cystine lyase alone reproduced the descriptors overall intensity, sour and sulfhydryl compound which were similar to the unblanched samples. Addition of lipase and lyase together resulted in the ammonia descriptor which was found in the unblanched sample. They concluded that most of the aroma descriptors detected in homogenized unblanched broccoli were due to the action of cystine lyase, however, lipase may also contribute.

Velasco et al. (1989) investigated the important enzyme(s) in off-aroma formation in sweet corn by using a similar approach. Lipoxygenase, catalase and peroxidase were partially purified from corn kernels. Unexpectedly, addition of each enzyme to homogenized blanched corn produced descriptors "overall aroma intensity", "corn cob", "cooked dough", "potato skin" and "cooked cabbage" which are similar to that of the unblanched sample. The investigators proposed that this might be due to the presence of an unidentified contaminating enzyme in each purified preparation, or to the

nonenzyme-catalyzed reactions. However, the investigators demonstrated that "overall aroma" intensity of the samples with added commercial highly purified lipoxygenase (from soybeans) was significantly different from the control (blanched corn homogenate), while the sample with added catalase (from bovine liver), and peroxidase (from horseradish) was not. The investigators believed that lipoxygenase, but not catalase and peroxidase, is important in off-aroma development in unblanched corn. They also suggested that other enzymes such as hydroperoxide isomerase and hydroperoxide lyase may be important. Therefore, identification of the specific enzyme(s) which play the most important role in off-aroma formation in sweet corn has yet to be clarified.

DESCRIPTIVE SENSORY ANALYSIS

Descriptive sensory analysis is a unique sensory method by which the attributes of a food material or product are identified, described, and quantified using human subjects who have been specially trained for this purpose (Einstein, 1991). Various descriptive analysis methods have been developed, however, the most widely used are: the Flavor Profile (Carncross and Sjostrom, 1950), General Foods Texture Profile (Brandt et al., 1963; Szczesniak, 1963; Szczesniak et al., 1963), Quantitative Descriptive Analysis (Stone et al. 1974; Stone and Sidel, 1985), and the Spectrum method (Meilgaard et al., 1987). The details of these methods have been reviewed in several papers (Stone and Sidel, 1985; Meilgaard et al., 1987; Powers, 1988; Einstein, 1991).

All descriptive analysis methods involve the detection (discrimination) and the description of both qualitative and quantitative sensory aspects of a product by a trained panel. The panelists must be able to detect and describe the perceived qualitative sensory attributes of a sample. The qualitative aspects of a sample can include all attributes, however, they are usually limited to attribute(s) of interest such as aroma, flavor, appearance, and texture. Panelists must learn to differentiate and rate the quantitative or intensity aspects of a sample and to define to what degree each characteristic or qualitative note is present in that sample (Meilgaard et al., 1987). Usually, the panelists develop a set of terms or words that form the basis of their ballot. The panelists

must agree as to the meaning of these words, and indicate how much of each sensory quality is perceived (Stone and Sidel, 1985). In helping panelists develop appropriate terminology and establish an intensity range, reference standards are considered useful tools (Rainey, 1986).

Generally, descriptive analysis methods consist of the following procedures: panel selection, language development through training, sample evaluation, statistical data analysis and interpretation. Descriptive analysis is useful to obtain detailed descriptions of sensory attribute(s) such as aroma, flavor, texture of products (Meilgaard et al., 1987). Descriptive analysis provides a comprehensive qualitative and quantitative description, which results in reliable measurements and reproducible data using human subjects. It provides valuable information about the sensory properties of the samples which are not obtainable from any other source. However, descriptive analysis is extremely time consuming, expensive, and dependent on both a highly trained panel and the skill of its leader (Einstein, 1991).

CHAPTER 3:
LIPOXYGENASE IN SWEET CORN GERMS: ISOLATION AND SOME PHYSICOCHEMICAL PROPERTIES

Chockchai Theerakulkait and Diane M. Barrett*

Department of Food Science and Technology,
Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602
and
*Department of Food Science and Technology,
Cruess Hall, University of California, Davis, CA 95616-8598

ABSTRACT

Lipoxygenase (LPO) activity in the germ fraction of sweet corn (*Zea mays* L. var. Jubilee) was determined and compared with that in the degermed fraction. Lipoxygenase activity per gram of the germs was found to be about three times as great as that of the degermed fraction. Optimized procedures for isolation of lipoxygenase from the germ fraction were developed. Lipoxygenase was isolated by preparation as an acetone powder, extraction with 0.2 M Tris-HCl, pH 8.0 (4°C), fractionation with 40-60% saturated ammonium sulfate and dialysis. The optimum pH and temperature for activity of partially purified lipoxygenase were 6-7 and 50°C, respectively. The enzyme appeared to be stable in the range of pH 5-8 and approximately 90% of original activity was inactivated after heating in pH 7 buffer at 70°C for 3 min.

Key Words: lipoxygenase, sweet corn, germs, isolation, extraction, physicochemical properties

INTRODUCTION

Lipoxygenase (Linoleate : oxygen oxidoreductase, EC 1.13.11.12; previously known as lipoxidase), LPO, is an important endogenous enzyme that remains active in vegetables under freezing conditions. It has been suggested as the cause of off-flavor development in various unblanched and underblanched frozen vegetables including sweet corn (Wagenknecht, 1959; Lee, 1981; Williams et al., 1986; Ganthavorn and Powers, 1989; Velasco et al., 1989; Sheu and Chen, 1991). Moreover, off-flavor and off-aroma development are considered the main causes of rapid quality deterioration in harvested sweet corn (Smittle et al., 1972). Lipoxygenase may be more important and more appropriate than peroxidase for use as the index of proper heat treatment (blanching), because the enzyme chosen as the blanching index should play a major role in quality deterioration (Williams et al., 1986).

According to Wagenknecht (1959) and Lee (1981), in unblanched sweet corn, lipoxygenase induced off-flavors in the fraction containing the germ. Previous work on corn germ lipoxygenase focused on the mature seed of field corn (Gardner, 1970; Egmond et al., 1972; Veldink et al., 1972; Gardner and Weisleder, 1970; Gardner et al., 1975). However, other sweet corn lipoxygenase studies have not emphasized the germ fraction (Wagenknecht, 1959; Lee, 1981; Velasco et al., 1989); nor have previous investigators evaluated lipoxygenase isolated from sweet

corn (*Zea mays* L. var. Jubilee) germs at the stage of maturity appropriate for the freezing industry.

The objectives of this study were: to determine the lipoxygenase activity in the germ fraction of sweet corn compared to that in the degermed fraction; to develop a procedure for rapid isolation of corn germs; to optimize the appropriate procedures and conditions for extraction and isolation of lipoxygenase from the sweet corn germ fraction; and to investigate some physicochemical properties of lipoxygenase from sweet corn germs.

MATERIALS & METHODS

Materials

Freshly harvested sweet corn (*Zea mays* L. var. Jubilee), at the stage of maturity selected by the company for freezing, was obtained from the Agri-Pac Co., Woodburn, Oregon. The corn was immediately transported on ice to the pilot plant of the Dept. of Food Science and Technology, Oregon State University, Corvallis, Oregon. The fresh sweet corn was temporarily stored at 4°C prior to germ separation, which was carried out over a period of 3 days. A portion of sweet corn was frozen immediately in liquid nitrogen and stored at -80°C until determination of lipoxygenase activity in the germ and degermed fractions was undertaken.

Linoleic acid, Tween-20, Triton X-100, ammonium sulfate, and bovine serum albumin were purchased from Sigma Chemical Co., St Louis, MO. All other chemicals were reagent grade. Deionized distilled water was used in all experiments.

Lipoxygenase activity of sweet corn germ and degermed fractions

Sweet corn ears were frozen in liquid nitrogen and intact kernels were removed from the cobs. While still frozen, corn kernels were separated into germ and degermed fractions and weighed. Each fraction was blended in liquid nitrogen. The liquid nitrogen powder

of each fraction was homogenized in 0.2 M Tris-HCl, pH 8.0 (4°C) at a ratio of 1:10 (w/v) using a Tissumizer (Tekmar Co., Cincinnati, OH), and a powerstat setting of 50 for 3 minutes. The extract was centrifuged at 17,000 x g (4°C) for 1 hour. The lipoxygenase activity in each supernatant was determined spectrophotometrically by monitoring the formation of conjugated dienes at 234 nm as described below.

Lipoxygenase activity assay

Lipoxygenase activity was determined spectrophotometrically at 234 nm by a modification of the procedure described by Chen and Whitaker (1986). The substrate solution was prepared by mixing 157.2 µL of pure linoleic acid, 157.2 µL of Tween-20 and 10 mL of deionized distilled water. The solution was clarified by adding 1 mL of 1.0 N sodium hydroxide and diluting to 200 ml with 0.2 M sodium phosphate buffer, pH 7.0; giving a 2.5 mM final concentration of linoleic acid. The substrate solution was flushed with oxygen gas at least 2 minutes to give an initial absorbance at 234 nm of 0.3-0.4, and was allowed to equilibrate in a water bath at 25°C before use in the activity assay. The total reaction volume was 3 mL, which contained 2.7 mL of substrate solution and 0.3 mL of enzyme solution. The initial rate of conjugated diene formation was read over the linear change in absorbance at 234 nm.

One unit of enzyme activity is defined as an increase in absorbance of 0.001 at 234 nm per minute under the assay

conditions. The extinction coefficient (ε_m) for the conjugated diene of linoleic acid was 23,000 M^{-1} cm^{-1} (Gibian and Vandenberg, 1987). A double beam spectrophotometer (Shimadzu, UV-160, Shimadzu Corporation, Kyoto, Japan) and 1 cm path length cuvette were used.

Protein determination

Protein was determined by the bicinchoninic acid (BCA) method (Smith et al., 1985) using crystalline bovine serum albumin as a standard.

Rapid isolation of sweet corn germs

Rapid isolation of the germ fraction from sweet corn on the cob was developed using a modification of the method of Fong and Smith (1985) as shown in Fig. 3.1. The precooled fresh sweet corn were husked, inspected, and cut by a TUC (The United Company, Westminster, MD) corn cutter to remove the top portion of the kernels (above the germ tissue). The cutter was then carefully adjusted to cut kernels again at the base of the kernels where they attach to the cob. The precooled, cut corn kernels were gently crushed with a rolling pin to release the intact germs and then sieved. Three stainless steel sieves (U.S. standard sieve series, the W. S. Tyler Co., Cleveland, OH) having mesh numbers 1/4 (6.3 mm opening), 8 (2.36 mm), and 20 (0.83 mm) were stacked such that the largest pore size was on the top. The crushed kernels were spread on

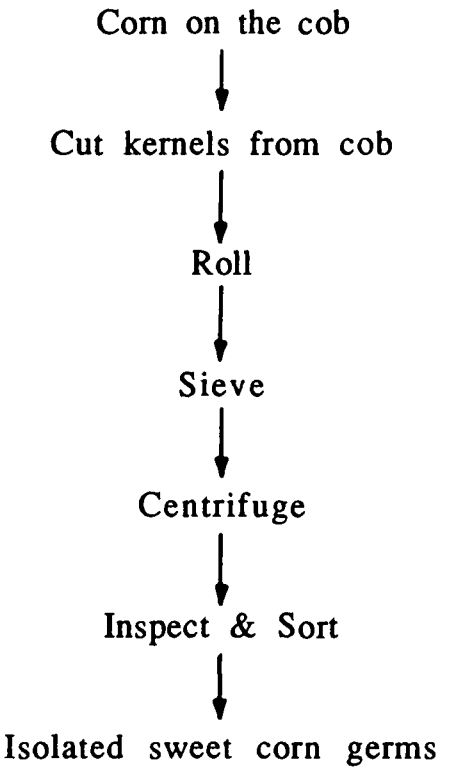

Fig. 3.1- Rapid isolation of sweet corn germs.

top of the sieve stack and shaken in cold 50 mM sodium phosphate buffer, pH 7. The enriched fraction of whole germs was trapped on sieve number 8. The crushed kernels containing germs that remained on sieve number 1/4 were crushed again with a rolling pin and sieved in cold buffer as before. The enriched fractions of germs were combined and carefully spread on a screen with an opening of 3.17 mm, and gently sprayed with cold buffer to allow the germs to pass through the sieve.

The enriched fraction of germs was further isolated by suspending it in approximately 35% (w/v) sucrose in a 50 mM sodium phosphate buffer pH 7, where most of the germs and some other tissue debris floated. All floating tissues were collected and centrifuged in approximately 30% (w/v) sucrose in 50 mM sodium phosphate buffer pH 7 at 2,000 x g (4°C) for 5 minutes to separate the germs from the remainder of the debris. The isolated germ fraction was inspected and sorted from the debris of non-embryonic tissues before freezing in liquid nitrogen. The frozen isolated germs were stored at -80°C until used.

Optimization of sweet corn germ lipoxygenase isolation

Preparation of acetone powder

An optimized procedure for preparation of acetone powder from sweet corn germs was developed as shown in Fig. 3.2. Frozen corn germs were blended in liquid nitrogen using a stainless steel

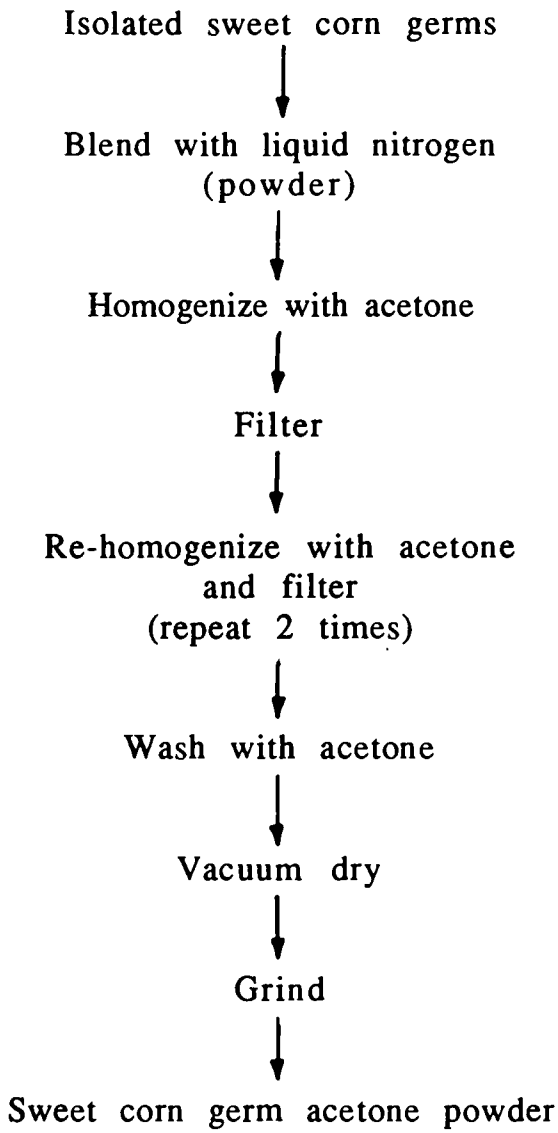

Fig. 3.2- Acetone powder preparation from sweet corn germs.

Waring blender. The liquid nitrogen powder was then homogenized in cold acetone (-23°C) in a ratio of 1:20 (w/v) using a Tissumizer (powerstat setting at 100) for 2 minutes in a cold room (4°C). The slurry was filtered through a Buchner funnel with Whatman paper filter number 1. The residue was re-homogenized with 10 volumes of cold acetone for 1 minute and filtered; and this step was repeated. The final residue was washed with 5 volumes of cold acetone, vacuum dried at room temperature, ground and stored at -23.3°C until used.

Optimization of extraction

The following optimized procedure for extraction of lipoxygenase from sweet corn germ acetone powder was developed: 2 grams of acetone powder were homogenized with 0.2 M Tris-HCl, pH 8.0 (4°C) in a ratio of 1:10 (w/v) for 3 minutes using a Tissumizer (powerstat setting at 50). The enzyme extract was centrifuged at 17,000 x g for 60 minutes (4°C).

In order to determine the optimum conditions for extraction, various factors affecting extraction efficiency of lipoxygenase from acetone powder were investigated (Table 3.1). Experiments were performed as above, except the conditions of extraction buffers were changed as indicated. All experiments were performed at 4°C and with at least 2 replications.

Table 3.1- Factors evaluated for optimization of extraction conditions for lipoxygenase.

Factors	**Conditions evaluated**
Buffer pH (0.1 M)	4.5 (Sodium acetate) 7.0 (Sodium phosphate) 8.0 (Tris-HCl) 9.0 (Borate)
Buffer types (0.1 M, pH 8.0)	Sodium phosphate Tris-HCl Borate
Buffer concentrations	0.05, 0.1, and 0.2 M
Surfactant	Triton X-100 (0, 0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 % v/v)
Metal chelator	EDTA (5 mM)
Protease inhibitor	PMSF (5 mM)
Reducing agent	DTT (5 mM)

Ammonium sulfate fractionation and dialysis

The enzyme extract was 40% saturated with ammonium sulfate with continuous stirring at 4°C for 1 hour. After centrifugation at 17,000 x g at 4°C for 30 minutes, the supernatant was 60% saturated with ammonium sulfate with continuous stirring at 4°C for 1 hour. The resulting precipitate after centrifugation was dissolved in a minimum volume of 50 mM Tris-HCl buffer, pH 8.0 (4°C) and dialyzed using a Spectra/Por membrane (molecular weight cutoff 10,000 daltons) against 2 liters of the same buffer overnight at 4°C with one change of buffer. The dialyzed solution was centrifuged at 17,000 x g (4°C) for 1 hour. The supernatant of isolated lipoxygenase was stored at -23.3°C. The activity of lipoxygenase and protein content were determined at each step of isolation.

pH optimum for activity and pH stability

For the pH optimum for activity study, the lipoxygenase activity of isolated sweet corn germ lipoxygenase was determined spectrophotometrically in the range from pH 3.0 to 9.0. The buffer systems used were 0.2 M citrate phosphate buffer, pH 3.0 to 5.0; 0.2 M sodium phosphate buffer, pH 6.0 to 7.0; and 0.2 M Tris-HCl, pH 8.0 to 9.0.

In the pH stability study, the enzyme was diluted 1:10 (v/v) with buffers of pH values ranging from 3.0 to 10.0 and incubated in a 13 x 100 mm capped test tube in a water bath (25°C) for 30 minutes,

then assayed for lipoxygenase activity. The buffer systems used for the pH 3.0 to 9.0 range were the same as those for the pH activity study. The buffer for pH 10.0 was 0.2 M Tris-HCl buffer.

Temperature optimum for activity and temperature stability

For the temperature optimum for activity study, the lipoxygenase activity of isolated sweet corn germ lipoxygenase was determined spectrophotometrically in 0.2 M sodium phosphate buffer, pH 7.0 at 10, 20, 25, 30, 35, 40, 50, 60 and 70°C.

In the temperature stability study, the enzyme solution was diluted 1:10 (v/v) with 0.2 M sodium phosphate buffer, pH 7.0. Aliquots of the diluted enzyme sample were placed in 13 x 100 mm capped test tubes and incubated in a water bath at 60°C and 70°C for various times up to 30 minutes. Each tube was removed at a specific time, immediately cooled in ice water and assayed for lipoxygenase activity.

RESULTS & DISCUSSION

Lipoxygenase activity of sweet corn germ and degermed fractions

The distribution of lipoxygenase activity in sweet corn kernels is shown in Fig. 3.3. Lipoxygenase activity per gram of tissue (wet weight) in the germ fraction was approximately 3 times as great as in the degermed fraction. Previous work on distribution of lipoxygenase in sweet corn on the cob also reported that lipoxygenase activity was highest in the section of kernel that contained germ tissue (Wagenknecht, 1959; Lee, 1981; Lee et al., 1989). In inbred yellow dent corn, the activity of lipoxygenase was also reported to be generally higher in the germ (embryo) than in the endosperm tissues throughout kernel development (Belefant and Fong, 1991). Gardner (1970) reported that lipoxygenase was localized mainly in the seed germ tissue of mature hybrid corn. Lipoxygenase activity was also found primarily in the germ tissue of cereal seeds such as wheat (Auerman et al., 1971; Von Ceumern and Hartfiel, 1984), barley (Lulai and Baker, 1976; Lulai et al., 1981), and rice (Yamamoto et al., 1980); however, the subcellular location in germ tissue is as yet unknown (Gardner, 1988).

The higher lipoxygenase activity per unit weight in the germs of sweet corn (var. Jubilee) suggests that this may be the major site for the enzymatic oxidation of polyunsaturated fatty acids. Off-flavor and off-aroma may ultimately develop in this region since

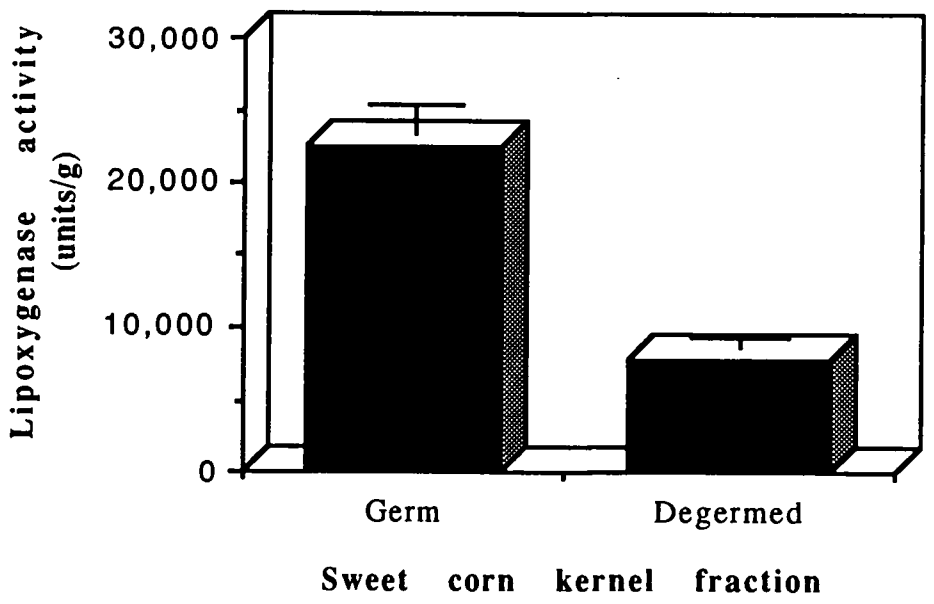

Fig. 3.3- Lipoxygenase activity in the germ and degermed fraction of sweet corn. Data are means ± SD.

polyunsaturated fatty acids have also been found to be higher in the germs of corn kernels (Wagenknecht, 1959; Gardner and Inglett, 1971; Flora and Wiley, 1972; Pascual and Wiley, 1974; Puangnak, 1976; Weber, 1978a and 1978b).

Rapid isolation of sweet corn germs

Large quantities of sweet corn germs were required for the study, and because of the seasonal availability of fresh corn and the tedious and time consuming nature of the manual isolation method, a rapid method of isolation was developed as previously described. The yield of isolated germs was about 2-3% wet weight of the cut kernels. This technique allowed the rapid isolation of sweet corn germs. Sucrose that was used during germ separation was tested for its effect on lipoxygenase activity and it was found to have no inhibitory effect on the enzyme.

Optimization of lipoxygenase isolation from sweet corn germs

Preparation of acetone powder

An optimized procedure for preparation of acetone powder from sweet corn germs was developed as shown in Fig. 3.2. The preparation of an acetone powder of corn germs from liquid nitrogen

powder was considered to be a mean of concentrating the enzyme as well as removing lipids, carotenoid pigments and other impurities present in corn germs. Some undesirable impurities that might act as inhibitors of lipoxygenase, e.g. natural antioxidants present in corn germs, may also have been removed. In addition this technique was used to solubilize membrane associated enzymes (Penefsky and Tzagoloff, 1971). Previous attempts at lipoxygenase isolation from whole kernel sweet corn (Velasco et al., 1989), mature seed corn (Poca et al., 1990), wheat germ (Nicholas et al., 1982), and most of the lipid containing plants such as sunflower seed (Leoni et al., 1985), English pea (Chen and Whitaker, 1986), flaxseeds (Rabinovitch-Chable et al., 1992) also made acetone powders prior to extraction with an appropriate buffer.

Optimization of extraction

Various factors which may affect the efficiency of lipoxygenase extraction from sweet corn germ acetone powder were investigated. Conditions for the extraction of lipoxygenase were chosen based on the following preliminary observations: (1) use of a Tissumizer for extraction was more efficient and appropriate than using either a magnetic stirrer or Waring blender; (2) three minutes was sufficient for effective extraction of lipoxygenase using the Tissumizer with the powerstat at 50 and a ratio of the acetone powder to buffer of 1:10 (w/v) (4°C).

The extraction buffers compared were pH 4.5, 7.0, 8.0 and 9.0. When extraction was carried out at pH 8.0, this yielded both the highest total lipoxygenase activity and the highest specific activity compared with the other pH buffers (Fig. 3.4). The effect of the buffer types used at pH 8.0 and the concentration of Tris-HCl at pH 8.0 were also investigated. The Tris-HCl buffer was found to be the most efficient in extraction of lipoxygenase. Sodium phosphate and borate buffers yielded about 92% and 86% lipoxygenase activity relative to that using a Tris-HCl buffer. Moreover, lipoxygenase activity was the highest using Tris-HCl (pH 8.0) at 0.2 M. Tris-HCl buffers prepared at 0.1 M and 0.05 M only yielded a lipoxygenase activity of 92% and 91% relative to the 0.2 M buffer. Therefore, 0.2 M Tris-HCl was selected as the buffer of choice for extraction of lipoxygenase from sweet corn germ acetone powder.

The effect of a surfactant (Triton X-100), a metal chelator (EDTA), a reducing agent (DTT), and a protease inhibitor (PMSF) on lipoxygenase extraction were also examined. Previous work by Grossman et al. (1969), Pinsky et al. (1971) and Boyes et al. (1992) showed that using Triton X-100 in the extraction buffer increased the yield of lipoxygenase activity in the extract of some plant tissues. Various concentrations of Triton X-100 up to 2.0% (v/v), were added to the 0.2 M Tris-HCl extraction buffer. Results showed that Triton X-100 actually decreased lipoxygenase activity in the sweet corn germ extract (Fig. 3.5). Triton X-100 at concentrations of 0.05%, 1.0% and 2.0% decreased lipoxygenase activity to about 64%, 32% and 5% of that in the control extract without Triton. Similarly, in some

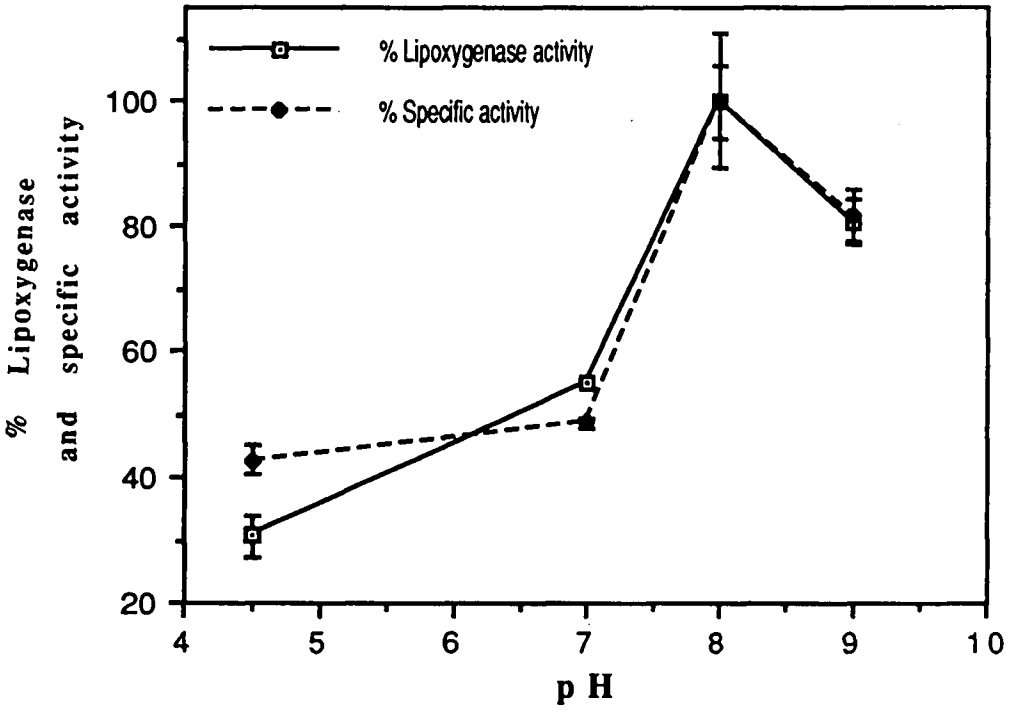

Fig. 3.4- Lipoxygenase and specific activity in extracts prepared with different pH buffers. Data are means ± SD.

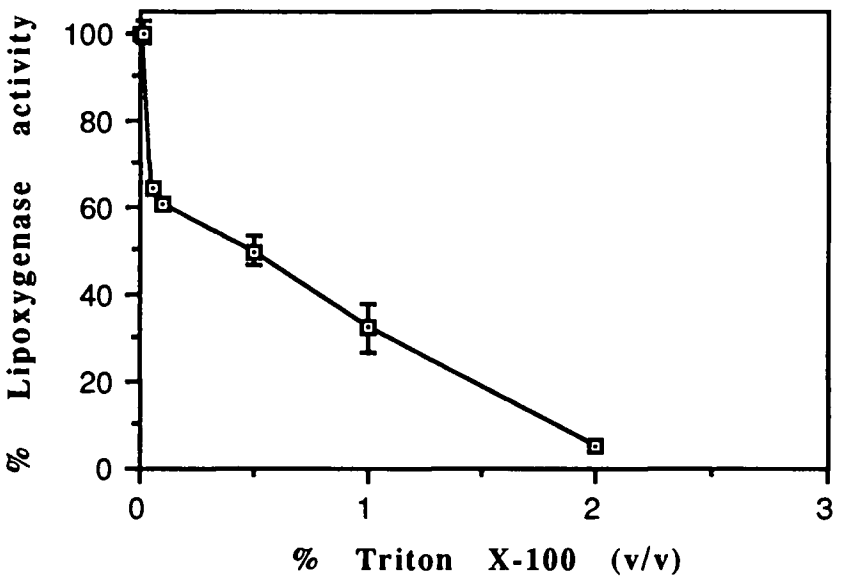

Fig. 3.5- Effect of Triton X-100 at various concentrations on percent recovery of lipoxygenase activity. Data are means ± SD.

plants such as soybean, pea, spinach and cauliflower, adding Triton X-100 to the extraction buffer has been shown to decrease lipoxygenase activity recovery (Pinsky et al., 1971).

The decreased yield of lipoxygenase activity in the extract using the extraction buffer with Triton X-100 may be due to the denaturation of the enzyme by detergent. Moreover, this may result from replacement of natural lipid by detergent which could cause the loss of enzymic activity. However, since adding Triton X-100 increased the yield of lipoxygenase in extracts of some plants, it could be due to the action of the detergent in solubilizing membrane-bound enzyme.

EDTA, a metal chelating agent, is typically added to extraction buffers to bind metal ions and divalent cations that may inhibit enzymes during extraction. Lipoxygenases in different plants such as cowpeas (Den and Mendoza, 1982), and chickpeas (Sanz et al., 1992) were reported to be inhibited by metal ions. However, extraction buffers to which 5 mM EDTA had been added showed about an 8% decrease in lipoxygenase activity.

EDTA has been reported to be an inhibitor of lipoxygenase in some plants e.g. broad beans (Al-Obaidy and Siddiqi, 1981) and soybeans (Chan, 1973). Lipoxygenase activity in plants such as sunflower seeds (Leoni et al., 1985) and barley (Lulai and Baker, 1976), however, was not affected by EDTA. Decreases in activity due to EDTA might be due to the presence of a non-heme iron atom at the active site as is true in soybean lipoxygenase (Chan, 1973; Roza and Francke, 1973; Pistorius and Axelrod, 1974; Navaratnam et al.,

1988; Nelson, 1988; Draheim et al., 1989). EDTA might form a complex with the iron in lipoxygenase and result in a decrease in activity.

The addition of a reducing agent (DTT) at a concentration of 5 mM in the extraction buffer decreased the yield of lipoxygenase activity to about 54% of that in the extract without DTT. This indicates that a reducing environment is not required to stabilize activity of the lipoxygenase during the extraction. The reducing conditions may in fact change or keep lipoxygenase molecules in the inactive form. Moreover, addition of 5 mM PMSF as a serine protease inhibitor was not beneficial for the extraction of lipoxygenase from sweet corn germs. Therefore, none of these agents were added to the 0.2 M Tris-HCl extraction buffer.

Ammonium sulfate fractionation and dialysis

The optimal percent ammonium sulfate saturation for the fractionation of lipoxygenase from the extract of sweet corn germ acetone powder was first determined by increasing the saturation in 10% increments from 20-100% saturation. Results show that the highest lipoxygenase activity recovery and specific activity was found between 40-60% ammonium sulfate (Fig. 3.6). This range was the same as that used to isolate lipoxygenase from green beans (Adams and Ongley, 1989); cow peas (Den and Mendoza, 1982); and soybeans (Mitsuda et al., 1967; Steven et al., 1970). However, other percent saturation ranges for ammonium sulfate have been reported

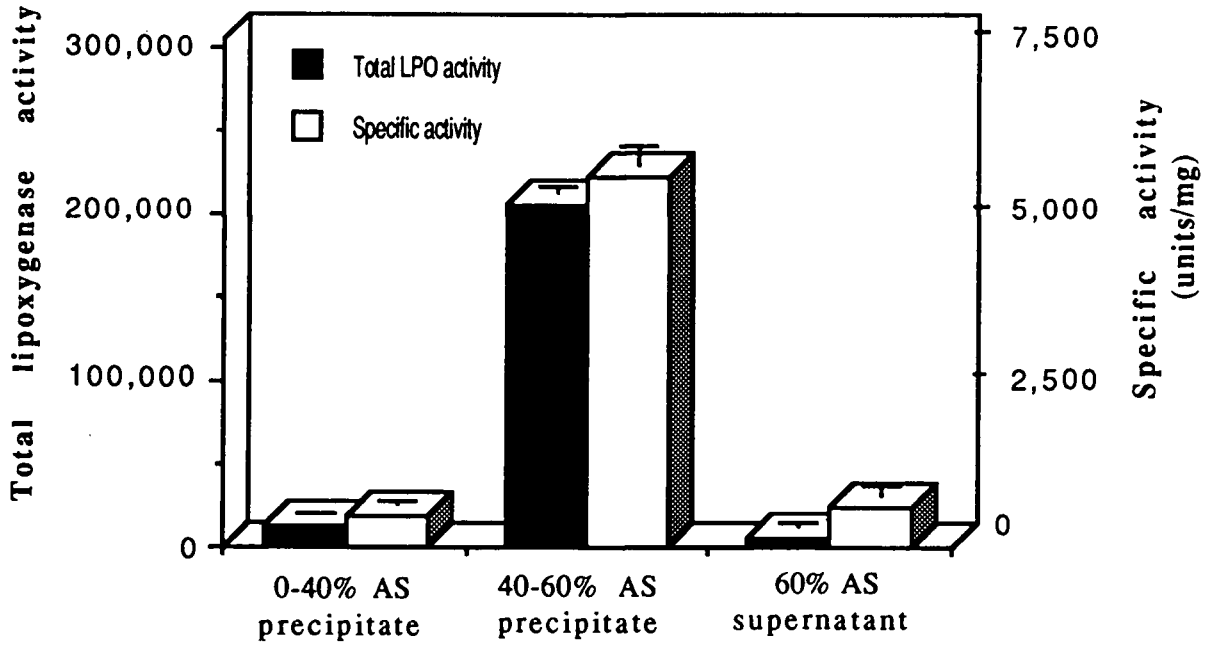

Fig. 3.6- Lipoxygenase activity and specific activity fractionated with ammonium sulfate (AS) from sweet corn germ acetone powder extract. Data are means ± SD.

for isolation of lipoxygenase from corn. For example, Belefant and Fong (1991) used 20-50% for corn embryos. Gardner and Weisleder (1970) used 42-53% for mature corn germ. Velasco et al. (1989) used 10-50% for sweet corn kernels and Poca et al. (1990) used 40-80% for mature seed of corn. Fig. 3.7 illustrates the procedure for isolation of lipoxygenase from sweet corn germs. The recovery and purification fold of isolated lipoxygenase after fractionation and dialysis were 62.1% and 3- fold, respectively (Table 3.2).

pH optimum for activity and pH stability

The optimum pH for the activity of lipoxygenase isolated from sweet corn germs was approximately 6.0-7.0 (Fig. 3.8). Very little activity was observed below pH 4.0 and above pH 8.0. This is similar to the pH optimum reported by Gardner (1988) for the lipoxygenase isozyme of mature maize germs which had a broad pH optimum between pH 6.0 and 7.2 and essentially no activity above pH 8.2. Belefant and Fong (1991) also found that lipoxygenase in the embryos of inbred yellow dent corn had an optimum pH of 6.8-7.0. However, Poca et al. (1990) reported that the pH optimum for two lipoxygenase isozymes of mature maize seeds had a broad pH optimum which ranged from pH 6.0 to 8.2 for isozyme-1 and from pH 7.0 to 9.0 for isozyme-2.

The optimum pH of lipoxygenase in the germ tissue of cereal grains has been reported to be 6.0-6.5 for wheat germ (Nicholas et al., 1982), 6.5-7.0 for rice germ (Yamamoto et al., 1980), and 6.5 for

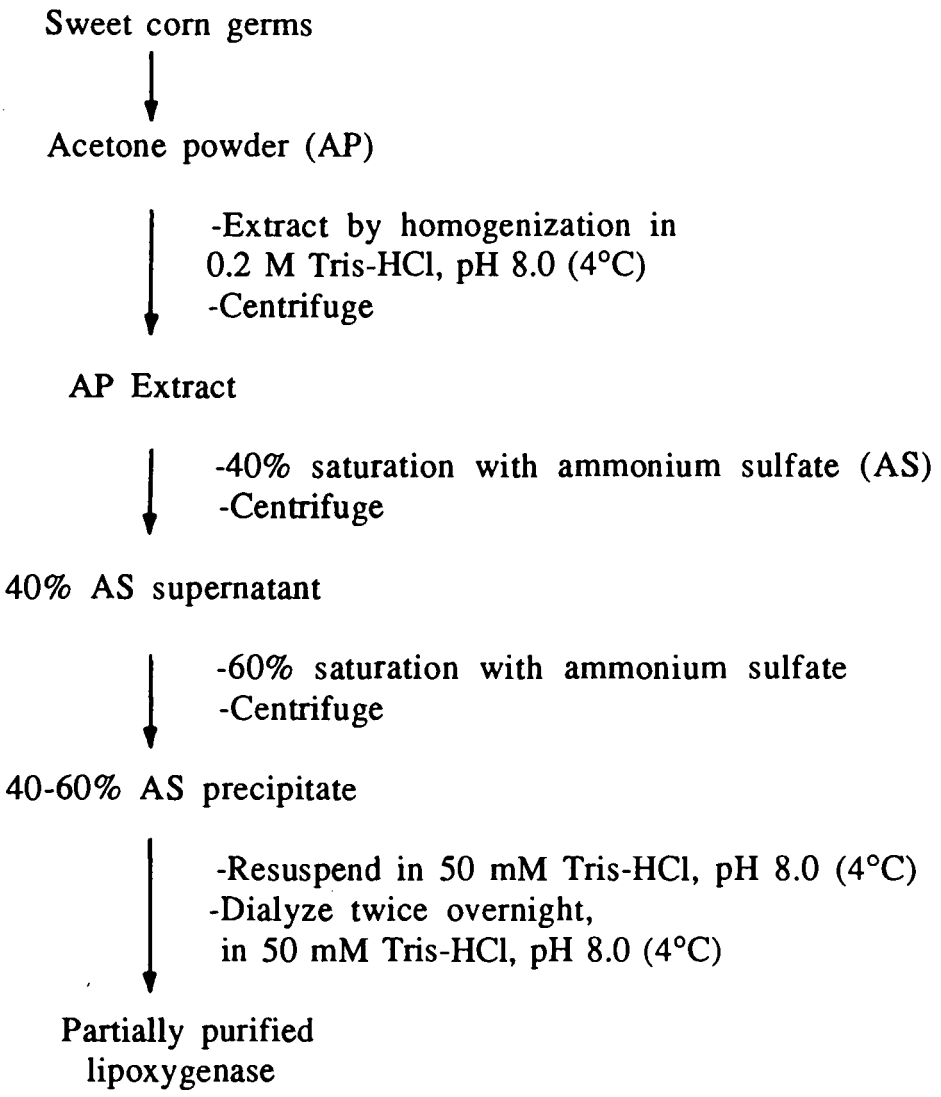

Fig. 3.7- Partial purification of lipoxygenase from sweet corn germs.

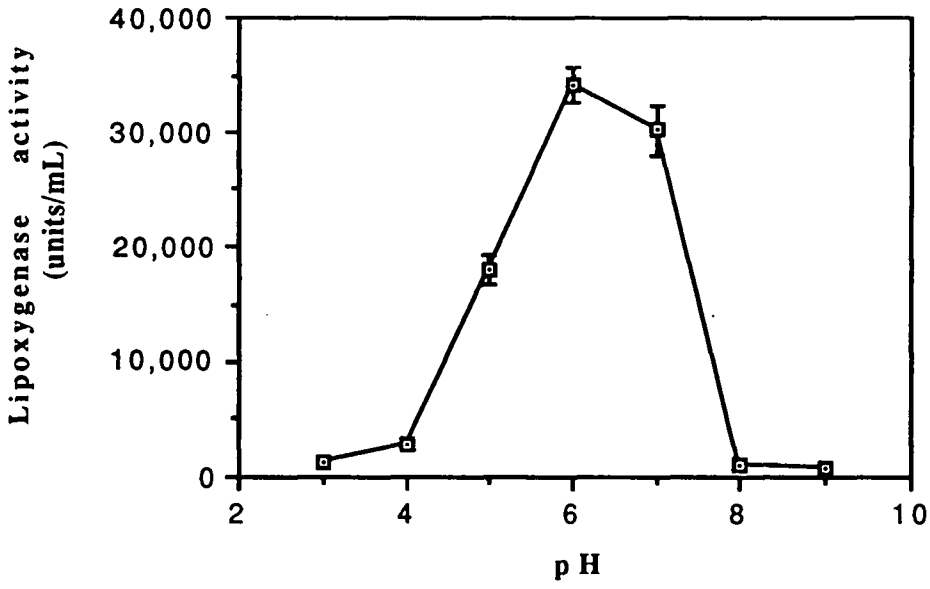

Fig. 3.8- pH activity profile for partially purified lipoxygenase from sweet corn germs. Data are means ± SD.

Table 3.2- Summary of partial purification of lipoxygenase from sweet corn germs.

Step	Total activity (units/g AP)	Specific activity (units/mg protein)	Degree of purification	% Recovery[a]
Acetone powder(AP) extract	137,250	2,338	1.0	100.0
40% Ammonium sulfate supernatant	105,044	2,809	1.2	76.5
40-60% Ammonium sulfate precipitate	102,375	5,543	2.4	74.6
After dialysis	95,297	7,021	3.0	62.1

[a] Relative to lipoxygenase activity in AP extract

germinated barley germ (Doderer et al., 1992). Lipoxygenase activity of many other plants, e.g. broad beans (Al-obaidy and Siddiqi, 1981), egg plant (Grossman et al., 1972), navy beans (Koch et al., 1971), soybean lipoxygenase-2 (Diel and Stan, 1978; Dreesen et al., 1982), pea isozyme-1 (Chen and Whitaker, 1986) and kiwifruit (Boyes et al., 1992) have also been found to have a pH optimum ranging from 6.0-7.5. However, lipoxygenase activity of soybean lipoxygenase-1 was found to be an optimum of pH 8.0-9.5 (Diel and Stan, 1978) and Reynolds and Klein (1982) found that dry English pea seeds (var. Little Marvel) contained a small amount of a type-1 lipoxygenase with an optimum pH of 9.0-10.0.

The pH stability study was performed by diluting isolated lipoxygenase 1:10 (v/v) with buffers of pH 3.0 to 10.0 and incubating the solution at 25°C for 30 minutes, then assaying for enzyme activity. Sweet corn germ lipoxygenase seems to be most stable in the range of pH 5.0-8.0; however, the activity was almost entirely lost at pH 10.0 (Fig. 3.9). This result is similar to that reported for pea lipoxygenase isozyme-1 which was stable at pH 4.5-8.0 at 25°C for 30 minutes (Chen and Whitaker, 1986). Ganthavorn and Powers (1989) reported that asparagus lipoxygenase was also stable at pH 4.5-8.0 at 2°C for 3 days. Al-obaidy and Siddiqi (1981) found that broad bean lipoxygenase was stable between pH 4.0 and 8.0, however, the time and temperature of their study were not reported.

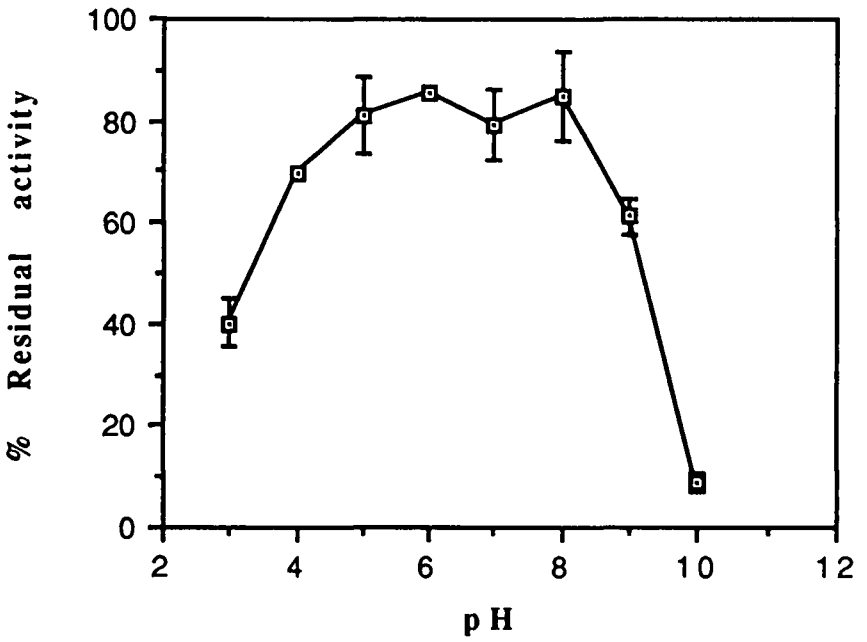

Fig. 3.9- pH stability curve for partially purified lipoxygenase from sweet corn germs. Data are means ± SD.

Temperature optimum for activity and temperature stability

For the temperature optimum for activity study, the activity of sweet corn germ lipoxygenase was determined spectrophotometrically in pH 7.0 0.2 M sodium phosphate buffer at 10, 20, 25, 30, 35, 40, 50, 60 and 70°C. The optimum temperature for lipoxygenase activity was found to be approximately 50°C (Fig. 3.10). This was similar to the optimum temperature reported for the activity of three major lipoxygenase isozymes of wheat germ which had optimums of approximately 45°C and only trace activities at 65°C (Shiiba et al., 1991), and for barley lipoxygenase which had an optimum of 47°C (Lulai and Baker, 1976). However, this result was higher than the optimum temperature found for plants such as sunflower seeds (Leoni et al., 1985) with an optimum temperature approximately 35°C; soybeans, broad beans and cowpeas with a maximum activity around 30°C (Tappel et al., 1953; Al-Obaidy and Siddiqi, 1981; Den and Mendoza, 1982).

Results of the temperature stability study (Fig. 3.11) indicate that approximately 90% of the original activity of isolated lipoxygenase from sweet corn germs was inactivated after heating at 70°C for 3 minutes. Lipoxygenase was almost completely inactivated after 20 minutes at 70°C. Approximately 50 and 90% of original lipoxygenase activity was inactivated after heating at 60°C for 3 and 20 minutes, respectively.

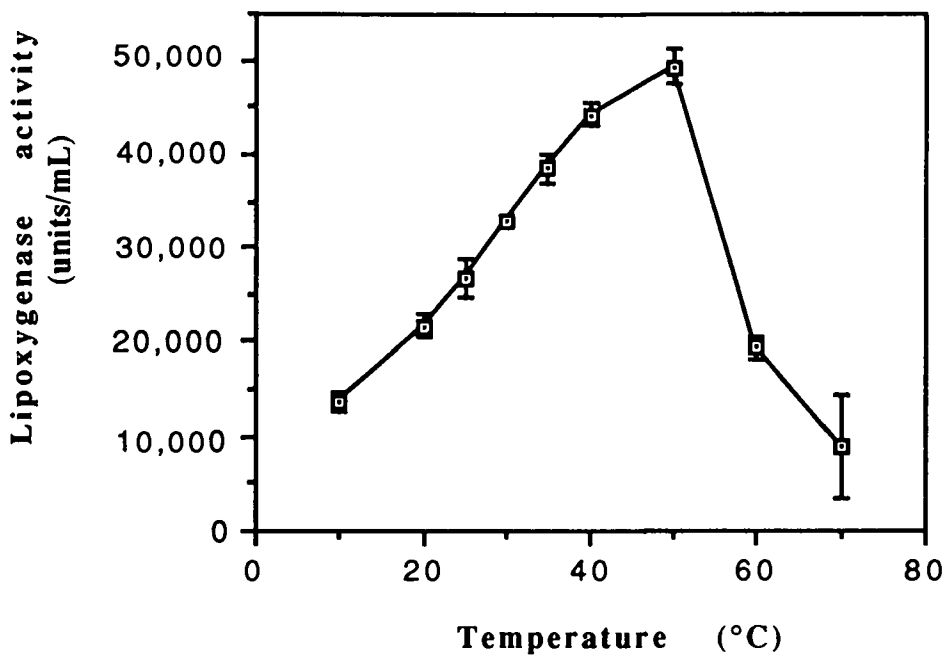

Fig. 3.10- Temperature activity profile for partially purified lipoxygenase from sweet corn germs. Data are means ± SD.

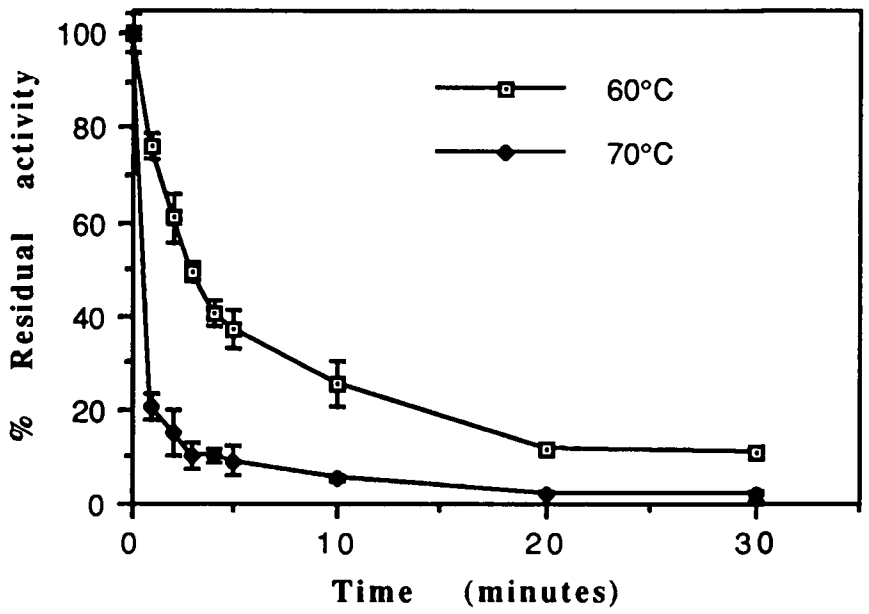

Fig. 3.11- Temperature stability for partially purified lipoxygenase from sweet corn germs. Data are means ± SD.

After heating for 10 minutes, the residual lipoxygenase activities were about 25% at 60°C and 5% at 70°C respectively. Lipoxygenase in sweet corn germs seemed to be less heat stable than that in English green pea homogenate where residual activities after heating at 60°C for 10 minutes were about 30% (Williams et al., 1986). Poca et al. (1990) reported that isolated lipoxygenase isozyme activities from mature maize seeds remained at about 50% after heating for 5 min at approximately 45°C for isozyme-1 and at 65°C for isoenzyme-2. Results for thermal inactivation of lipoxygenase enzymes obtained by different investigators may not be directly comparable because of various factors, e. g. technique for heating and assaying enzymes, criteria used for inactivation, enzyme concentration, amount and type of impurities such as other proteins, substrates of the enzyme, and reaction products (McConnell, 1956; Svensson and Eriksson, 1972; Alsoe and Alder-Nissen, 1988; Ganthavorn and Powers, 1989).

The pH 7 buffer was chosen to study the heat stability of the enzyme since pH 7 is approximately the physiological pH of corn. However, the lipoxygenase activity in germs in nature might be more heat stable than in solution due to the natural protective environment such as the kernel structure and compounds which may act as stabilizers present in the germs.

CONCLUSIONS

Lipoxygenase was found to be in higher concentration in the germ compared with the degermed fraction of sweet corn at maturity selected for freezing, suggesting that industry should pay more attention to the germ fraction. The germ may be an important site for off-flavor and off-aroma formation in unblanched sweet corn due to enzymatic oxidation. The conditions for extraction and isolation of lipoxygenase from the germs of sweet corn were optimized for the first time. Its physicochemical properties such as pH and temperature optimum for activity, pH and temperature stability were also useful for providing a better understanding of lipoxygenase in germs. Hopefully, continued research will lead to understanding of its possible role in sweet corn quality and finally whether lipoxygenase is more appropriate to use than peroxidase as the blanching index in the frozen sweet corn industry.

ACKNOWLEDGMENTS

This paper was presented at the 51st Annual Meeting of the Instituted of Food Technologists, Dallas, TX., June 2-5, 1991.

Author Theerakulkait would like to express sincere appreciation for the International Rotary Foundation in providing a Rotary Freedom From Hunger scholarship for 3 years. Authors would like to thank you for contribution and support from Mr. Dennis Spink of the Agri-Pac Co., Woodburn, Oregon, in providing the sweet corn samples for the research, and Agricultural Research Foundation (Oregon Processed Vegetable Commission) for financial support.

REFERENCES

Adams, J.B. and Ongley, M.H. 1989. The behavior of green bean lipoxygenase on chromatography and isoelectric focusing. Food Chem. 31: 57-71.

Al-obaidy, H.M. and Siddiqi, A.M. 1981. Properties of broad bean lipoxygenase. J. Food Sci. 46: 622-626.

Alsoe, K.O. and Alder-Nissen, J. 1988. Thermal denaturation of lipoxygenase from soya beans in the presence of its natural substrate. Lebensm.-Wiss. u. Technol. 21: 8-12.

Auerman, L.Y., Popov, M.P., Dubtsov, G.G., and Samsonov, M.M. 1971. Lipoxygenase activity of wheat grain and flour. Prikl. Biokhim. Mikrobiol. 7: 678-681.

Belefant, H. and Fong, F. 1991. Lipoxygenases in developing *Zea mays* kernels. Plant Physiol. Biochem. 29(2): 99-104.

Boyes, S., Perera, C., and Young, H. 1992. Kiwifruit lipoxygenase: preparation and characteristics. J. Food Sci. 57(6): 1390-1394.

Chan, H.W.S. 1973. Soya-bean lipoxygenase: an iron-containing dioxygenase. Biochim. Biophys. Acta. 327: 32-35.

Chen, A.O. and Whitaker, J.R. 1986. Purification and characterization of a lipoxygenase from immature English peas. J. Agric. Food Chem. 34: 203-211.

Den, T.V. and Mendoza, E.M.T. 1982. Purification and characterization of two lipoxygenase isozymes from cowpea [*Vigna unguiculata* (L.) Walp.]. J. Agric. Food Chem. 30: 54-60.

Diel, E. and Stan, H-J. 1978. Purification and characterization of two isozymes of lipoxygenase from soybeans. Planta 142: 321-328.

Doderer, A., Kokkelink, I.K., van der Veen, S., Valk, B.E., Schram, A. W., and Douma, A.C. 1992. Purification and characterization of two lipoxygenase isozymes from germinating barley. Biochim. Biophys. Acta. 1120: 97-104.

Draheim, J.E., Carroll, R.T., McNemar, T.B., Dunham, W.R., Sands, R. H. and Funk, M.O.J. 1989. Lipoxygenase isozymes: a spectroscopic and structural characterization of soybean seed enzymes. Arch. Biochem. Biophys. 269(1): 208-218.

Dreesen, T.D., Dickens, M. and Koch, R.B. 1982. Partial purification and characterization of a Ca^{2+}- stimulated lipoxygenase from soybean seeds. Lipids. 17(12): 964-969.

Egmond, M.R., Vliegenthart, J.F.G. and Boldingh, J. 1972. Stereospecificity of the hydrogen abstraction at carbon atom n-8 in the oxygenation of linoleic acid by lipoxygenases from corn germs and soya beans. Biochem. Biophys. Res. Commun. 48(5): 1055-1060.

Flora, L.F., and Wiley, R.C. 1972. Effect of various endosperm mutants on oil content and fatty acid composition of whole kernel corn (*Zea mays* L.). J. Am. Soc. Hortic. Sci. 97(5): 604-607.

Fong, F., and Smith, J.D., 1985. Rapid isolation and purification of immature zygotic embryos. Maize Gen. Coop. Newslett. 59: 30-31.

Ganthavorn, G. and Powers, J.R. 1989. Partial purification and characterization of Asparagus Lipoxygenase. J. Food Sci. 52: 371-73.

Gardner, H.W. 1970. Sequential enzymes of linoleic acid oxidation in corn germ: lipoxygenase and linoleate hydroperoxide isomerase. J. Lipid Res. 11: 311-321.

Gardner, H.W. 1988. Lipoxygenase pathway in cereals. Ch. 6, In *Advances in Cereal Science and Technology*, Vol. 9, Y. Pomeranz (Ed.), 161-215. American Association of Cereal Chemists, Incorporated, St. Paul, Minnesota.

Gardner, H.W. and Inglett, G.E. 1971. Food products from corn germ: enzyme activity and oil stability. J. Food Sci. 36: 645-648.

Gardner, H.W., Kleiman, R., Christianson, D.D., and Weisleder, D. 1975. Positional specificity of γ-ketol formation from linoleic acid hydroperoxides by a corn germ enzyme. Lipids. 10(10): 602-608.

Gardner, H.W. and Weisleder, D. 1970. Lipoxygenase from *Zea mays*: 9-D-hydroperoxy-trans-10,cis-12-octadecadienoic acid from linoleic acid. Lipids. 5: 678-683.

Gibian, M.J. and Vandenberg, P. 1987. Product yield in oxygenation of linoleate by soybean lipoxygenase: the value of the molar extinction coefficient in spectrophotometric assay. Anal. Biochem. 163: 343-349.

Grossman, S., Ben-Aziz, A., Budowski, P., Ascarelli, I., Gertler, A., Birk, Y. and Bondi, A. 1969. Enzymic oxidation of carotene and linoleate by alfalfa: extraction and separation of active fractions. Phytochemistry 8: 2287-2293.

Grossman, S., Trop, M., Avtalion, R. and Pinsky, A. 1972. Egg plant lipoxygenase: isolation and partial characterization. Lipids. 7: 467-473.

Koch, R.B., Brumfiel, B.L., and Brumfiel, M.N. 1971. Calcium requirement for lipoxygenase catalyzed linoleate oxidation. J. Am. Oil Chem. Soc. 48: 532-538.

Lee, C.Y., Smith, N.L. and Hawbecker, D.E. 1989. An improved blanching technique for frozen sweet corn on-the-cob. Tech. innov. in freezing and refrigeration. UC Davis July 9-12: 85-90.

Lee, Y.C. 1981. Lipoxygenase and off-flavor Development in some frozen foods. Korean J. Food Sci. Technol. 13: 53-56.

Leoni, O., Iori, R. and Palmieri, S. 1985. Purification and properties of lipoxygenase in germinating sunflower seeds. J. Food Sci. 50: 88-92.

Lulai, E.C. and Baker, C.W. 1976. Physicochemical characterization of barley lipoxygenase. Cereal Chem. 53: 777-786.

Lulai, E.C. and Baker, C.W. and Zimmerman, D.C. 1981. Metabolism of linoleic acid by berley lipoxygenase and hydroperoxide isomerase. Plant Physiol. 68: 950-955.

McConnell, J.E.W. 1956.. Enzyme-heat resistance and regeneration: review of literature on thermal inactivation of enzymes in foods. National Canners Association. 1-12.

Mitsuda, H., Yasumoto, K., Yamamoto, A., and Kusano, T. 1967. Study on lipoxygenase part I. preparation of crystalline enzyme and assay by polarographic method. Agric. Biol. Chem. 31(1): 115-118.

Navaratnam, S., Feiters, M.C., Al-Hakim, M., Allen, J.C., Veldink, G.A., and Vliegenthart, J.F.G. 1988. Iron environment in soybean lipoxygenase-1. Biochim. Biophys. Acta. 956: 70-76.

Nelson, M.J. 1988. Catecholate complexes of ferric soybean lipoxygenase 1. Biochem. 27: 4273-4278.

Nicholas, J., Autran, M., and Drapron, R. 1982. Purification and some properties of wheat germ lipoxygenase. J. Sci. Food Agric. 33: 53-63.

Pascual, L.R. and Wiley, R.C. 1974. Fatty acids in the major lipid fractions of maturing sweet corn (*Zea mays* L.). J. Am. Soc. Hortic. Sci. 99(6): 573-576.

Penefsky, H.S., and Tzagoloff, A. 1971. Extraction of water-soluble enzymes and protein from membrane. Ch. 19, In *Meth. Enzymol.*, Vol. 22, W.B. Jakoby (Ed.), 204-219. Academic Press, Inc., New York.

Pinsky, A., Grossman, S. and Trop, M. 1971. Lipoxygenase content and antioxidant activity of some fruits and vegetables. J. Food Sci. 36: 571-572.

Pistorius, E.K. and Axelrod, B. 1974. Iron, an essential component of lipoxygenase. J. Biol. Chem. 249: 3183-3186.

Poca, E., Rabinovitch-Chable, H., Cook-Moreau, J., Montserrat, P., and Rigaud, M. 1990. Lipoxygenases from *Zea mays* L. purification and physicochemical characteristics. Biochim. Biophys. Acta. 1045: 107-114.

Puangnak, W. 1976. Effect of hybrid, maturity and kernel structure on lipid content, composition and aroma development of sweet corn. M.S. thesis. University of Maryland, College Park.

Rabinovitch-Chable, H., Cook-Moreau, J. Breton, J-C. and Rigaud, M. 1992. Purification of lipoxygenase and hydroperoxide dehydrase in flaxseeds: interaction between these enzymatic activities. Biochem. Biophys. Res. Commun. 188(2): 858-864.

Reynolds P.A. and Klein, B.P. 1982. Purification and characterization of a type-1 lipoxygenase from pea seeds. J. Agric. Food Chem. 30: 1157-1163.

Roza, M. and Francke, A. 1973. Soyabean lipoxygenase: An iron-containing enzyme. Biochim. Biophys. Acta. 327: 24-31.

Sanz, L.C., Perez, A.G. and Olias, J.M. 1992. Purification and catalytic properties of chickpea lipoxygenases. Phytochemistry 31(9): 2967-2972.

Sheu, S.C. and Chen, A.O. 1991. Lipoxygenase as blanching index for frozen vegetable soybeans. J. Food Sci. 56(2): 448-451.

Shiiba, K., Negishi, Y., Okada, K. and Nagao, S. 1991. Purification and characterization of lipoxygenase isozymes from wheat germ. Cereal Chem. 68(2): 115-122.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fugimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150: 76-85.

Smittle, D.A., Thornton, R.E. and Dean, B.B. 1972. Sweet corn quality deterioration: A survey of industry practices and possible methods of control. Wash. Agric. Expt. Stat. Circ. 555.

Stevens, F.C., Brown, D.M., and Smith, E.L. 1970. Some properties of soybean lipoxygenase. Arch. Biochem. Biophys. 136: 413-421.

Svensson, S.G. and Eriksson, C.E. 1972. Thermal inactivation of lipoxygenase from peas (*Pisum sativum* L.) II. influence of substrate and reaction products. Lebensm.-Wiss. u. Technol. 5(4): 124-127.

Tappel, A.L., Lundberg, W.O. and Boyer, P.D. 1953. Effect of temperature and antioxidants upon the lipoxidase-catalyzed oxidation of sodium linoleate. Arch. Biochem. Biophys. 42: 293-304.

Velasco, P.J., Lim, M.H., Pangborn, R.M. and Whitaker, J.R. 1989. Enzymes responsible for off-flavor and off-aroma in blanched and frozen stored vegetables. Appl. Biochem. Biotechnol. 11: 118-127.

Veldink, G.A., Garssen, G.J., Vliegenthart, J.F.G., and Boldingh, J. 1972. Positional specificity of corn germ lipoxygenase as a function of pH. Biochem. Biophys. Res. Commun. 47(1): 22-26.

Von Ceumern, S. and Hartfiel, W. 1984. Activity of lipoxygenase in cereals and possibilities of enzyme inhibition. Fette Seifen Anstrichm. 86: 204-208.

Wagenknecht, A.C. 1959. Lipoxidase activity and off-flavor in underblanched frozen corn-on-the-cob. Food Res. 24: 539-547.

Weber, E.J. 1978a. Corn lipids. Cereal Chem. 55(5): 572-584.

Weber, E.J. 1978b. The lipids of corn germ and endosperm. J. Am. Oil Chem. Soc. 56: 637-641.

Williams, D.C., Lim, M.H., Chen, A.O., Pangborn, R.M., and Whitaker, J.R. 1986. Blanching of vegetables for freezing - which indicator enzyme to choose. Food Technol. 40(6): 130-140.

Yamamoto A., Fujii, Y., Yasumoto, K. and Mitsuda, H. 1980. Partial purification and study of some properties of rice germ lipoxygenase. Agric. Biol. Chem. 44(2): 443-445.

CHAPTER 4:
PARTIAL PURIFICATION AND CHARACTERIZATION OF SWEET CORN GERM LIPOXYGENASE

Chockchai Theerakulkait and Diane M. Barrett*

Department of Food Science and Technology,
Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602
and
*Department of Food Science and Technology,
Cruess Hall, University of California, Davis, CA 95616-8598

ABSTRACT

Lipoxygenase (LPO) from the germ fraction of sweet corn (*Zea mays* L. var. Jubilee) was purified by acetone powder preparation; extraction with 0.2 M Tris-HCl, pH 8; 40-60% fractionation with ammonium sulfate; and conventional column chromatography on Sephacryl S-300 HR and Fast Protein Liquid Chromatography (FPLC) on a Mono Q column. The purification was 124 fold with 26.3 percent recovery. Further purification was achieved by FPLC chromatofocusing on a Mono P column and size exclusion chromatography on Superose-12. The apparent molecular weight and isoelectric point (pI) determined by FPLC on Superose-12 and Mono P columns were 90,500 and 5.06, respectively. Enzymatic formation of conjugated dienes by lipoxygenase was inhibited by both synthetic (BHA, BHT) and natural (quercetin, chlorogenic acid) phenolic antioxidants at a concentration of 0.2 mM with 57.2, 16.3, 61.4 and 32.3 percent inhibition, respectively. The activation energy for thermal inactivation of sweet corn germ lipoxygenase in the ammonium sulfate preparation at 55 to 70°C was 56.3 kcal/mol.

Key Words: lipoxygenase, purification, sweet corn, germ, FPLC, molecular weight, isoelectric point, phenolic antioxidants, thermal inactivation

INTRODUCTION

It has been suggested that lipoxygenase (Linoleate : oxygen oxidoreductase, EC 1.13.11.12) catalyzes a reaction leading to off-flavor development in unblanched and underblanched frozen vegetables, including sweet corn (Wagenknecht, 1959; Lee, 1981; Williams et al., 1986; Ganthavorn and Powers, 1989; Velasco et al., 1989; Sheu and Chen, 1991). Lipoxygenase may, therefore, be more important and more appropriate than peroxidase for use as the blanching index in the sweet corn freezing process. Previous studies suggested that in unblanched sweet corn, lipoxygenase induced off-flavors in the fraction containing the germ (Wagenknecht, 1959; Lee, 1981). The lipoxygenase activity of sweet corn germs should receive more attention, however, this activity has not been thoroughly investigated. To the best of our knowledge, research on lipoxygenase from sweet corn germs has been very limited; the only previous work was done by Belefant and Fong (1991), who partially purified lipoxygenase from sweet corn germs by precipitation with 20-50% ammonium sulfate and dialysis. The purification fold and percent recovery of the enzyme were not given.

Previous work in our laboratory showed that lipoxygenase activity per unit weight of germs was greater than that in the degermed fraction of sweet corn kernels (Theerakulkait and Barrett, manuscript in prep.). Optimized procedures and conditions for extraction and isolation of lipoxygenase from the germs have been developed, and some physicochemical properties of lipoxygenase in

sweet corn germs have also been described (Theerakulkait and Barrett, manuscript in prep.). Previous work only resulted in a 3 fold purification, however, and the enzyme was still contaminated with peroxidase activity. Therefore, the isolated lipoxygenase required further purification in order to be used for further investigation of its characteristics and for use in study of its possible role in off-aroma formation. Moreover, fast protein liquid chromatography (FPLC), a novel protein purification technique which was developed in the past decade and has been used to purify lipoxygenases in other plants such as soybean (Kato et al., 1992; Shkarina et al., 1992) and kidney bean (Sanz et al., 1993) will be employed. FPLC has not been used to purify lipoxygenase from sweet corn.

The objectives of this work were: to develop a procedure to further purify lipoxygenase from sweet corn germs by a combination of conventional chromatography and FPLC, and to determine characteristics including molecular weight (MW), isoelectric point (pI), inhibition by synthetic and natural phenolic antioxidants, and thermal inactivation.

MATERIALS & METHODS

Materials

Freshly harvested sweet corn (*Zea mays* L. var. Jubilee) at the optimal maturity for freezing was obtained from the National Frozen Food Co., Albany, Oregon. The corn was immediately transported to the pilot plant of the Dept. of Food Science and Technology, Oregon State University, Corvallis, Oregon. The fresh sweet corn was temporarily stored at 4°C prior to germ separation, which was carried out over a period of 3 days.

The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): linoleic acid, ammonium sulfate, Tween-20 (polyoxoethylene-sorbitan monolaureate), bovine serum albumin, gel filtration media including: Sepharose CL 6B (fractionation range of 10,000 to 4,000,000 for globular protein), Sephacryl S-300 HR column (fractionation range of 10,000 to 1,500,000 for globular protein), anion exchanger diethylaminoethyl (DEAE)-Sephacel, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), chlorogenic acid, and protein molecular weight standards including: cytochrome c from horse heart (MW 12,400), carbonic anhydrase from bovine erythrocytes (MW 66,000), soybean lipoxygenase-1 (MW 100,000), alcohol dehydrogenase from yeast (MW 150,000), apoferritin from horse spleen (MW 443,000) and blue dextran (MW 2,000,000). Quercetin was a gift from the laboratory of Dr. R. E.

Wrolstad. All other chemicals were reagent grade. Deionized distilled water was used in all experiments.

Polybuffer exchanger (PBE) 94 chromatofocusing media, Polybuffer 74, prepacked FPLC columns including Mono Q HR 5/5 (5 x 0.5 cm ID.), Mono P HR 5/20 (20 x 0.5 cm ID.), Superose-12 HR 10/30 (30 x 1.0 cm ID.), and prepacked disposable PD-10 columns (Sephadex G-25 M) were from Pharmacia-LKB, Uppasala, Sweden. The FPLC system (Pharmacia-LKB) consisted of a LCC-500 plus controller, two P-500 high precision pumps, Frac-200 fraction collector, UV-M monitor, MV-7 injection valve, FPLC manager software version 1.10, loops of variable size up to 2 mL, and 50 mL superloop, Basic Time AT computer, and an Epson FX 286 wide carriage printer. Filters of 0.45 μm pore size, type HA were obtained from the Millipore Corp., Bedford, MA. Ultrafiltration membranes (YM 30, MW cutoff 30,000 daltons) were purchased from Amicon Division, W.R. Grace & Co., Beverly, MA.

Isolation and purification of sweet corn germ lipoxygenase

The germ fraction was rapidly isolated from sweet corn on the cob, frozen in liquid nitrogen and stored at -80°C prior to use. The germs were prepared as acetone powder, extracted with 0.2 M Tris-HCl, pH 8.0 (4°C) and fractionated by 40-60% ammonium sulfate saturation as described by Theerakulkait and Barrett (manuscript in prep.). The lipoxygenase precipitate was kept at -23.3°C until use in purification.

All purification steps were carried out at 4°C, except column chromatography using the FPLC system, which was performed at room temperature (22°C). Protein elution was monitored by measuring absorbance at 280 nm. Lipoxygenase activity in each fraction was determined spectrophotometrically at 234 nm as described below. All buffers were filtered and degassed prior to use with the FPLC system.

Preliminary experiments

Preliminary attempts at using conventional column chromatography included: gel filtration, ion exchange, and chromatofocusing. Preliminary experiments for purification of sweet corn germ lipoxygenase utilizing conventional chromatography consisted of the following chromatographic steps:

Gel filtration on Sepharose CL 6B: The 40-60% ammonium sulfate precipitate was dissolved in 50 mM Tris-HCl, pH 8.0 (4°C) and centrifuged at 17,000 x g for 20 min. The supernatant was applied to the top of a Sepharose CL 6B column (1.6 X 70 cm) previously equilibrated with 50 mM Tris-HCl, pH 8.0 (4°C) in order to remove salt and fractionate by size. Fractions of 2 mL were eluted from the column with the same buffer at a flow rate of 30 mL per hr.

Anion exchange on a DEAE-Sephacel: The fractions containing more than 10-20% of the lipoxygenase activity in the most active fraction

were pooled and applied to the top of a DEAE-Sephacel column (2.6 X 40 cm) equilibrated with 20 mM Tris-HCl, pH 8.0 (4°C). The protein was eluted with the same buffer until the absorbance at 280 nm returned to the base line. Further elution was carried out with a linear salt gradient of 0 to 0.2 M NaCl in 20 mM Tris-HCl, pH 8.0 (4°C).

Chromatofocusing on PBE-94: The active lipoxygenase fractions from the DEAE-Sephacel column were pooled, salt was removed and the buffer exchanged with 25 mM imidazole-HCl buffer, pH 7.4 by diafiltration using a YM 30 ultrafiltration membrane. The enzyme solution was then applied to the top of a PBE 94 chromatofocusing column (0.9 X 48 cm) pre-equilibrated with 25 mM imidazole-HCl buffer, pH 7.4. The protein was eluted by applying Polybuffer 74, pH 4.0 to form a pH gradient of 7.0 to 4.0. The pH of the pooled active lipoxygenase was sequently adjusted to approximately 7 by adding 0.1 N NaOH.

Purification of sweet corn germ lipoxygenase

Gel filtration on Sephacryl S-300 HR: The ammonium sulfate precipitate was dissolved in 50 mM Tris-HCl, pH 8.0 (4°C), and centrifuged at 17,000 x g for 20 min. The supernatant was applied to the top of a Sephacryl S-300 HR column (1.6 X 98 cm) previously equilibrated with 50 mM Tris-HCl, pH 8.0 (4°C). Fractions of 2 mL were eluted from the column with the same buffer, at a flow rate of

60 mL per hr. The fractions containing at least 30% of the lipoxygenase activity in the most active fraction were pooled and subjected to further purification by FPLC on Mono Q column.

FPLC anion exchange on Mono Q: The pooled active fraction from the Sephacryl S-300 HR column was filtered through a 0.45 μm filter and degassed. The filtered and degassed sample was applied to the 50 mL superloop and injected via the Pharmacia MV-7 valve into a 1 mL Mono Q HR 5/5 column previously equilibrated with 20 mM Tris-HCl, pH 7.5. After loading the sample, elution was performed by a linear 0 to 0.2 M NaCl gradient at a flow rate of 1 mL/min using two buffers: buffer A: 20 mM Tris-HCl, pH 7.5, and buffer B: 0.5 M NaCl in 20 mM Tris-HCl, pH 7.5. The gradient elution program was the following: 17 mL at 0% B (for loading the sample); to 40% B in 30 mL; to 100% B in 10 mL; 10 mL at 100% B; followed by 10 mL at 0% B. The total volume for each run was 77 mL. Each 1 mL fraction from the Mono Q column was automatically collected by a Pharmacia Frac-200 fraction collector. The collected fractions were kept on ice prior to assay for lipoxygenase activity. The fractions containing more than 30% of the lipoxygenase activity in the most active fraction were pooled, and lipoxygenase activity and protein content were determined. The pooled active fraction was desalted and buffer exchanged with deionized distilled water using a prepacked PD-10 gel filtration column. The enzyme was lyophilized and stored in the desiccator at -23.3°C.

Because of an increase in back pressure, the Mono Q column was washed extensively after 3 to 4 runs by treating the column overnight with a solution of approximately 1 mg/mL pepsin in 0.1 M acetic acid containing 0.5 M NaCl at 37°C. After the enzymatic cleaning, chemical scrubbing was performed.

FPLC chromatofocusing on Mono P: The sweet corn germ lipoxygenase could be further purified by chromatofocusing on a prepacked FPLC Mono P HR 5/20 column using Polybuffer 74 with a pH range of 3.8-6.3. The pooled active lipoxygenase fractions from the Mono Q column were first desalted and buffer exchanged with 25 mM histidine-HCl, pH 6.3 (starting buffer) using a prepacked PD-10 gel filtration column. The sample was then loaded onto the Mono P column previously equilibrated with starting buffer, and protein fractions were eluted with Polybuffer 74, pH 3.8. The Polybuffer 74 solution was prepared by dilution with deionized distilled water in a ratio of 1:9.5 (v/v); the pH was adjusted to 3.8 with 1 N HCl and then made to a final dilution of 1:10 (v/v) with deionized distilled water.

The flow rate was 1 mL/min and 1 mL fractions from the Mono P column were collected. The pH of each fraction was immediately measured using a Corning semi-micro combination electrode with a Beckman ϕ32 pH meter and the active lipoxygenase fractions were adjusted to approximately pH 7 with 0.1 N NaOH. All fractions were kept on ice and subjected to lipoxygenase activity determination. The fractions containing more than 50% of lipoxygenase activity in the most active fraction were pooled.

FPLC gel filtration on Superose-12: The pooled active fraction from the Mono P column was injected onto a FPLC Superose-12 gel filtration column previously equilibrated with 50 mM ammonium acetate buffer, pH 7.0. The protein was eluted with the same buffer. The flow rate was 0.5 mL/min and 0.5 mL fractions were collected. All fractions were kept on ice and lipoxygenase activity was determined. The fractions containing more than 50% of the lipoxygenase activity in the most active fraction were pooled.

Characteristics of sweet corn germ lipoxygenase

Molecular weight determination

The molecular weight (MW) of purified lipoxygenase from the Mono Q column was determined using FPLC on Superose-12. The column was calibrated with protein molecular weight standards. The equilibration and elution were performed using 50 mM sodium phosphate buffer, pH 7.0, containing 0.10 M NaCl. One hundred microliters of each standard protein were applied to the column, and eluted at a flow rate of 0.5 ml/min. Elution profiles of each protein were monitored at 280 nm and the respective elution volumes (V_e) were determined. The void volume (V_o) of the column was determined using blue dextran.

The 100 μL sample of purified lipoxygenase from the Mono Q column was applied to the column in the same manner as the standard proteins were and 0.5 mL fractions were collected. The

elution profile of the protein was monitored at 280 nm. The fractions were kept on ice, assayed for lipoxygenase activity, and the elution volume was determined. Molecular weight of the enzyme was estimated from its point of elution using a plot of V_e/V_o versus logarithm of molecular weight of standard proteins in a manner similar to that described by Whitaker (1963) for conventional gel filtration.

Isoelectric point determination

The isoelectric point (pI) of purified sweet corn germ lipoxygenase was estimated by FPLC chromatofocusing on a prepacked Mono P column using Polybuffer 74 in a pH range of 3.8-6.3. The purified lipoxygenase from the Mono Q column was dissolved in 25 mM histidine-HCl, pH 6.3. Two mL of the sample were loaded onto the equilibrated Mono P column and protein fractions were eluted with Polybuffer 74, pH 3.8, using a flow rate of 1 mL/min. Each 1 mL fraction from the Mono P column was collected, the pH immediately measured, and the fractions with a pH below 5.6 were adjusted to approximately pH 7 with 0.1 N NaOH. The elution profile of the protein was monitored at 280 nm, all fractions were kept on ice and the lipoxygenase activity was determined. Isoelectric point of the enzyme was estimated by its pH of elution which is closed to its pI.

Inhibition by phenolic antioxidants

The phenolic antioxidants BHA, BHT, chlorogenic acid and quercetin were dissolved in absolute ethanol to a concentration of 20 mM. The effect of each antioxidant on the formation of conjugated dienes by lipoxygenase was determined by adding 30 µL of each antioxidant stock solution to 2.96 ml of substrate solution in the cuvette. Ten µL of purified enzyme solution were then added and the change of absorbance at 234 nm (25°C) was monitored as described above. The final volume of the reaction was 3 mL and the concentration of antioxidant was 0.2 mM. A control was performed by adding 30 µL of absolute ethanol instead of the antioxidant stock solution. Percent lipoxygenase inhibition was calculated by comparing units of lipoxygenase activity in the presence of antioxidants relative to that of the control.

Thermal inactivation

The 40-60% ammonium sulfate precipitate was re-dissolved in a 0.2 M sodium phosphate buffer, pH 7.0 and desalted using a prepacked PD-10 column. The ammonium sulfate preparation was used for the thermal inactivation study. Fifty µL aliquots of the sample were placed in borosilicate tubes (OD. 3 mm) capped with Teflon lined caps. The tubes were centrifuged for 30 seconds at 12,000 x g using a microcentrifuge (Microspin 12, Sorvall Instruments, The Du Pont Co., Wilmington, DE) in order to allow the

enzyme solution to fill the bottom of the tubes. Tubes were heated in a circulating water bath at 55, 60, 65 and 70°C for a specified time, immediately cooled in ice water, then centrifuged for 30 seconds and assayed for lipoxygenase activity.

Lipoxygenase activity assay

Lipoxygenase activity was determined spectrophotometrically by monitoring the formation of conjugated dienes at 25°C as described by Theerakulkait and Barrett (manuscript in prep.). One unit of enzyme activity is defined as an increase in absorbance of 0.001 at 234 nm per minute under the assay conditions. The molar extinction coefficient (ε_m) for the conjugated diene of linoleic acid was 23,000 M^{-1} cm^{-1} (Gibian and Vandenberg, 1987). A double beam spectrophotometer (Shimadzu, UV-160, Shimadzu Corporation, Kyoto, Japan) and 1 cm path length cuvette were used.

Protein determination

Protein was determined by the dye-binding method (Bradford, 1976) using crystalline bovine serum albumin (BSA) as a standard. The elution profile of the protein for conventional column chromatography was monitored by measuring absorbance at 280 nm using the double beam spectrophotometer and for Fast Protein Liquid Chromatography (FPLC) using a UV-M Monitor that could be observed via the computer monitor.

RESULTS & DISCUSSION

Preliminary experiments for lipoxygenase purification

In a previous study, the lipoxygenase in sweet corn germs was isolated as an acetone powder preparation, extracted with 0.2 M Tris-HCl, pH 8.0, fractionated with 40-60% ammonium sulfate, and dialyzed (Theerakulkait and Barrett, manuscript in prep.). The purification of lipoxygenase, however, was only 3 fold and this preparation was still contaminated with peroxidase activity. Therefore, its purity was not considered to be sufficient for use in the further study of its possible role in off-aroma formation in a sweet corn model system. Various conventional column chromatography systems such as gel filtration, ion exchange, hydrophobic interaction and chromatofocusing were studied in preliminary attempts to further purify the enzyme.

After testing various possible methods, one strategy for purification of sweet corn germ lipoxygenase utilizing conventional chromatography was developed in our laboratory. This system consisted of the following chromatographic steps: (1) gel filtration on Sepharose CL 6B; (2) anion exchange on DEAE-Sephacel; and further purification by (3) chromatofocusing on PBE-94.

Results indicated that lipoxygenase eluted at approximately 0.1 M NaCl and it could be separated from the majority of the peroxidase isozyme by anion exchange chromatography on DEAE-Sephacel column (data not shown). The major peroxidase isozyme passed

through the column before the salt gradient was applied. Moreover, by sequencing the chromatographic steps such that gel filtration preceded ion exchange, higher recovery and purification of lipoxygenase were achieved than using the opposite sequence. Salt in the ammonium sulfate preparation did not require dialysis for removal prior to application to the column. In addition, the pooled active fraction from the gel filtration step did not require concentration (ultrafiltration) before application to the anion exchange column. Therefore, the loss of activity experienced during the dialysis and ultrafiltration steps could be omitted and the time requirement for the procedure was reduced. The results of further purification of lipoxygenase using chromatofocusing on PBE-94 showed that lipoxygenase was found to elute at approximately pH 4.5 to 5.5 (data not shown).

Purification of sweet corn germ lipoxygenase

Based on the investigation of lipoxygenase behavior during the preliminary purification experiments and the desire to increase yield and purity while decreasing time required, a purification procedure for sweet corn germ lipoxygenase was developed using a combination of conventional column chromatography with FPLC (Fig. 4.1).

Gel filtration on Sephacryl S-300 HR: Gel filtration on a Sephacryl S-300 HR column was used to remove salt from the solubilized

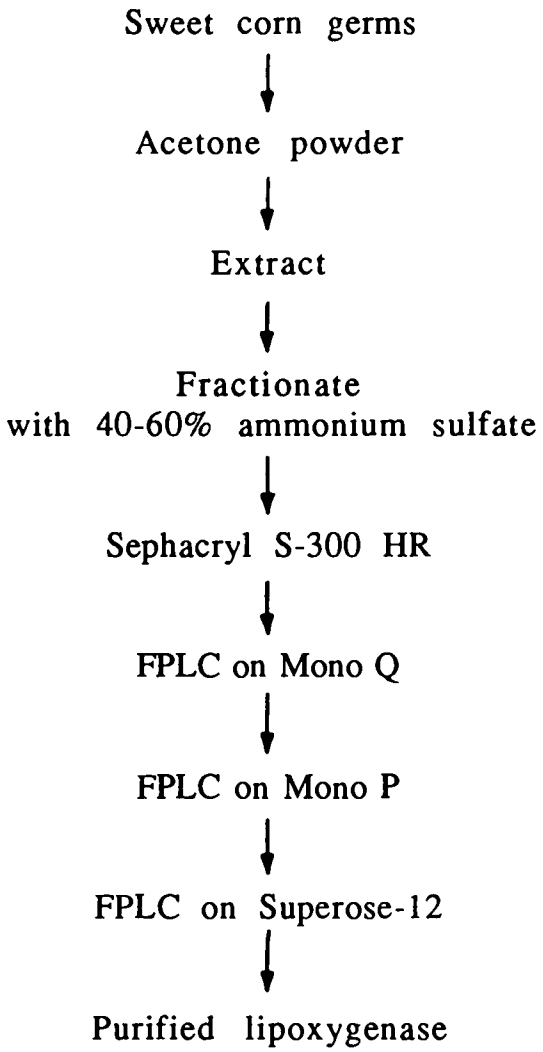

Fig. 4.1- Flow sheet of purification of lipoxygenase (LPO) from sweet corn germs.

ammonium sulfate preparation as well as to fractionate the proteins by size. One single peak of lipoxygenase activity was obtained using Sephacryl S-300 HR column, and most of the proteins in the solubilized 40-60% ammonium sulfate precipitate separated from lipoxygenase were of higher molecular weight (Fig. 4.2). The purification and percent recovery of lipoxygenase from this step were 5.4 fold and 48.9%, respectively (Table 4.1).

Other investigators have used conventional gel filtration chromatography as a means to purify corn lipoxygenase. Belefant and Fong (1991) partially purified lipoxygenase from commercially available sweet corn germs by precipitation with 20-50% ammonium sulfate and dialysis, however the degree of purification and percent recovery of the enzyme were not given. Velasco et al. (1989) partially purified lipoxygenase from sweet corn kernels by 10-50% ammonium sulfate fractional precipitation, and dialysis, followed by conventional size exclusion chromatography on a Ultrogel AcA34 column. Veldink et al. (1972) partially purified lipoxygenase from mature maize germs with 30-60% ammonium sulfate fractional precipitation, followed by conventional size exclusion chromatography on a Sephadex-G 100 (Superfine) column. Purification fold and percent recovery of lipoxygenase activity were not reported by the investigators.

Various size exclusion chromatographic techniques have also been used for the step subsequent to ammonium sulfate precipitation in other plants. For example, Sanz et al. (1992a) purified lipoxygenase from chickpeas using Sephacryl S-300

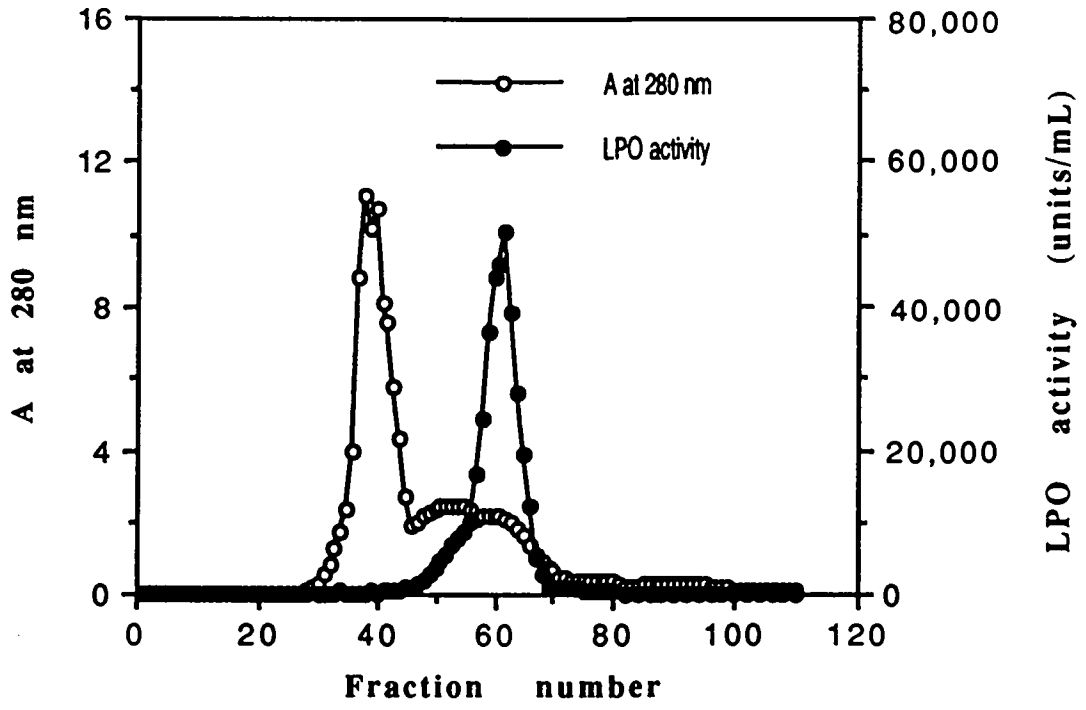

Fig 4.2- Sephacryl S-300 HR chromatographic purification of sweet corn germ lipoxygenase from 40-60% ammonium sulfate preparation.

Table 4.1- Purification of lipoxygenase (LPO) from sweet corn germs[a]

Step in Purification	Total activity (units/g AP)	Specific activity (units/mg protein)	% Recovery[b]	Purification fold[c]
Acetone Powder (AP) extract	164,038	3,764	100	1
40% Ammonium sulfate supernatant	114,583	5,914	69.9	1.6
40-60% Ammonium sulfate precipitate	111,875	10,805	68.2	2.9
Gel filtration (S-300HR)	80,163	20,304	48.9	5.4
Anion Exchange, FPLC (Mono Q)	43,166	466,047	26.3	124
Chromatofocusing, FPLC (Mono P)[d]	27,626	1,642,105	16.8	436

[a] Based on purification from 6 g acetone powder (values are means of 3 replications)

[b] Relative to lipoxygenase activity in AP extract

[c] Based on specific activity of AP extract

[d] Based on purification of pooled LPO active fraction from Mono Q (approx. 2.5 g acetone powder, 1 replication)

subsequent to 40-65% ammonium sulfate fractionation. However, purification fold and percent recovery at this step were not reported. Hidaka et al. (1986) used Sephadex G-100 after 25-70% ammonium sulfate fractionation for purification of lipoxygenase from pumpkin. The degree of purification and percent recovery of the enzyme at this step were found to be 5.7 fold and 35%, respectively.

FPLC anion exchange on Mono Q: Preliminary experiments indicated that conventional anion exchange chromatography on a DEAE-Sephacel column successfully separated lipoxygenase from the major peroxidase isozyme. Moreover, most of previous work attempting to purify lipoxygenase from other plants often included anion exchange chromatography in the purification scheme; for instance, DEAE-cellulose for immature English peas (Chen and Whitaker, 1986), DEAE-Trisacryl for mature maize (Poca et al., 1990), DEAE-Sepharose CL-6B for wheat germ (Shiiba, 1991), DEAE-cellulose for chickpea (Sanz et al., 1992a) and DEAE-Sepharose for germinating barley (Doderer, 1992). Using anion exchange could separate lipoxygenase from peroxidase and also provides other advantages including: unrestricted sample volume, considered as concentrating step, high capacity and resolution.

In order to obtain sufficient quantities of pure, active enzyme in a reasonable amount of time, the purification was improved by using FPLC anion exchange on Mono Q instead of DEAE-Sephacel after gel filtration. In contrast to DEAE-Sephacel, which is a weak anion exchange matrix, Mono Q is a strong anion exchanger that remains

fully ionized over the entire pH range from 2 to 12 and binds negatively charged components through quaternary amine groups. The very narrow particle size distribution of Mono Q resin results in low back pressure, allowing a short elution time and higher resolution. The chromatogram shows that one peak of lipoxygenase activity was eluted at 0.09 M NaCl on the FPLC Mono Q column (Fig. 4.3). This elution point was very close to that found during preliminary purification of lipoxygenase on DEAE-Sephacel anion exchange chromatography where lipoxygenase was eluted at approximately 0.1 M NaCl. Chen and Whitaker (1986) used DEAE-cellulose anion exchange chromatography as one step of their purification scheme for lipoxygenase isozymes from immature English green peas. The major peak for their lipoxygenase isozyme was also eluted at 0.1 M NaCl.

The percent recovery and purification of pooled active lipoxygenase from the Mono Q column were 26.3% and 124 fold, respectively (Table 4.1). The use of FPLC on Mono Q resulted in considerably high recovery and resolution; served to concentrate the enzyme; and required relatively short amount of time and could be performed at room temperature.

The purity of sweet corn germ lipoxygenase after the Mono Q column was considered to be suitable for our objectives, which were to partially characterize and further study its involvement in off-aroma formation. It was free from peroxidase activity. Moreover, the stability and recovery of the enzyme were deemed adequate for these purposes at this stage of purification. When the amount of

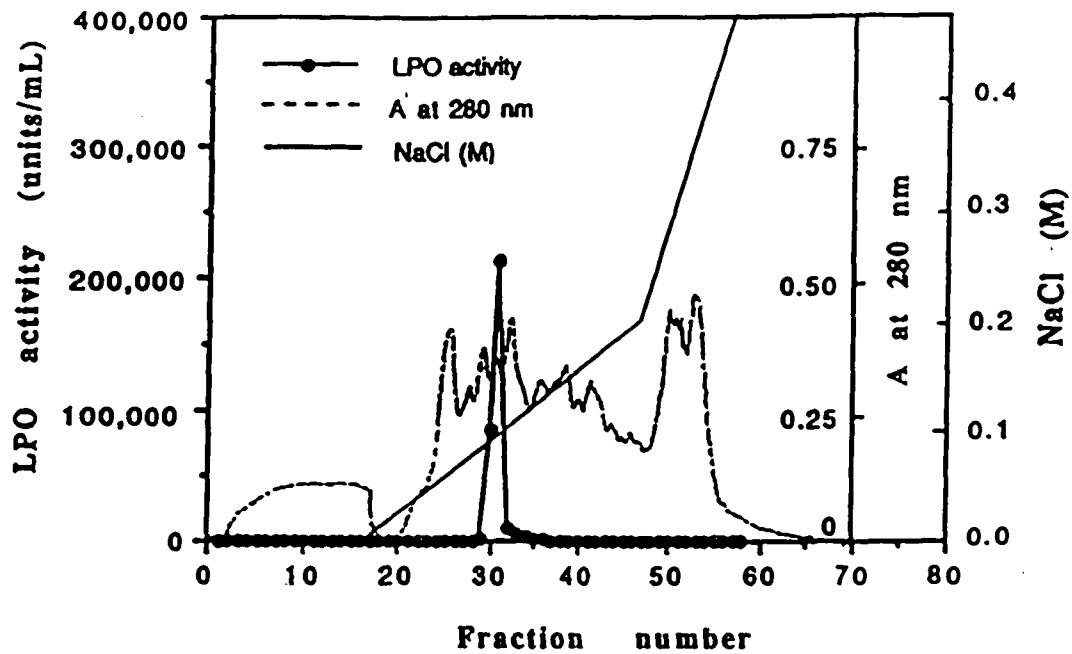

Fig. 4.3- FPLC (Mono Q) chromatographic purification of sweet corn germ lipoxygenase of active fraction pooled from Sephacryl S-300 HR

sample applied to the Mono Q column was reduced, the resolution was better. When only the most active fractions from the S-300 HR column were pooled and applied to Mono Q column, the purification fold increased but the percent recovery decreased.

To the best of our knowledge, our work represents the first time that FPLC on Mono Q has been used to purify lipoxygenase from sweet corn germs. Other than our study, the only published work was by Benefant and Fong (1991) who partially purified lipoxygenase from isolated sweet corn germs using only ammonium sulfate precipitation and dialysis. However, the variety of sweet corn used, percent recovery and purification fold for lipoxygenase were not reported by these researchers. FPLC on Mono Q has been used in purifying lipoxygenase in other plants such as soybeans (Kato et al., 1992; Shkarina et al., 1992) and kidney beans (Sanz et al., 1993).

FPLC on Mono P and Superose-12: If a higher degree of lipoxygenase purity is necessary for studies of amino acid composition, the enzyme may be further purified by FPLC chromatofocusing on Mono P, followed by FPLC size exclusion chromatography on Superose-12. The procedure for further purification using Mono P was developed as a result of the preliminary experiments with chromatofocusing on a PBE-94 column. Since the sample used for chromatofocusing requires a very low ionic strength, salt (NaCl) in the active fraction from DEAE-Sephacel had to be removed. In the conventional system on PBE-94, diafiltration was used to remove the salt and to exchange the buffer with starting buffer (25 mM imidazole-HCl, pH 7.4). This

procedure, however, seemed to result in the loss of a moderate amount of activity. In the FPLC system on Mono P, the prepacked disposable PD-10 column was used for desalting and buffer exchanging before application on the Mono P column. This resulted in a relatively small loss of lipoxygenase activity, the small volume of sample from the Mono Q column was sufficient for use with the PD-10 column and the time requirement was also relatively short.

Preliminary results from the PBE-94 elution profile indicated that lipoxygenase eluted in the pH range of 4.5 to 5.5 (data not shown). This information provided us with a guideline for selecting the appropriate pH range for separation on the Mono P column. A pH range between 4 and 6 was chosen rather than 4 and 7 since a narrower pH interval provided better resolution. The FPLC Mono P chromatogram shows that lipoxygenase eluted in a single peak at approximately pH 5 (Fig. 4.4). The active lipoxygenase fraction from the Mono P column was adjusted to approximately pH 7 with 0.1 N NaOH because the enzyme stability appeared to be better at this pH. The percent recovery and purification of pooled active lipoxygenase from the Mono P column were 16.8 % and 436 fold, respectively (Table 4.1).

FPLC size exclusion on a Superose-12 column was used to remove the residual Polybuffer 74 in the pooled active lipoxygenase fractions. One peak of lipoxygenase activity eluted on the FPLC Superose-12 column (Fig. 4.5), however, the stability of the purified lipoxygenase at this stage was much less than that which eluted from

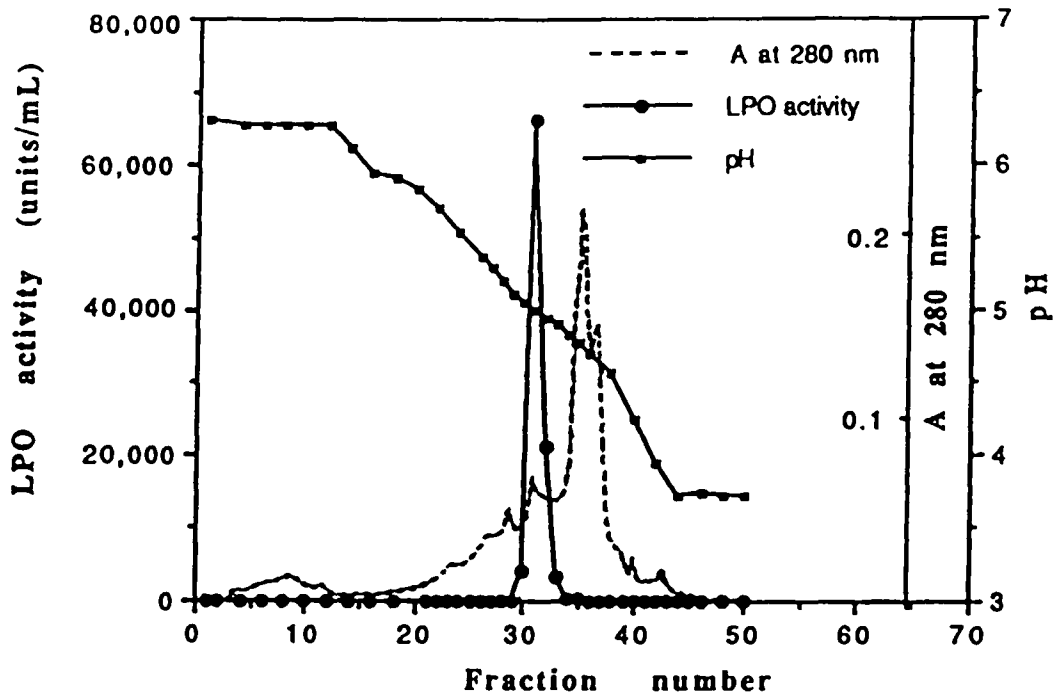

Fig. 4.4- FPLC (Mono P) chromatographic purification of sweet corn germ lipoxygenase of active fraction pooled from Mono Q

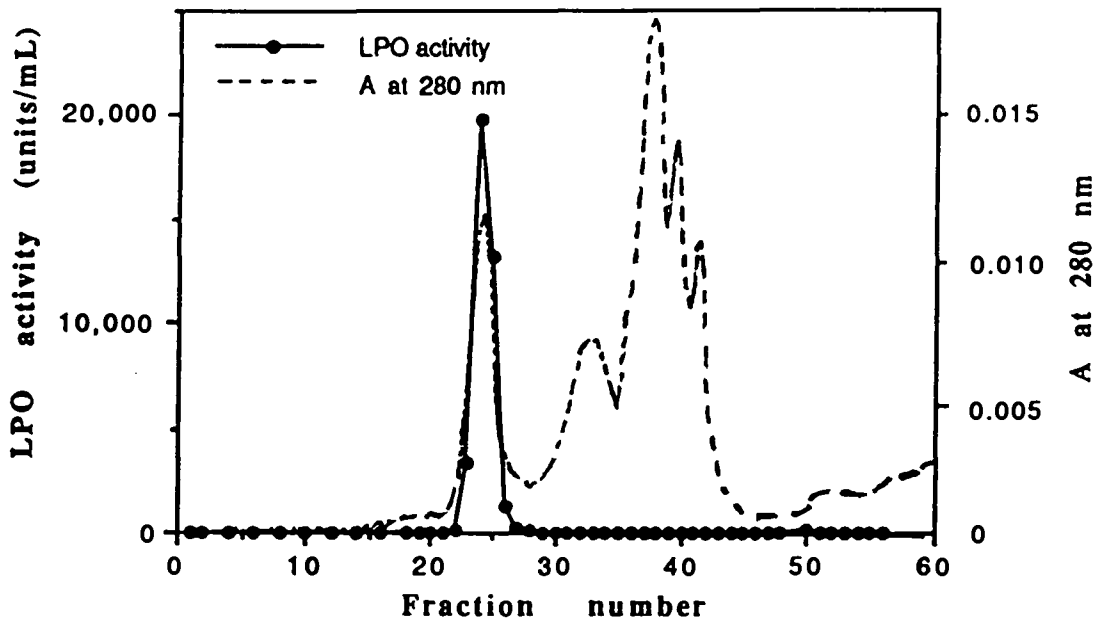

Fig. 4.5- FPLC (Superose-12) chromatographic purification of sweet corn germ lipoxygenase of active fraction pooled from Mono P

the Mono Q. This might be due to the removal of some proteins which may minimize the effect of deleterious contaminants and provide a stabilizing environment for the enzyme. Moreover, it may be due to a dilution effect. This is one reason that lipoxygenase which eluted from the Mono Q column will be used for partial characterization and for investigation of its involvement in off-aroma formation in the future.

The summary scheme for purification of lipoxygenase from sweet corn germs is shown in Fig. 4.1. Lipoxygenase from the germs was isolated by acetone powder preparation; extraction with 0.2 M Tris-HCl, pH 8.0; and 40-60% ammonium sulfate fractionation. It was purified via gel filtration chromatography on Sephacryl S-300 HR and FPLC anion exchanger on Mono Q column, and further purification could be achieved by FPLC chromatofocusing on a Mono P column and size exclusion chromatography on Superose-12.

A single peak of lipoxygenase activity was seen during purification on size exclusion, anion exchange and chromatofocusing column chromatography (both conventional and FPLC system) indicating that possibly only one major lipoxygenase isozyme was in the germ of mature sweet corn (var. Jubilee) evaluated. Belefant and Fong (1991) suggested that only one isozyme of lipoxygenase with an optimum pH at 6.8 accounted for the majority of lipoxygenase found in the germs of inbred yellow dent corn. These researchers found, however, there were at least three lipoxygenase isozymes with distinct pH activity optimums in the whole kernel during kernel development. Poca et al. (1990) reported that mature maize seeds

after 5 days of germination contained at least two lipoxygenase isozymes, L1 and L2, and these had two distinct activity peaks during purification on a DEAE-Trisacryl column.

In general, past studies have shown that the number of activity peaks found in typical column chromatography was most likely to indicate the number of isozymes. For example, three soybean lipoxygenase isozymes were reported with three activity peaks on DEAE-A50 (Christopher et al., 1972); two lipoxygenase isozymes from cowpeas with two activity peaks on DEAE-Sephadex A-50 (Den and Mendoza, 1982); three major wheat germ lipoxygenase isozymes with three major activity peaks on CM-Sepharose CL-6B (Shiiba et al., 1991); and chickpeas contained two lipoxygenase isozymes which also showed two activity peaks on DEAE-Cellulose column (Sanz et al., 1992a).

Some characteristics of sweet corn germ lipoxygenase

Molecular weight determination

To determine the apparent molecular weight (MW) of lipoxygenase purified from sweet corn germs, an FPLC Superose-12 HR 10/30 column was calibrated with different standard protein molecular weight markers in the range between 12,400 to 443,000 (Fig. 4.6). The estimated apparent molecular weight of sweet corn germ lipoxygenase purified from Mono Q was 90,500 $\pm$ 350 (mean $\pm$

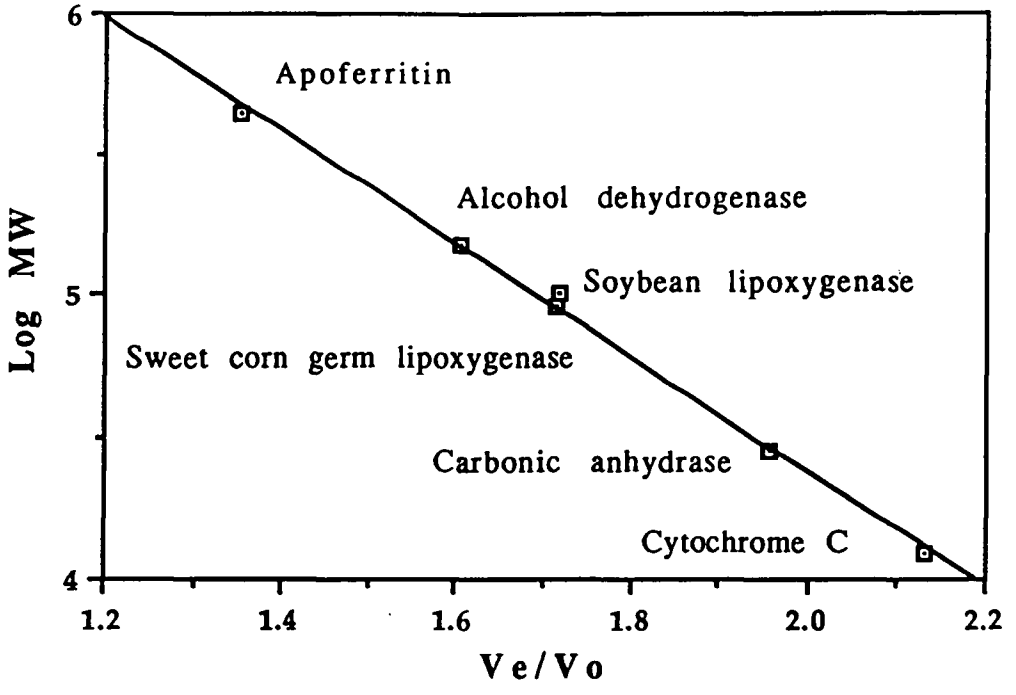

Fig. 4.6- Calibration curve of logarithm of molecular weight (MW) vs. Ve/Vo for estimation of the apparent MW of sweet corn germ lipoxygenase using FPLC on Superose-12 (Ve= elution volume, Vo=void volume)

SD), as determined from its point of elution using a plot of V_e/V_o versus logarithm of molecular weight of the standard proteins. The elution profile showed only a single peak of lipoxygenase activity on FPLC Superose-12.

The MW of lipoxygenase from sweet corn germs appeared to be close to that from germinating maize seeds which had a molecular weight of 100,000 for L1 and of 90,000 for L2 (Poca et al., 1990). This result was also close to that in other plants such as soybean lipoxygenase isozymes with MWs from 94,000 to 102,000 (Veldink et al., 1977; Shibata et al., 1987; Hildebrand et al., 1988; Shibata et al., 1988; Yenofsky et al., 1988); chickpea lipoxygenase L-1 and L-2 with a MW of 96,000 to 97,000 (Sanz et al., 1992b), cucumber cotyledon lipoxygenase L-1 and L-2 with MW 90,000 and 96,000 (Matsui et al., 1993); rice embryo lipoxygenase-3 with MW 89,000 or 93,000 (Ohta et al., 1986), ungerminated and germinating barley lipoxygenase with a MW of approximately 90,000 (van Aarle et al., 1991; Doderer et al., 1992), immature English pea lipoxygenase isozyme-1 with MW of 100,000 (Chen and Whitaker, 1986); and wheat germ lipoxygenase with a range of MW 90,000-95,000 (Nicholas et al., 1982).

However, the molecular weight of sweet corn germ lipoxygenase appeared to be higher than that of peanuts, pea seeds, cowpeas, and avocado which had molecular weights of 73,000, 74,000, 68,000 and 74,000 (L-1 and L-2), and 74,000, respectively (Haydar and Hadziyev, 1973; Sanders et al., 1975; Den and Mendoza, 1982; Marcus et al., 1988). Sweet corn germ lipoxygenase was lower

than that of flaxseed with a molecular weight of 130,000 (Rabinovitch-Chable et al., 1992). Determination of the molecular weight of the enzyme by different methods might result in some differences, for instance, soy bean lipoxygenase isozymes L1, L2, and L3 had a molecular weight of 94,000, 97,000, and 96,500, respectively, based on their amino acid sequence (Shibata et al., 1987; Shibata et al., 1988) but MW ranged from 100,000-102,000 when determined on gel filtration (Veldink et al., 1977).

Isoelectric point determination

The isoelectric point (pI) of purified sweet corn germ lipoxygenase was estimated by FPLC chromatofocusing on a Mono P column using Polybuffer 74 over a pH range from 3.8-6.3. Only one peak of lipoxygenase activity with a pI of 5.06 $\pm$ 0.06 was determined, as shown in Fig. 4.7 This pI was similar to that of chickpea lipoxygenase isozyme-1 with a pI of 4.92 (Sanz et al., 1992b); lipoxygenase isozyme-1 from germinating barley embryos with a pI of 4.9 (Yabuuchi, 1976), and lipoxygenase isozyme-3 from wheat germ with a pI of 5.1 (Nicholas et al., 1982). It was lower than the pIs of lipoxygenase isozymes L1, L2(a) and L2(b) from germinating maize seed reported by Poca et al. (1990) with pI values of 6.40, 5.55 and 5.70, respectively.

The pI was also lower than that found for lipoxygenase in some other plants, e.g. avocado, 5.9 (Marcus et al., 1988); soybeans, 5.7 to 6.4 (Hildebrand et al., 1988); wheat germ L1 and L2, 6.25 and 5.4

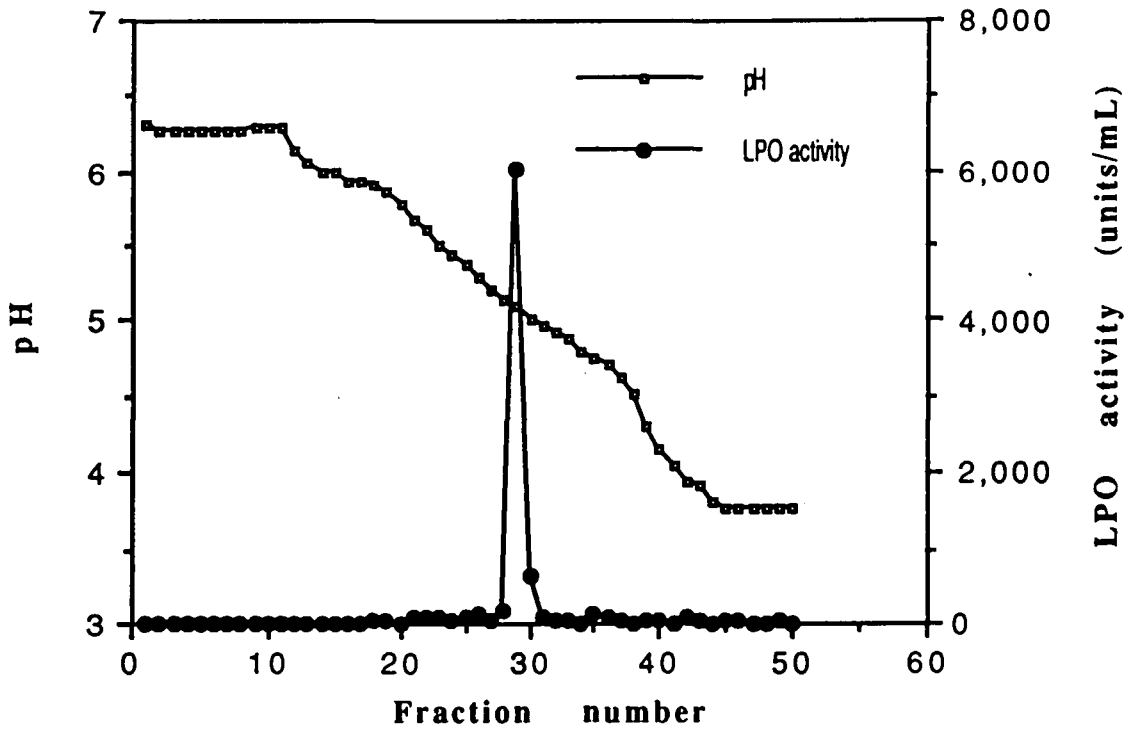

Fig. 4.7- FPLC (Mono P) chromatogram for estimation of an isoelectric point (pI) of sweet corn germ lipoxygenase.

(Nicholas et al., 1982); horse beans, 5.78 and 5.9 (Nicholas and Drapron, 1977); and germinating barley embryo L-2, 6.6 (Yabuuchi, 1976). However, the pI of lipoxygenase from sweet corn germ (5.06) was higher than the pI of type-1 lipoxygenase from dry English pea seeds, 4.05 (Reynolds and Klein, 1982); and lipoxygenase L-2 from chickpea, 4.74 (Sanz et al., 1992b).

The pI of lipoxygenase from sweet corn germs was less basic than that from some plants, perhaps due to more acidic amino acid residues and/or less basic amino acid residues in the molecule of the enzyme.

Inhibition by phenolic antioxidants

The inhibition of purified sweet corn germ lipoxygenase by 0.2 mM concentrations of various phenolic compounds (quercetin, chlorogenic acid, BHA, and BHT) is shown in Fig. 4.8. Lipoxygenase-catalyzed conjugated diene formation was inhibited in a range of 16.3 to 61.4 % by all these compounds. Quercetin and BHA inhibited conjugated diene formation more effectively than chlorogenic acid and BHT, with percentage inhibitions of 61.4, 57.2, 32.3 and 16.3%, respectively. Yasomoto et al. (1970) reported that quercetin and BHA were more effective at inhibition of the soybean lipoxygenase reaction than BHT. King and Klein (1987) found that, on an equimolar basis, BHA and quercetin inhibited the conjugated diene formation by soybean lipoxygenase more effectively than chlorogenic

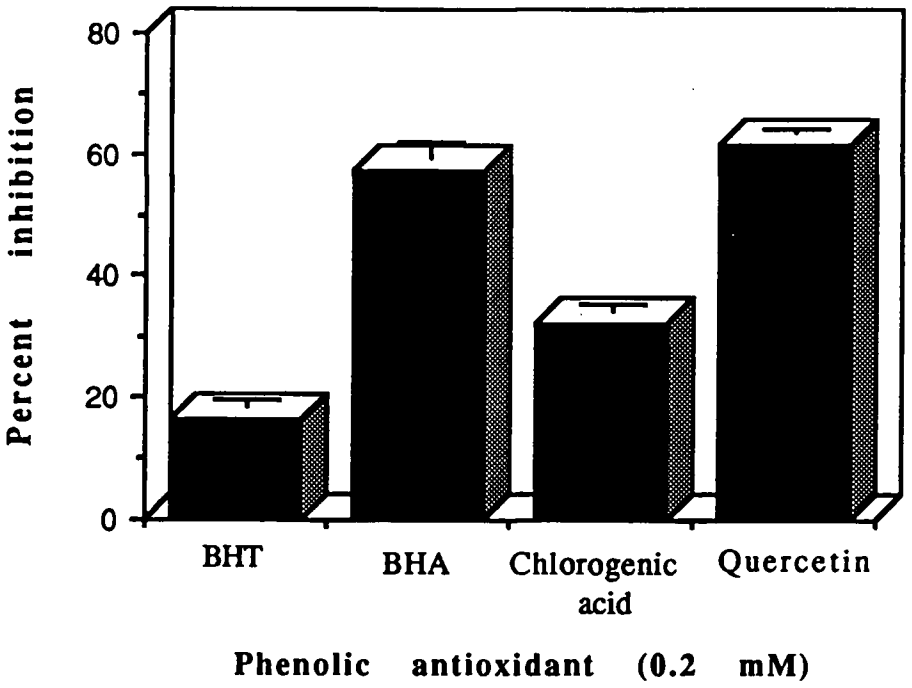

Fig. 4.8- Percent inhibition of sweet corn germ lipoxygenase activity by phenolic antioxidants. Data are means ± SD.

acid and BHT. BHA and quercetin were also found to be effective inhibitors of pea lipoxygenase (Rhee and Watts, 1966).

Of the two synthetic phenolic antioxidants tested, BHA was more effective than BHT in inhibiting lipoxygenase. BHA showed effective inhibitory effects on broad bean lipoxygenase-catalyzed linoleate oxidation (Al-Obaidy and Siddiqi, 1981). The inhibitory effect of BHT and BHA on lipoxygenase may result from breaking the free radical chain reaction through removal of either the linoleic acid radicals or the linoleic acid peroxide radicals in a manner similar to the non-enzymatic system described by Labuza (1971).

Of the two natural phenolic antioxidants, quercetin showed more of an inhibitory effect than chlorogenic acid on sweet corn germ lipoxygenase. Takahama (1985) suggested that quercetin inhibited soybean lipoxygenase-1-dependent linoleic acid peroxidation by reduction of the linoleic acid radical formed as an intermediate during lipoxygenation. Quercetin possibly acted as a primary antioxidant (chain breaking) as in the non-enzymatic systems (Letan, 1966). Quercetin has also been reported to have a stronger inhibitory effect than chlorogenic acid, an acidic phenolic compound, on the carotene bleaching activity of lipoxygenase (Oszmianski and Lee, 1990).

Quercetin is a polyhydroxylated flavonol with five free hydroxyl groups found naturally in plants such as green onion, green pepper pods and seeds, and potato peels. It has antioxidative activity (Pratt, 1965; Torel et al., 1986; Faure et al., 1990) with the highest inhibitory effects on soybean lipoxygenase activity when

compared to other isoflavonones from soybeans (Naim et al., 1976). However, chlorogenic acid, which has been found to be the most abundant phenolic acid in plant extract (Larson, 1988; Hayase and Kato, 1984) was also reported to have antioxidative activity in plants such as cacao bean husk and sweet potatoes (Naito et al., 1982; Hayase and Kato, 1984).

The results indicate that natural phenolic antioxidants, especially quercetin, showed an effective inhibitory effect on sweet corn germ lipoxygenase and were similar in potency to the synthetic antioxidant, BHA. The inhibition of lipoxygenase activity by natural phenolic antioxidants could be useful to the food industry for prevention of enzymatic lipid oxidation. The safety of synthetic antioxidants has been questioned (Haigh, 1986; Imida et al., 1983; Maeura et al., 1984) and some natural phenolic antioxidants, especially quercetin, have been found to be potent anticarcinogens in animals (Leighton et al. 1991; Verma, 1991; Weisburger, 1991) and may also inhibit the induction of human cancer (Verma, 1991; Yoshida et al., 1990).

Thermal inactivation

The thermal inactivation of partially purified lipoxygenase from sweet corn germs in the desalted 40-60% ammonium sulfate preparation was investigated in the range of 55 to 70°C. Activation energy (E_a) for the thermal inactivation of lipoxygenase was calculated from the Arrhenius plot (Fig. 4.9) and found to be 56.3 $\pm$

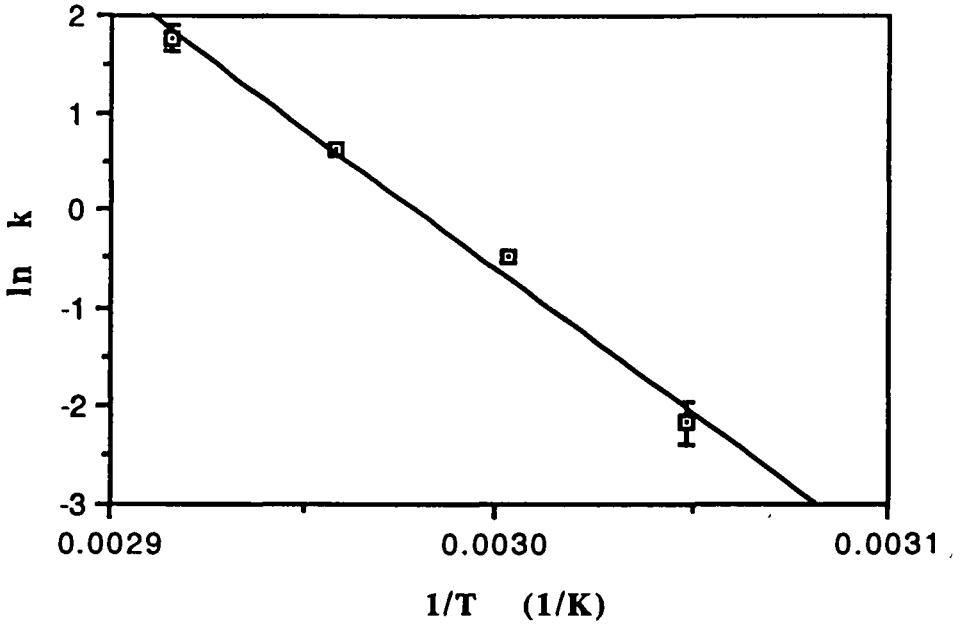

Fig. 4.9- Arrhenius plot of natural logarithm of inactivation rate (ln k) of sweet corn germ lipoxygenase in 40-60% ammonium sulfate preparation vs. the reciprocal of heating temperature (1/T).

6.6 kcal/mol. This was close to the E_a reported for inactivation of asparagus lipoxygenase in 50% ammonium sulfate and in CMC (Carboxymethylcellulose) purified preparations with values of 48.9 and 47.5 kcal/mol, respectively (Ganthavorn et al., 1991). The result was also in the range of 50-150 kcal/mol as suggested by Whitaker (1972) for the activation energies required for denaturation of enzymes.

Park et al. (1988) reported E_a for inactivation of two potato lipoxygenase isozymes were 40.8 and 46.5 kcal/mol. The E_a of lipoxygenase from sweet corn germ appears to be lower than that for soybeans and peas with reported values of 100 and 140 kcal/mol, respectively (Svensson and Eriksson, 1974; Schwimmer, 1981). The E_a was higher than that for lipoxygenase from immature English peas which had a value of 24.6 kcal/mol (Chen and Whitaker, 1986). This suggests that lipoxygenase from different sources might have different thermal stabilities. However, factors such as techniques of heating and assaying the enzymes, criteria for inactivation, enzyme concentration, impurities such as other proteins, substrates of the enzyme, and reaction products may also affect the thermal inactivation of enzymes (McConnell, 1956; Farkas and Goldsmith, 1962; Svensson and Eriksson, 1972 and 1974; Alsoe and Alder-Nissen, 1988; Ganthavorn and Powers, 1989).

CONCLUSIONS

Lipoxygenase was purified for the first time from the germs of sweet corn using a combination of conventional column chromatography and FPLC. This purification method would be useful for purifying lipoxygenase from other plant germs in order to investigate other characteristics such as the role of lipoxygenase in off-aroma formation. Understanding the characteristics of the enzyme would be helpful for food scientists and technologists to explain and control the phenomena related to quality changes of sweet corn caused by the enzyme. The results indicate that there may be only one major isozyme of lipoxygenase present in the germs of sweet corn (var. Jubilee) at the maturity selected for freezing. Lipoxygenase activity was effectively inhibited by both synthetic and natural phenolic antioxidants, in particular, BHA and quercetin. Therefore, the blanching heat treatments might be reduced by combining the heat treatment with the addition of a phenolic antioxidant, in particular a natural phenolic antioxidant.

ACKNOWLEDGMENTS

Author Theerakulkait would like to express sincere gratitude to the International Rotary Foundation for providing him with a Rotary Freedom From Hunger scholarship for 3 years. He also would like to express sincerely appreciation to the committee on Grants-in-Aid of Research, Sigma Xi, The Scientific Research Society for awarding him a grant in partial support of the research. Authors would like to thank the National Frozen Food Co., Albany, Oregon, for contribution and support in providing the sweet corn samples for the research, and Agricultural Research Foundation (Oregon Processed Vegetable Commission) for some financial support.

The authors also would like to thank very much the following persons: Dr. Reg McParland, Central Service Laboratory for Gene Research and Biotechnology, Oregon State University, Corvallis, OR; Dr. Dean Malencik at Dr. Sonia Anderson's laboratory, Dept. of Biochem. & Biophys., Oregon State University, Corvallis, OR; Dr. John Whitaker, Dept. of Food Sci. & Tech, U. of California at Davis, Davis, CA for their contributions, suggestions and support.

REFERENCES

Al-obaidy, H.M. and Siddiqi, A.M. 1981. Inhibition of broad bean lipoxygenase. J. Food Sci. 46: 597-605.

Alsoe, K.O. and Alder-Nissen, J. 1988. Thermal denaturation of lipoxygenase from soya beans in the presence of its natural substrate. Lebensm.-Wiss. u. Technol. 21: 8-12.

Belefant, H. and Fong, F. 1991. Lipoxygenases in developing *Zea mays* kernels. Plant Physiol. Biochem. 29(2): 99-104.

Bradford, M.N. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72: 248-254.

Chen, A.O. and Whitaker, J.R. 1986. Purification and characterization of a lipoxygenase from immature English peas. J. Agric. Food. Chem. 34: 203-211.

Christopher, J.P., Pistorius, E.K., and Axelrod, B. 1972. Isolation of a third isozyme of soybean lipoxygenase. Biochim. Biophys. Acta. 284: 54-62.

Den, T.V. and Mendoza, E.M.T. 1982. Purification and characterization of two lipoxygenase isozymes from cowpea [*Vigna unguiculata* (L.) Walp.]. J. Agric. Food Chem. 30: 54-60.

Doderer, A., Kokkelink, I.K., van der Veen, S., Valk, B.E., Schram, A. W., and Douma, A.C. 1992. Purification and characterization of two lipoxygenase isozymes from germinating barley. Biochim. Biophys. Acta. 1120: 97-104.

Farkas, D.F. and Goldsmith, S.A. 1962. Studies on the kinetics of lipoxidase inactivation using thermal and ionizing energy. J. Food Sci. 27: 262-276.

Faure, M., Lissi, E., Torres, R., and Videla, L.A. 1990. Antioxidant activities of lignans and flavonoids. Phytochemistry 29(12): 3773-3775.

Ganthavorn, C., Nagel, C.W. and Powers, J.R. 1991. Thermal inactivation of asparagus lipoxygenase and peroxidase. J. Food Sci. 56(1): 47-49, and 79.

Ganthavorn, G. and Powers, J.R. 1989. Partial purification and characterization of Asparagus Lipoxygenase. J. Food Sci. 52: 371-373.

Gibian, M.J. and Vandenberg, P. 1987. Product yield in oxygenation of linoleate by soybean lipoxygenase: the value of the molar extinction coefficient in spectrophotometric assay. Anal. Biochem. 163: 343-349.

Haigh, R. 1986. Safety and necessity of antioxidants: EEC approach. Food Chem. Toxicol. 24: 1031-1034.

Hayase, F. and Kato, H. 1984. Antioxidative components of sweet potatoes. J. Nutr. Sci. Vitaminol. 30: 37-46.

Haydar, M. and Hadziyev, D. 1973. A study of lipoxidase in pea seeds and seedlings. J. Sci. Food. Agric. 24: 1039-1053.

Hidaka, T., Katsuki, S. and Nagata, Y. 1986. Partial purification and properties of pumpkin lipoxygenase with carotene-bleaching activity. J. Food Biochem. 10: 55-73.

Hildebrand, D.F., Hamilton-Kemp, T.R., Legg, C.S. and Bookjans, G. 1988. Plant lipoxygenase: occurrence, properties and possible functions. Curr. Top. Plant Biochem. Physiol. 7: 201-219.

Imida, K., Fukushima, S., Shivaiet, T., Ohtani, M., Nakanishi, K., and Ito, N. 1983. Promoting activities of butylated hydroxyanide and butylated hydroxytoluene on 2-stages urinary bladder carcinogenesis and inhibition of γ-glutamyl transpeptidase-positive foci development in the liver of rats. Carcinogenesis. 4: 895.

Kato, T., Ohta, H., Tanaka, K. and Shibata, D. 1992. Appearance of new lipoxygenases in soybean cotyledons after germination and evidence for expression of a major new lipoxygenase gene. Plant Physiol. 98: 324-330.

King, D.L. and Klein, B.P. 1987. Effect of flavonoids and related compounds on soybean lipoxygenase-1 activity. J. Food Sci. 52(1): 220-221.

Labuza, T.P. 1971. Kinetics of lipid oxidation in foods. CRC Crit. Rev. Food Technol. 2: 355-405.

Larson, R.A. 1988. The antioxidants of higher plants. Phytochemistry 27(4): 969-978.

Lee, Y.C. 1981. Lipoxygenase and off-flavor Development in some frozen foods. Korean J. Food Sci. Technol. 13: 53-56.

Leighton, T., Ginther, C., Nortario, V., Harter, W. 1991. Quercetin and quercetin glycosides: genetic regulation, metabolic processing, and *in vitro* effects on oncogenic transformation. Presented at the 202nd National Meeting of the American Chemical Society, New York, NY, Symposium on Phenolic Compounds in Foods and Health, AGFD Abstract No. 116.

Letan, A. 1966. The relation of structure to antioxidant activity of quercetin and some of its derivatives. I. primary activity. J. Food Sci. 31: 518-523.

Maeura, Y., Weisburger, J. H., and Williams, G. 1984. Dose-dependent reduction of N-2-fluorenylacetamide-induced liver cancer and enhancement of bladder cancer in rats by butylated hydroxytoluene. Cancer Res. 44: 1604-1610.

Marcus, L., Prusky, D. and Jacoby, B. 1988. Purification and characterization of avocado lipoxygenase. Phytochemistry 27(2): 323-327.

Matsui K., Irie, M., Kajiwara, T., Kakuno, T. and Hatanaka, A. 1993. Rapid degradation of cucumber cotyledon lipoxygenase. Phytochemistry 32(6): 1387-1391.

McConnell, J.E.W. 1956. Enzyme-heat resistance and regeneration: review of literature on thermal inactivation of enzymes in foods. National Canners Association. 1-12.

Naim, M., Gestetner, B., Bondi, A. and Birk, Y. 1976. Antioxidative and antihemolytic activities of soybean isoflavones. J. Agric. Food Chem. 24: 1174-1176.

Naito, S., Yamaguchi, N. and Yokoo, Y. 1982. Fractionation of antioxidants from cacao bean husk (studies on natural antioxidant. part IV). J. Jpn. Soc. Food. Sci. Technol. 29: 529-533.

Nicholas, J., Autran, M., and Drapron, R. 1982. Purification and some properties of wheat germ lipoxygenase. J. Sci. Food Agric. 33: 53-63.

Nicholas, J. and Drapron, R. 1977. Some physicochemical properties of horse bean (*Vicia faba* L.). Ann. Technol. Agric. 26: 119-132.

Ohta, H, Ida, S., Mikami, B. and Morita, Y. 1986. Purification and characterization of rice lipoxygenase component 3 from embryo. Agric. Biol. Chem. 50(12): 3165-3171.

Oszmianski, J. and Lee, C.Y. 1990. Inhibitory effect of phenolics on carotene bleaching in vegetables. J. Agric. Food Chem. 38: 688-690.

Park, K-H., Kim, Y-M. and Lee, C-W. 1988. Thermal inactivation kinetics of potato tuber lipoxygenase. J. Agric. Food Chem. 36(5): 1012-1015.

Poca, E., Rabinovitch-Chable, H., Cook-Moreau, J., Montserrat, P., and Rigaud, M. 1990. Lipoxygenases from *Zea mays* L. purification and physicochemical characteristics. Biochim. Biophys. Acta. 1045: 107-114.

Pratt, D.E. 1965. Lipid antioxidants in plant tissue. J. Food Sci. 30: 737-741.

Rabinovitch-Chable, H., Cook-Moreau, J. Breton, J-C. and Rigaud, M. 1992. Purification of lipoxygenase and hydroperoxide dehydrase in flaxseeds: interaction between these enzymatic activities. Biochem. Biophys. Res. Commun. 188(2): 858-864.

Reynolds P.A. and Klein, B.P. 1982. Purification and characterization of a type-1 lipoxygenase from pea seeds. J. Agric. Food Chem. 30: 1157-1163.

Rhee, K.S. and Watts, B.M. 1966. Effect of antioxidants on lipoxygenase activity in model systems and pea (*Pisum sativum*) slurries. J. Food Sci. 31: 699-674.

Sanders, T.H., Pattee, H.E. and Singleton, J.A. 1975. Lipoxygenase isozymes of peanut. Lipids. 10(11): 681-685.

Sanz, L.C., Perez, A.G. and Olias, J.M. 1992a. Purification and catalytic properties of chickpea lipoxygenases. Phytochemistry 31(9): 2967-2972.

Sanz, L.C., Perez, A.G., Rios, J.J. and Olias, J.M. 1992b. Physico-chemical properties of chickpea lipoxygenase. Phytochemistry 31(10): 3381-3384.

Sanz, L.C., Perez, A.G., Rios, J.J. and Olias, J.M. 1993. Positional specificity of ketodienes from linoleic acid aerobically formed by lipoxygenase isozymes from kidney bean and pea. J. Agric. Food Chem. 41(5): 696-699.

Schwimmer, S. 1981. Source Book of Food Enzymology. AVI Publishing Co., Inc., Westport, CT.

Sheu, S.C. and Chen, A.O. 1991. Lipoxygenase as blanching index for frozen vegetable soybeans. J. Food Sci. 56(2): 448-451.

Shibata, D., Steczko, J., Dixon, J.E., Andrews, P.C., Hermodson, M., and Axelrod, B. 1988. Primary structure of soybean lipoxygenase L-2. J. Biol. Chem. 263: 6816-6821.

Shibata, D., Steczko, J., Dixon, J.E., Hermodson, M., Yazdanparast, R. and Axelrod, B. 1987. Primary structure of soybean lipoxygenase L-1. J. Biol. Chem. 262: 10080-10085.

Shiiba, K., Negishi, Y., Okada, K. and Nagao, S. 1991. Purification and characterization of lipoxygenase isozymes from wheat germ. Cereal Chem. 68(2): 115-122.

Shkarina, T.N., Kuhn, H. and Schewe, T. 1992. Specificity of soybean lipoxygenase-1 in hydrated reverse micelles of sodium bis (2-ethylhexyl) sulfosuccinate (areosol OT). Lipids. 27(9): 690-693.

Svensson, S.G. and Eriksson, C.E. 1972. Thermal inactivation of lipoxygenase from peas (*Pisum sativum* L.) II. influence of substrate and reaction products. Lebensm.-Wiss. u. Technol. 5(4): 124-127.

Svensson, S.G. and Eriksson, C.E. 1974. Thermal inactivation of lipoxygenase from peas (*Pisum sativum* L.) III. activation energy obtained from single heat treatment experiments. Lebensm.-Wiss. u. Technol. 7: 142-144.

Takahama, U. 1985. Inhibition of lipoxygenase-dependent lipid peroxidation by quercetin: mechanism of antioxidative function. Phytochemistry 24(7): 1443-1446.

Theerakulkait, C. and Barrett, D.M. Lipoxygenase in sweet corn germ: isolation and some physicochemical properties. In preparation for J. Food Sci.

Torel, J., Cillard, J. and Cillard, P. 1986. Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry 25(2): 383-385.

van Aarle, P.G.M., de Barse, M.M.J., Veldink, G.A. and Vliegenthart, F.G. 1991. Purification of lipoxygenase from ungerminated barley: characterization and product formation. FEBS Lett. 280 (1): 159-162.

Velasco, P.J., Lim, M.H., Pangborn, R.M. and Whitaker, J.R. 1989. Enzymes responsible for off-flavor and off-aroma in blanched and frozen stored vegetables. Appl. Biochem. Biotechnol. 11: 118-127.

Veldink, G.A., Garssen, G.J., Vliegenthart, J.F.G., and Boldingh, J. 1972. Positional specificity of corn germ lipoxygenase as a function of pH. Biochem. Biophys. Res. Commun. 47(1): 22-26.

Veldink, G.A., Vliegenthart, J.F.G. and Boldingh, J. 1977. Plant lipoxygenase. Prog. Chem. Fats Other Lipids. 15: 131-166.

Verma, A.K. 1991. Anticarcinogenicity of the flavonol quercetin. Presented at the 202nd National Meeting of the American Chemical Society, New York, NY, Symposium on Phenolic Compounds in Foods and Health, AGFD Abstract No. 113.

Wagenknecht, A.C. 1959. Lipoxidase activity and off-flavor in underblanched frozen corn-on-the-cob. Food Res. 24: 539-547.

Weisburger, J.H. 1991. Mechanisms underlying the mutagenic, carcinogenic, and chemopreventive effects of phenols and catechols.Presented at the 202nd National Meeting of the American Chemical Society, New York, NY, Symposium on Phenolic Compounds in Foods and Health, AGFD Abstract No. 86.

Whitaker, J.R. 1963. Determination of molecular weights of proteins by gel filtration on Sephadex. Anal. Chem. 35: 1950-1953.

Whitaker, J.R. 1972. Effect of temperature on enzyme-catalyzed reaction. Ch. 11, In *Principles of enzymology for the food sciences,* J.R. Whitaker (ed.), 319-349, Marcel Dekker, New York.

Williams, D.C., Lim, M.H., Chen, A.O., Pangborn, R.M., and Whitaker, J.R. 1986. Blanching of vegetables for freezing - which indicator enzyme to choose. Food Technol. 40(6): 130-140.

Yabuuchi, S. 1976. Occurrence of a new lipoxygenase isozyme in germinating barley embryos. Agric. Biol. Chem. 40: 1987-1992.

Yasumoto, K., Yamamoto, A. and Mitsuda, H. 1970. Effect of phenolic antioxidants on lipoxygenase reaction. Agric. Biol. Chem. 34: 1162-1168.

Yenofsky, R.L., Fine, M., and Lui, C. 1988. Isolation and characterization of a soybean (*Glycine max*) lipoxygenase-3 gene. Mol. Gen. Genet. 211: 215-222.

Yoshida, M., Sakai, T., Hosokawa, N., Marui, N., Matsumoto, K., Fujioka, A., Nishino, H. and Aoike, A. 1990. The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. FEBS Lett. 260(1): 10-13.

CHAPTER 5:
SWEET CORN GERM ENZYMES INVOLVED IN OFF-AROMA FORMATION

Chockchai Theerakulkait, Diane M. Barrett*, and Mina R. McDaniel

Department of Food Science and Technology,
Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602
and
*Department of Food Science and Technology,
Cruess Hall, University of California, Davis, CA 95616-8598

ABSTRACT

The aroma profile of a homogenate of frozen stored unblanched corn on the cob was determined by descriptive analysis. Mean overall intensity, and most descriptors describing undesirable characteristics, were significantly higher than that of a blanched control sample. To investigate the involvement of sweet corn germ enzymes in off-aroma formation, crude enzyme extract, purified lipoxygenase (LPO), and peroxidase (POD) were prepared from corn germs and added back to the homogenate of blanched sweet corn. The samples, including the homogenate of blanched and unblanched samples were incubated, heated, and evaluated by the trained descriptive panel. Adding lipoxygenase significantly increased "painty" and "stale/oxidized" off-aroma descriptors and significantly lowered "sweet" and "corn" aromas. Other enzymes in the germs may possibly be involved in formation of other off-aromas, particularly "cooked cabbage". There was evidence suggesting that peroxidase in sweet corn germs may not be important in off-aroma formation. Lipoxygenase seems to be more important and appropriate than POD for use as a blanching index.

Key Words: sweet corn, germ, enzymes, lipoxygenase, peroxidase, off-aroma.

INTRODUCTION

Off-flavor and off-aroma are the main causes of quality deterioration in sweet corn after harvesting and during frozen storage (Smittle et al., 1972; Wagenknecht, 1959; Lee, 1981; Velasco et al., 1989). The cause of off-aroma formation in frozen stored raw or underblanched vegetables, including sweet corn, is believed to be the result of enzymatic action during frozen storage (Joslyn, 1949; Wagenknecht, 1959; Lee, 1981; Williams et al., 1986; Ganthavorn and Powers, 1989; Velasco et al., 1989; Sheu and Chen, 1991).

Of all sweet corn enzymes, lipoxygenase (LPO) has been most often suggested as the cause of off-flavor development in raw and underblanched sweet corn (Wagenknecht, 1959; Lee, 1981; Velasco et al., 1989), particularly in the fraction containing the germ (Wagenknecht, 1959; Lee, 1981). In contrast, there is no evidence that peroxidase (POD), which has long been used as a blanching index for sweet corn and other vegetables prior to freezing, is directly associated with off-flavor or other types of quality deterioration in plant materials (Morris, 1958; Burnette, 1977; Williams, et al., 1986; Lim et al., 1989; Sheu and Chen, 1991). Velasco et at. (1989) separated POD, LPO and catalase from whole sweet corn kernels and studied their responsibility in off-aroma formation in sweet corn. They were unable to clarify, however, which enzyme(s) played the key role in off-aroma formation in sweet corn. Moreover, the involvement of enzymes isolated from the germ tissue, particularly LPO, in off-aroma formation has not been studied.

The main objective of this work was to investigate the involvement of enzymes from isolated germs of sweet corn, especially LPO, in off-aroma formation using descriptive analysis. The aroma profile of homogenate prepared from unblanched frozen stored corn on the cob was studied, and the possible role of POD from sweet corn germs in off-aroma formation was also investigated.

MATERIALS & METHODS

Materials

Freshly harvested sweet corn (*Zea mays* L. var. Jubilee) was obtained from the National Frozen Food Co., Albany, Oregon. The corn was immediately transported to the pilot plant of the Dept. of Food Science and Technology, Oregon State University, Corvallis, Oregon. The fresh sweet corn was dehusked by hand, and randomly separated into 4 portions: (1) unblanched intact kernels: the corn was frozen immediately in liquid nitrogen and the intact kernels were removed from the cob by hand and stored at -35°C, (2) blanched intact kernels: the corn on the cob was water blanched at 98°C for 30 min to ensure inactivation of enzymes, cooled in water and frozen immediately in liquid nitrogen. The intact kernels were then removed from the cob by hand and stored at -35°C until used as the control (heat inactivated enzyme activity) for the study, (3) unblanched frozen corn on the cob: the corn on the cob was frozen immediately in liquid nitrogen and stored at -23.3°C (-10°F) for up to 1.75 years for investigation of off-aroma, and (4) the remainder of the fresh corn was temporarily stored at 4°C prior to germ separation, which was carried out over a period of 3 days.

Linoleic acid, ammonium sulfate, Tween-20 (polyoxoethylene-sorbitan monolaureate), and gel filtration media (Sephacryl S-300 HR) were purchased from Sigma Chemical Co. St Louis, MO. All other chemicals were reagent grade. Deionized distilled water was used in

all purification experiments. A prepacked Fast Protein Liquid Chromatography (FPLC) column of Mono Q HR 5/5 (5 X 0.5 cm ID.), and a prepacked disposable PD-10 (Sephadex G-25 M) column were obtained from Pharmacia-LKB, Uppasala, Sweden. The FPLC system (Pharmacia) was as previously described by Theerakulkait and Barrett (manuscript in prep., a).

Reference standards for aroma descriptors were linseed oil (Grumbacher, Artists Oil Medium by M. Grumbacher, Inc., New York, NY), corn tortilla mix (Quaker Masa Harina De Maiz, manufactured by the Quaker Oats Co., Chicago, IL), canned whole kernel corn (Golden sweet, family style, Del Monte brand, distributed by Del Monte Co., San Francisco, CA), dried straw and hay (Dept. of Animal Science, Oregon State University, Corvallis, OR), and fresh sweet corn and cabbage (purchased at local markets, Corvallis, OR). Reference standards for aroma intensity were safflower oil (Saffala Quality Foods Inc., Los Angeles, CA), orange drink (Hi-C, Coca Cola Foods, Houston, TX), grape juice (Welch's, Concord, MA), and cinnamon bubble gum (Plen T-Pak Big Red, WM. Wrigley Jr. Co., Chicaco, IL).

Enzyme preparation and purification

Rapid isolation of sweet corn germs

The germ fraction was rapidly isolated from sweet corn on the cob by a procedure described in Theerakulkait and Barrett

(manuscript in prep., a). The isolated germs were frozen in liquid nitrogen and stored at -80°C.

Preparation of crude enzyme extract

Isolated sweet corn germs were prepared as an acetone powder, and extracted with 0.2 M Tris-HCl, pH 8.0 (4°C) as described by Theerakulkait and Barrett (manuscript in prep., a). The supernatant of the crude extract was lyophilized and stored at -23.3°C. To prepare the extract for use, it was dissolved in 0.2 M sodium phosphate buffer, pH 7.0, and then the solubilized crude extract was centrifuged at 17,000 x g for 30 min (4°C). The supernatant was buffer exchanged with 50 mM phosphate buffer, pH 7.0, using a prepacked PD-10 gel filtration column. The extract was finally frozen in liquid nitrogen, stored at -23.3°C and used as the source of crude enzyme extract.

Purification of sweet corn germ LPO and POD

The schemes for LPO and POD purification from the sweet corn germs are shown in Fig. 5.1. All steps were carried out at 4°C, except column chromatography with the FPLC system, which was performed at room temperature. All buffers were filtered and degassed prior to use in the FPLC system.

LPO in sweet corn germs was purified as described by Theerakulkait and Barrett (manuscript in prep., b). The germs were

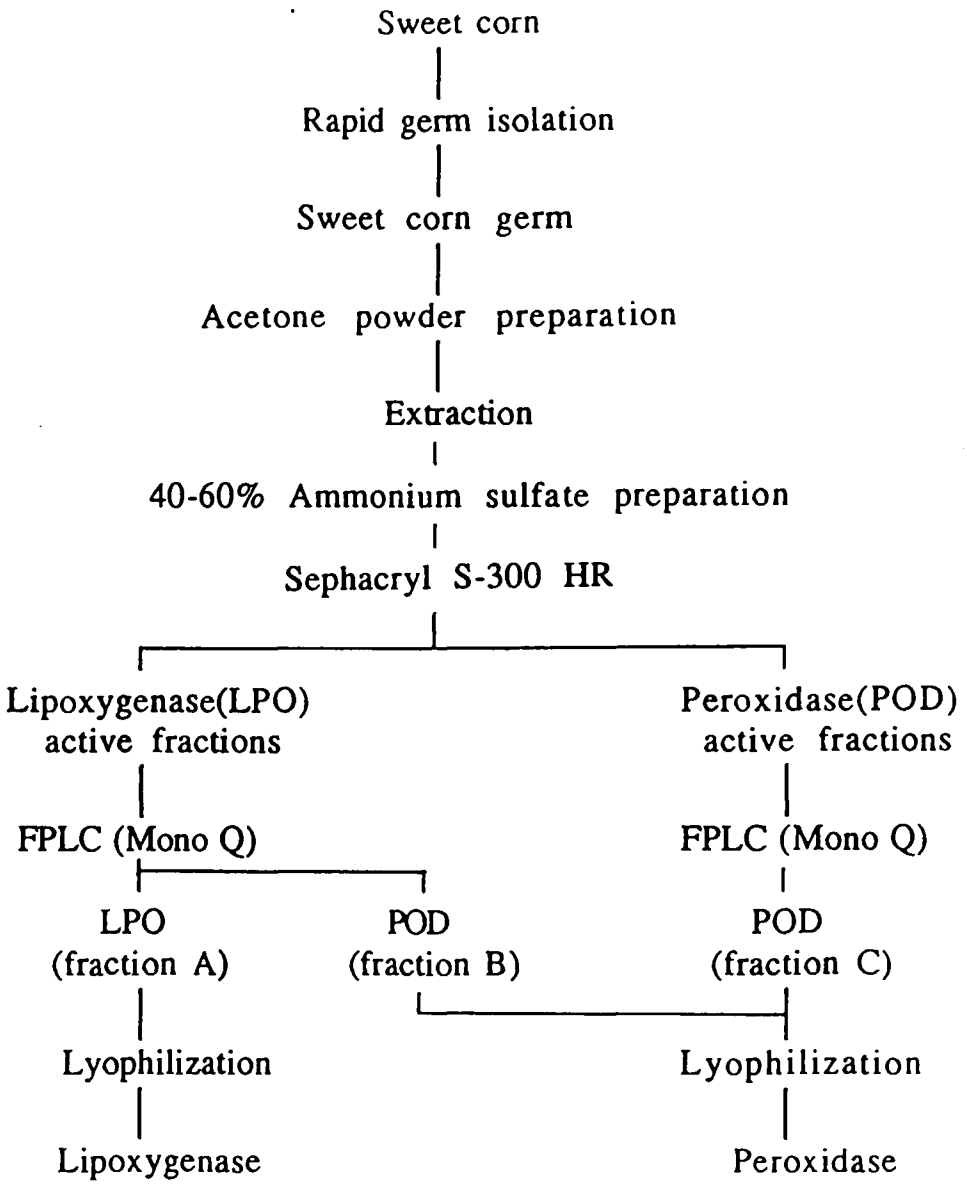

Fig. 5.1- Scheme for purification of lipoxygenase (LPO) and peroxidase (POD) from sweet corn germs.

prepared as an acetone powder, extracted with 0.2 M Tris-HCl, pH 8.0 (4°C), fractionated by 40-60% ammonium sulfate saturation, and purified by conventional column chromatography on Sephacryl S-300 HR and the FPLC on a Mono Q column. The pooled active LPO fraction (fraction A in Fig. 5.1) was desalted and buffer exchanged with deionized distilled water using a prepacked PD-10 column, lyophilized, and stored in a desiccator at -23.3°C.

During purification of LPO on the Mono Q column, the fractions containing POD activity, which eluted from the column prior to starting the NaCl gradient, were pooled (fraction B in Fig. 5.1), and POD activity and absorbance at 280 nm were determined. POD in the solubilized 40-60% ammonium sulfate preparation from sweet corn germs was also separated from LPO using a Sephacryl S-300 HR column. The fractions containing at least 30% of the POD activity in the most active fraction from the column were pooled. The pooled POD fraction was then subjected to further purification by FPLC on a Mono Q HR 5/5 eluted with a linear NaCl gradient, in the same manner as for LPO purification (Theerakulkait and Barrett, manuscript in prep., b). The protein elution profile was monitored at 280 nm.

The collected fractions were kept on ice and assayed for POD activity. Fractions which eluted from the Mono Q before starting the NaCl gradient and contained POD activity were pooled (fraction C in Fig. 5.1), and POD activity and absorbance at 280 nm were determined. Fraction C was combined with fraction B, lyophilized and stored in the desiccator at -23.3°C until used. To prepare the POD for

use, it was dissolved in 50 mM sodium phosphate buffer, pH 7.0, and buffer exchanged with phosphate buffer, pH 7.0, using a prepacked PD-10 column.

Enzyme activity assays

LPO activity was determined spectrophotometrically by monitoring the formation of conjugated dienes at 25°C as described by Theerakulkait and Barrett (manuscript in prep., a). One unit of enzyme activity is defined as an increase in absorbance of 0.001 at 234 nm per minute under the assay conditions. The molar extinction coefficient (ε_m) for the conjugated diene of linoleic acid was 23,000 M^{-1} cm^{-1} (Gibian and Vandenberg, 1987). A double beam spectrophotometer (Shimadzu, UV-160) and 1 cm path length cuvette were used.

POD activity was determined spectrophotometrically at 470 nm (25°C) by a modification of the procedure of Flurkey and Jen (1978). The substrate solution was freshly prepared by mixing 0.90 mL of guaiacol with approximately 180 mL of 0.2 M sodium phosphate buffer, pH 6.0, for about 20 min, adding 0.02 mL of 30% hydrogen peroxide, and mixing thoroughly. The solution was then adjusted to a volume of 200 mL with 0.2 M sodium phosphate buffer, pH 6.0. The total reaction volume was 3 mL, which contained 2.7 mL of substrate solution and 0.3 mL of enzyme solution. The initial rate of reaction was read over the linear change in absorbance at 470 nm.

One unit of enzyme activity is defined as an increase in absorbance of 0.001 at 470 nm per minute under the assay conditions.

Protein determination

The protein elution profile on conventional column chromatography was monitored by measuring absorbance at 280 nm using a double beam spectrophotometer. For the FPLC system a UV-M Monitor was used for observing absorbance at 280 nm via a computer monitor. The protein in pooled active fractions from the conventional column chromatography and FPLC systems was estimated by measuring absorbance at 280 nm. One unit of protein is defined as an absorbance of one at 280 nm.

Sweet corn germ enzymes in off-aroma formation

Panel selection and training

A seven-member panel (six females and one male) was selected from graduate students, staff and faculty of the Department of Food Science & Technology at Oregon State University based on interest, completion of training sessions, availability and consistent performance.

Panel training (15 one-hour sessions) was conducted over a five-week period. The initial sessions involved orientation and development of aroma descriptors. The panelists evaluated and developed their own aroma descriptors to describe the aroma of

sweet corn homogenate samples. In subsequent sessions, various reference materials were provided to help panelists develop terminology to properly describe the samples' aroma characteristics and to help standardize the use of each aroma descriptor. Appropriate reference standards for each selected aroma descriptor were selected and made available to train and assist panelists in evaluation. To reduce variability among panelists in rating aroma intensity, reference standards for aroma intensity were also provided for training. The reference standards were anchored at point 3 (30 mL of safflower oil), point 7 (20 mL of orange drink), point 11 (20 mL of grape juice), and point 13 (1 stick of cinnamon bubble gum) in covered wine glasses.

Further training sessions and group discussions resulted in panel agreement on the use of aroma descriptors. Appropriate sample preparation technique, serving container, sample size, testing technique, scaling method and ballot were established. Training was continued until results from the panel and individual panelists were consistent. The final aroma descriptors and definitions agreed on by the panelists, and the reference standards used for each aroma descriptor are shown in Table 5.1.

Experiment one- *Aroma profile of homogenate of blanched and unblanched frozen stored corn*

Sample preparation: Unblanched frozen corn on the cob stored at -23.3°C for 1.75 years and blanched (98°C, 30 min for corn on the

Table 5.1- Aroma descriptors, definitions, reference standards and their preparation and amount used for serving[a] for descriptive sensory evaluation of sweet corn homogenate samples.

Descriptors	Reference standards and preparations	Definitions
Overall aroma	-	The overall aroma impact (intensity) of all compounds perceived by nose.
Painty	Linseed oil used 15 mL of linseed oil (Grumbacher Artists Oil Medium)	Aroma quality associated with the deterioration of the oil fraction. It may be described as linseed oil, paint thinner, shoe polish.
Stale/oxidized	Wet masa harina prepared by mixing 1 cup of corn tortilla mix (Quaker Masa Harina De maiz) with 1/2 cup of hot water.	Cardboard, old corn flour or the dusty/musty aroma that does not include painty.
Cooked cabbage	Sliced cooked cabbage prepared by cooking 250 g of sliced cabbage with 500 mL of spring water on gas stove at high (10) for 4 min and at low (2) for 30 min, used 10 mL liquid portion and 15 g cooked cabbage	All characteristic notes associated with aroma of cooked cabbage, e.g. sour, cabbage, ferment.
Straw/hay	Chopped straw and hay prepared by chopping the dried straw and hay in a length about 1 to 2 cm, used 3 g chopped straw and 3 g chopped hay.	All characteristic notes associated with straw and hay.

Table 5.1- (continued)

Corn	Cooked fresh cut sweet corn prepared by cooking 75 g of fresh cut sweet corn with 5 mL spring water using microwave at full power for 1.5 min, used 30 g cooked cut corn.	The characteristic note of "corn" associated with cooked sweet corn.
Sweet	Liquid of canned whole kernel corn use 30 mL of liquid portion of canned whole corn kernels (Golden sweet, family style, Del Monte brand).	The characteristic note of "sweet" associated with canned sweet corn.
Cobby/husky	Diced fresh corn cob and fresh corn husk prepared by dicing fresh corn husk (thickness about 0.5 cm) and fresh corn cob (thickness about 0.1 cm), used 15 g for diced cob and 8 g for diced husk.	The characteristic note associated with diced fresh corn cob and husk.

[a] Served in 250 mL clear wine glasses covered with tight fitting aluminium lids.

cob) frozen intact sweet corn kernels stored at -35°C for 1.75 years were used for the study. The samples were prepared by a modification of the procedures described by Velasco et al. (1989). The unblanched samples were prepared by freezing in liquid nitrogen. The intact kernels were removed from the cob and homogenized in liquid nitrogen using a stainless steel Waring blender with the powerstat setting at 100. The corn liquid nitrogen powder (75 g) was weighed into a wide mouth (450 mL) freezer jar (Ball Brothers Co. Inc., West coast Div., El Monte, CA.), and allowed to stand at room temperature for about 30 min. Then, 15 mL of 50 mM sodium phosphate buffer, pH 7.0, was added. The control sample was prepared in the same manner, except the homogenate of blanched frozen corn kernel was used. Each sample was mixed thoroughly, covered with a watch glass, and heated at 93°C in a water bath for 30 min, stirring every 10 min. Eight grams of each sample were weighed into 250 mL black, tulip-shaped wine glasses coded with three digit random numbers, capped with aluminium lids and sealed with parafilm.

Sample testing: Samples were cooled by leaving them at room temperature (22°C) for at least 30 min. The samples (a set of two), with a randomly assigned order of presentation, were randomly presented to each panelist in individual booths. Each panelist was asked to remove the cover, sniff the aroma in the head space of the samples and then rate the intensity of each aroma descriptor for

each sample on the ballot from left to right (one at a time). Panelists sniffed water after each sample to avoid a cross-over effect.

Each aroma descriptor intensity was rated using a 16-point intensity scale, (0=none, 1=just detectable, 3=slight, 5=slight to moderate, 7=moderate, 9=moderate to large, 11=large, 13=large to extreme, and 15=extreme). Reference standards for each aroma descriptor and aroma intensity were available for the panelists to review in every test session. The testing was performed in 3 individual sessions (3 replications).

Experiment two- LPO involvement

Unblanched and blanched frozen intact sweet corn kernels stored at -35°C for 1.75 years were used for this study. The samples included: (1) blanched corn homogenate with added buffer, as a control, (2) homogenate to which was added purified LPO, (3) homogenate to which crude enzyme extract was added, and (4) unblanched corn homogenate with the same buffer added as in the control. Each of the first three samples were prepared by adding either 50 mM sodium phosphate buffer, pH 7.0, purified LPO solution, or crude enzyme extract in the same phosphate buffer, respectively, into liquid nitrogen powder of blanched corn in the ratio of 1 to 5 (v/w) in each freezer jar in a similar manner as previously described. The unblanched sample was prepared in the same manner as the control, except using the homogenate of frozen unblanched intact corn kernel. Total LPO activity in the samples

with added purified LPO solution and crude extract, and the unblanched sample were approximately the same level (675,000 units), while that in the control was insignificant.

Each sample was mixed thoroughly and incubated in a slow speed shaking water bath (at 30°C) for 3 hr and stirred every 30 min. The samples were heated at 93°C in a water bath for 30 min and then analyzed in the same way as described in the aroma profile study of blanched and unblanched corn homogenate, except a set of four (instead of two) samples were used for testing.

Experiment three- POD involvement

Investigation of the involvement of POD in off-aroma formation was performed in the similar manner as the LPO study, except that samples number 2 and 3 were: added purified POD (instead of LPO) and crude extract from sweet corn germs. Total POD activity in these two samples was approximately equal (650,000 units). Sample preparation and testing were also similar to that for LPO.

Statistical analysis

A randomized complete block design was used in all experiments. The block corresponded to each of the seven panelists in each replication. All experiments provided three replications over the treatments using the same panelists. Assessments by panelists were analyzed per aroma descriptor through three-way ANOVA's

with panelist (P), replication (R) and treatment (T) as factors. The interactions for each descriptor were also tested for significance. SAS version 6 (SAS Institute, Inc., 1987) was used for statistical analysis. A mixed effect linear model was used with panelist and replication a random effects (Lundahl and McDaniel, 1988), while treatment was considered a fixed effect. For the model containing all two- and three-factor interactions, the F-statistic for testing treatments (F_t) was calculated according to Steele and Torrie (1980) by the following formula:

$$F_t = \frac{MS(T) + MS(P*R*T)}{MS(R*T) + MS(P*T)}$$

However, since the replication-by-treatment interactions (R*T) for all descriptors in all experiments were not statistically significant, the appropriate F-statistic was simplified to:

$$F_t = \frac{MS(T)}{MS(P*T)}$$

The mean square for panelist-by-treatment interaction (P*T) was used as the error term for the test for treatment effect. Comparisons of treatment means of each aroma descriptor were conducted using Fisher's least significant difference test ($p \leq 0.05$).

The data were also analyzed by principal component analysis (PCA) using SAS (SAS Institute, Inc., 1987). The principal component scores for each axis were analyzed by ANOVA and by Fisher's least significant difference test ($p \leq 0.05$).

RESULTS & DISCUSSION

Purification of sweet corn germ LPO and POD

In order to study the involvement of sweet corn germ enzymes, including LPO and POD, in off-aroma formation, a crude enzyme extract was prepared. The isolation and purification of LPO and POD from germs was also required. The purification procedure of LPO from the germs was developed and optimized as described in our previous work (Theerakulkait and Barrett, manuscript in prep., b). The purification of LPO (pooled fraction A in Fig. 5.3) was 188 fold with 26.3 percent recovery (Table 5.2). Velasco et al. (1989) purified LPO from whole kernels of sweet corn and used for study of its possible role in off-aroma formation, however, the purification of LPO was only 10.3 fold.

It was considered of interest to determine whether sweet corn germ POD is involved in aroma deterioration, since POD has been long used as a blanching index for the frozen sweet corn industry (Morris, 1958). POD from the solubilized 40-60% ammonium sulfate preparation of sweet corn germs was simultaneously purified with LPO on Sephacryl S-300 HR (Fig. 5.2). The majority of the POD eluted from the column later than LPO, indicating that the molecular size of the major POD isozymes in sweet corn germs was smaller than that of LPO. On the FPLC Mono Q column, the majority of POD eluted from the column before starting the NaCl gradient both during purification of LPO (Fig. 5.3) and POD (Fig. 5.4), which indicates that the majority

Table 5.2- Purification of lipoxygenase (LPO) from sweet corn germs[a]

Step in Purification	Total activity (units/g AP)	Specific activity (units/unit[b] of protein)	Recovery[c] (%)	Purification (fold)
Acetone Powder (AP) extract	164,038	937	100	1
40% Ammonium sulfate supernatant	114,583	1,238	69.9	1.3
40-60% Ammonium sulfate precipitate	111,875	2,600	68.2	2.8
Gel filtration (S-300HR)	80,163	15,420	48.9	16.5
Anion Exchange, FPLC (Mono Q)	43,166	175,857	26.3	188

[a] calculated from starting with 6 g acetone powder (average from 3 replications).

[b] a unit of protein defined as an absorbance of 1.0 at 280 nm.

[c] relative to LPO activity in AP extract.

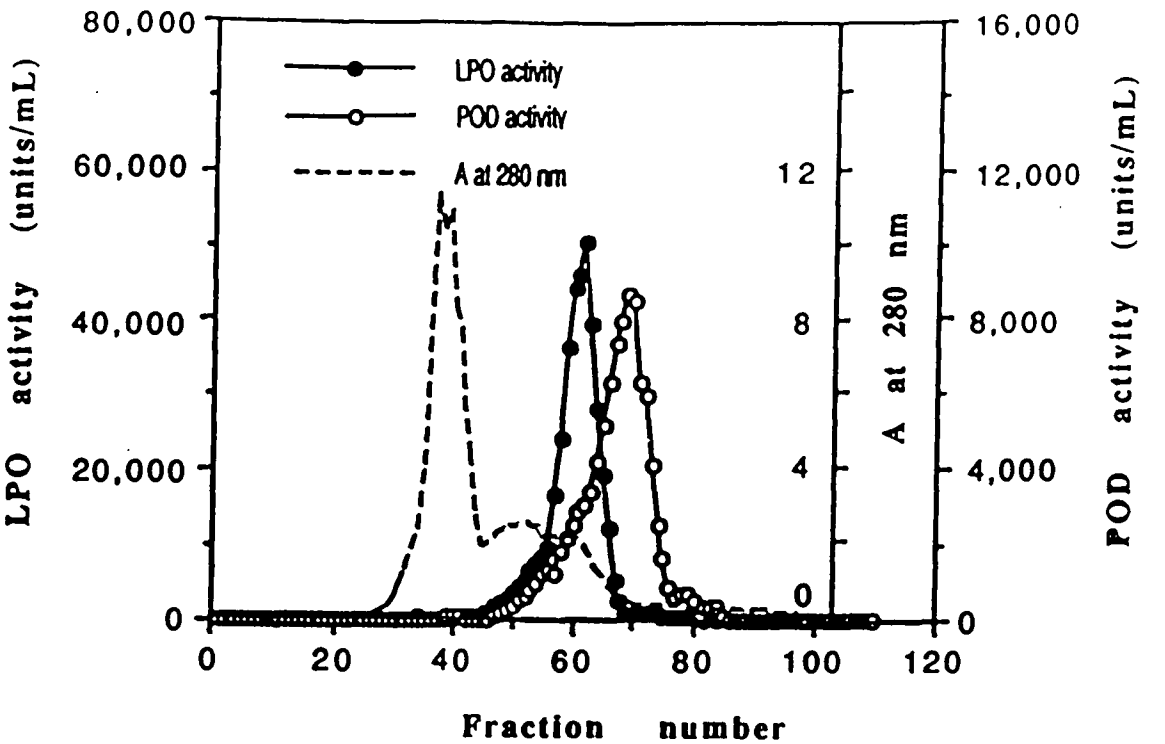

Fig. 5.2- Sephacryl S-300 HR chromatographic purification of sweet corn germ POD and LPO from 40-60% ammonium sulfate preparation.

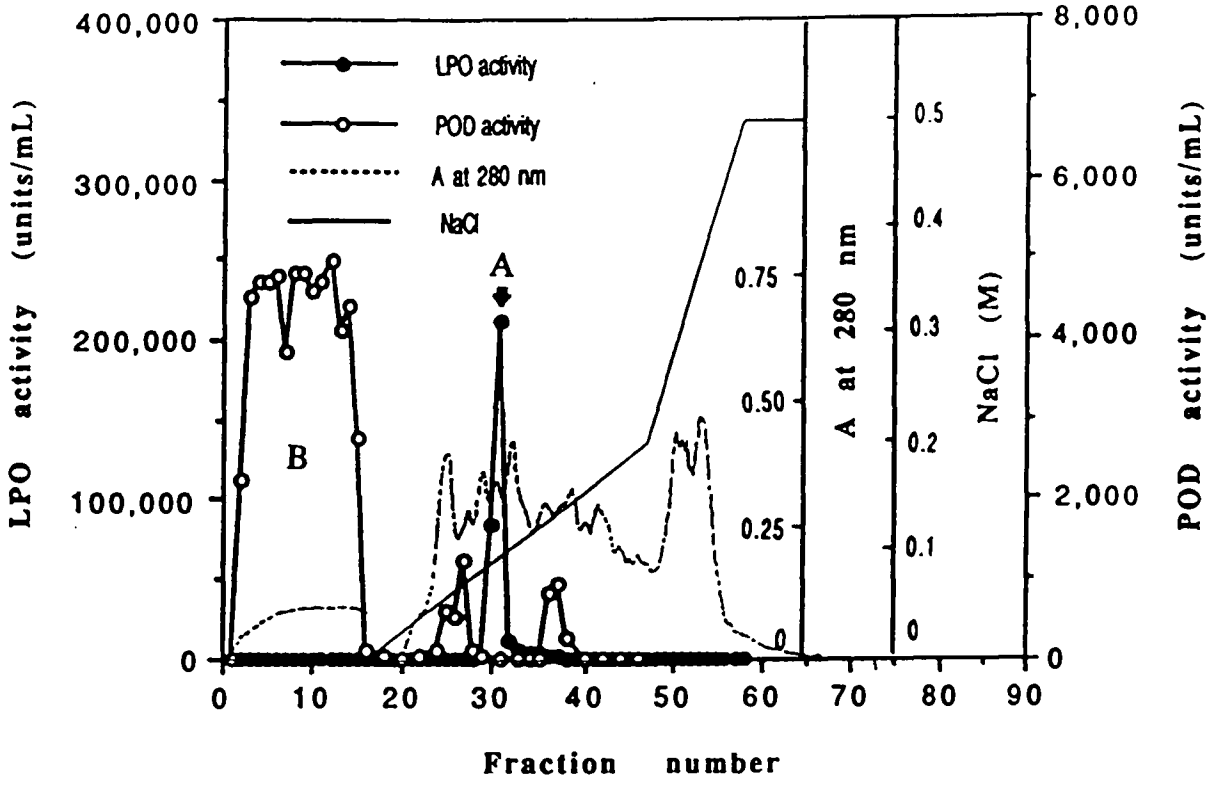

Fig. 5.3- FPLC (Mono Q) chromatographic purification of sweet corn germ POD and LPO of active LPO fractions pooled from Sephacryl S-300 HR.

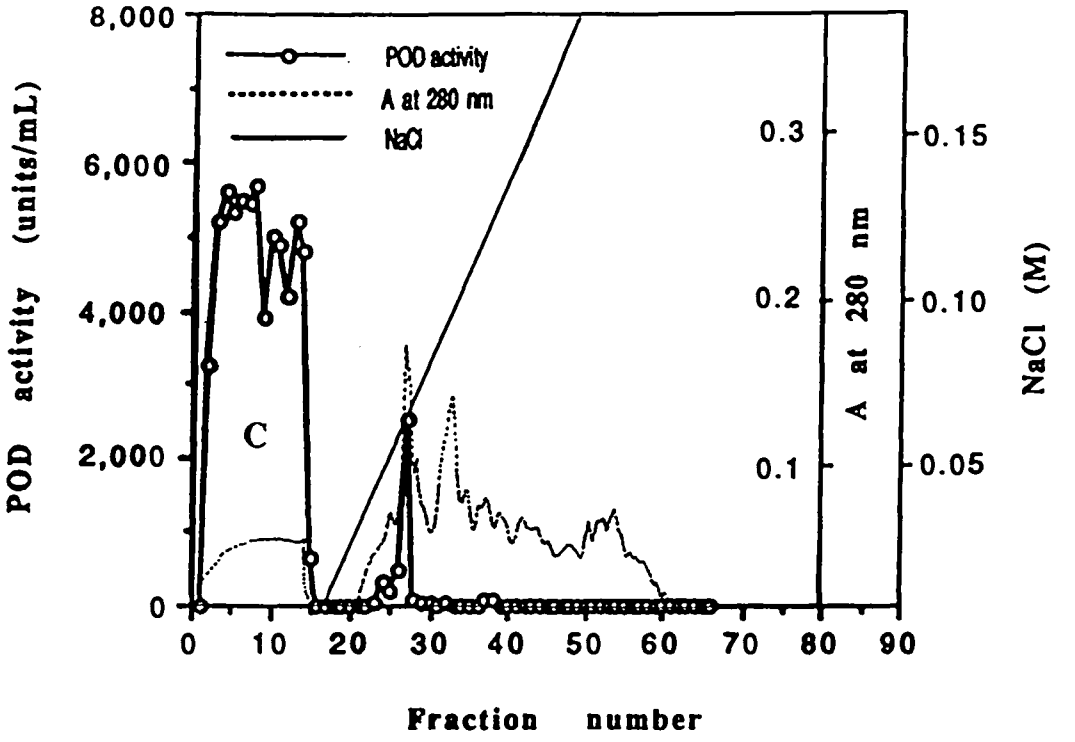

Fig. 5.4- FPLC (Mono Q) chromatographic purification of sweet corn germ POD of active POD fractions pooled from Sephacryl S-300 HR.

of POD is in the basic form. Due to the limited amount of POD purified from sweet corn germs, the two pooled POD fractions (Fraction B and C in Fig. 5.3 and 5.4, respectively) from the Mono Q column were combined, lyophilized and kept at -23.3°C until use. The purification and recovery of POD in pooled fraction was 58 fold and 27.6 %, respectively (Table 5.3). The POD at this stage of purification was free from LPO activity.

Sweet corn germ enzymes in off-aroma formation

Experiment one- Aroma profile of homogenate of blanched and unblanched frozen stored corn

Prior to investigating the involvement of sweet corn germ enzymes in the off-aroma formation in sweet corn, the aroma profile of a homogenate of blanched frozen intact corn kernels (control) was evaluated. The aroma profile of the control was described as slight to moderate "sweet" and "corn", just detectable to slight "cobby/husky", "painty", and "stale/oxidized", and just detectable "straw/hay", and "cooked cabbage" with moderate to large "overall aroma intensity" (Fig. 5.5). The overall aroma characteristics were relatively high in "desirable" aromas of sweet corn including "sweet" and "corn", while relatively low in "undesirable" or "off-aroma" characteristics including "stale/oxidized", "painty", "cobby/husky", "cooked cabbage" and "straw/hay" aroma descriptors.

Table 5.3- Purification of peroxidase (POD) from sweet corn germs[a]

Step in Purification	Total activity (units/g AP)	Specific activity (units/unit[b] of protein)	Recovery[c] (%)	Purification (fold)
Acetone Powder (AP) extract	84,372	482	100	1
40% Ammonium sulfate supernatant	49,117	530	58.2	1.1
40-60% Ammoniun sulfate precipitate	37,221	858	44.1	1.8
Gel filtration[d] (S-300HR)	30,412	4,068	36.0	8.5
Anion Exchange,[e] FPLC (Mono Q)	23,323	27,852	27.6	58

[a] calculated from starting with 6 g acetone powder (average from 3 replications).

[b] a unit of protein defined as an absorbance of 1.0 at 280 nm.

[c] relative to POD activity in AP extract.

[d] calculated from data of pooled active POD activity and LPO fractions.

[e] calculated from data of pooled POD from Mono Q (fraction B & C in Fig. 5.1).

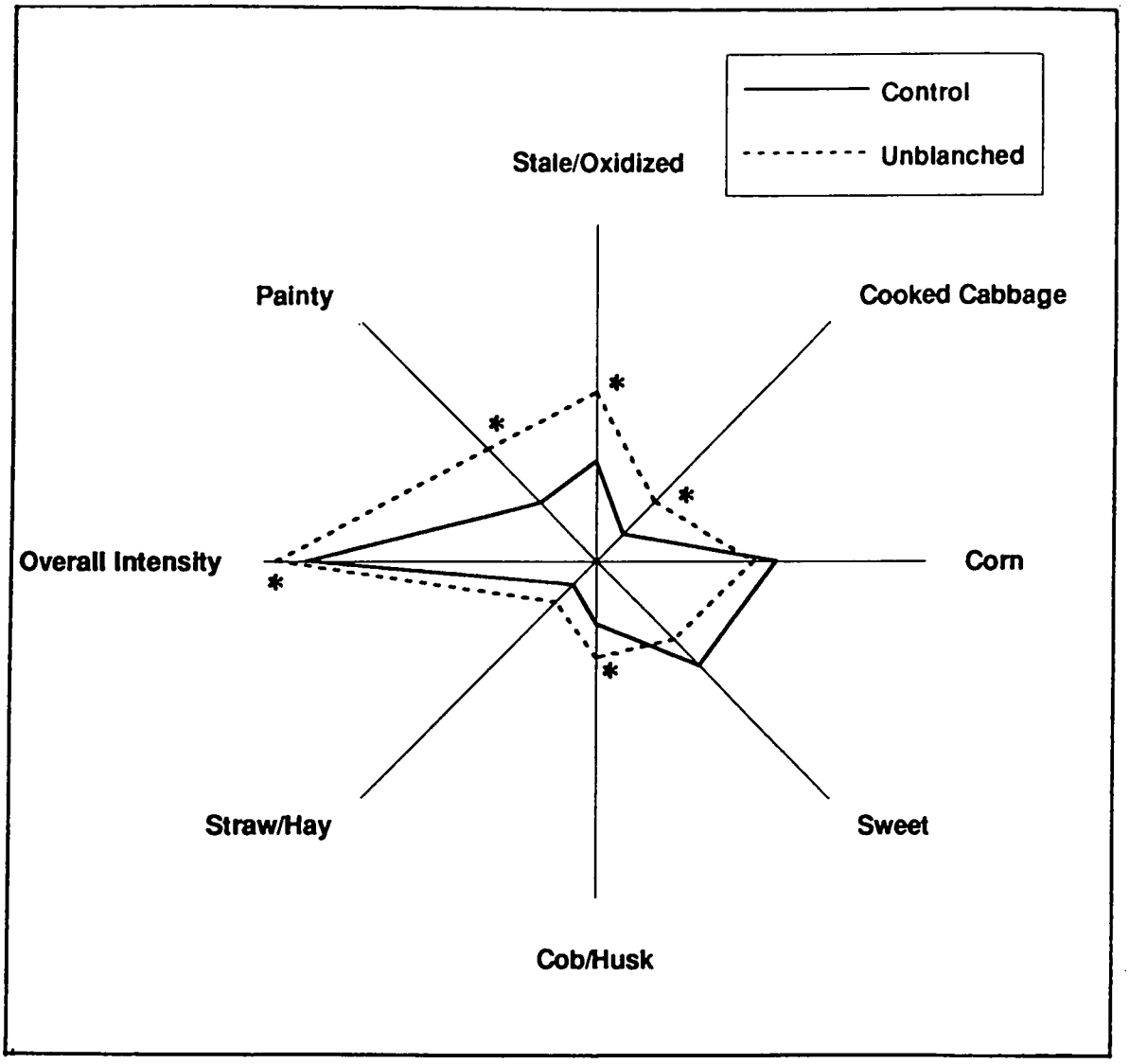

Fig. 5.5- A typical spider-web diagram of descriptive analysis for unblanched sweet corn homogenate samples compared with the control; the distance from the center is the mean value for that aroma descriptor. The mean designated with "*" are statistically significant difference from the control ($p \leq 0.05$).

The aroma profile of the homogenate prepared from frozen stored raw (unblanched) corn on the cob was compared with the control using a typical spider web plot (Stone et al., 1974) as shown in Fig. 5.5. Univariate ANOVA on each descriptor showed significant treatment differences between unblanched and control samples for most of the off-aroma descriptors except for "straw/hay" and "desirable" aroma descriptors "sweet" and "corn" (Table A 5.1). It implied that the difference between the samples were significantly detectable by the trained panel for most descriptors of off-aroma characteristics. Replication effects were not significant for any descriptors except "painty", indicating good reproducibility of the replicates (Table A 5.1).

When both treatment and panelist by treatment interactions are significant, it is important to examine whether the panelist by treatment interaction influences the conclusions drawn regarding the treatments. This was done by visualizing the line graph plot between each panelist's ratings against each treatment to search for systematic inconsistencies among the panelists contributing to variation. For example, in the case of "stale/oxidized" there was a significant effect of both treatment and panelist-by-treatment interaction. It was found that most of the panelists responded similarly, suggesting that the main treatment effect differences were important.

The mean aroma intensity of the homogenate of the unblanched sample was significantly higher than the control in "overall intensity" and in most off-aroma descriptors, while the mean

intensity of "desirable" aroma descriptors was lower than that of the control (Table A 5.2), but not significantly. Unblanched corn on the cob developed off-aroma during frozen storage as indicated by the off-aroma characteristics of its homogenate. The development of off-flavor and off-aroma in frozen raw (unblanched) or underblanched sweet corn stored in freezers for extended periods of time has also been reported when the corn was evaluated as cooked corn on the cob (Wagenknecht, 1959; Lee, 1981) or as cooked whole kernels (McDaniel et al., 1988). One of the causes of off-flavor and off-aroma formation of frozen stored raw or underblanched vegetables is believed to be the result of enzyme action during frozen storage (Joslyn, 1949). However, the question remains, which enzyme(s) play(s) the major role in this quality deterioration.

Experiment two- LPO involvement

In this experiment, the crude enzyme extract and purified sweet corn germ LPO were used to investigate their involvement in off-aroma formation. The PCA model of aroma descriptors showed that only the first two principal components (PC) were significant with 64.99 and 18.81 % of the total variation explained by PC1 and PC2, respectively (Table A 5.3). The loading of aroma descriptors for PC1 and PC2 indicated that PC1 can be defined as an "desirable" vs "undesirable" (off-aroma) descriptor axis (Fig. 5.6a). The overall intensity and off-aroma descriptors, especially "painty", were negatively correlated with the "desirable" aroma descriptors,

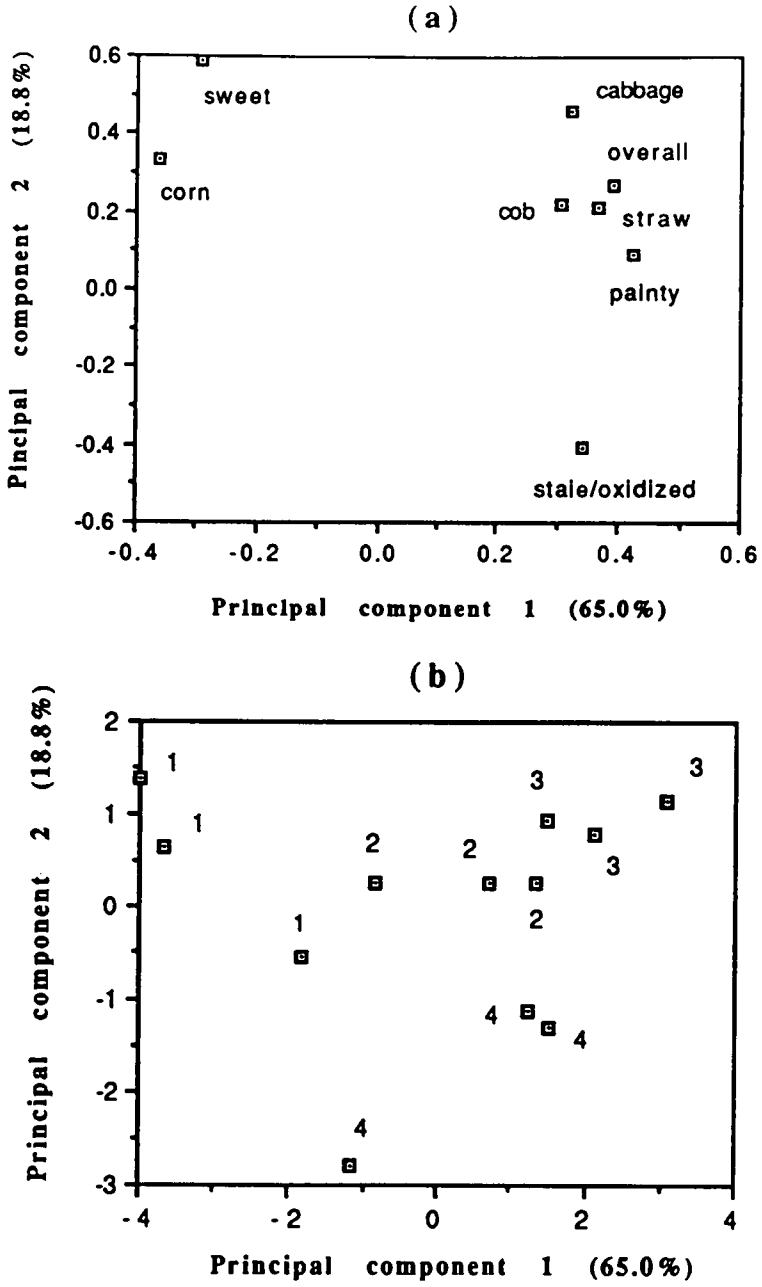

Fig. 5.6- (a) Loading of aroma descriptors used for descriptive analysis, and (b) principal component analysis plot of aroma intensity rating of aroma descriptors for the samples of sweet corn homogenate (1=control, 2= unblanched, 3= added crude enzyme extract and 4=added LPO; each contains 3 replications) on PC1 vs. PC2.

especially "corn" (Table A 5.3). PC2 might be defined as "stale/oxidized" vs "sweet" and "cooked cabbage" (Table A 5.3 and Fig. 5.6a).

ANOVA tests showed that there were significant differences between the mean PC1 and PC2 scores among the samples (Table A 5.4), with non-significant effects of the replications indicating good reproducibility. The reproducibility could also be observed from the grouping of three replications for each sample (Fig. 5.6b). The PCA plot of aroma intensity ratings indicated that the samples could be classified into 3 groups (Fig. 5.6b): control (1), unblanched and added crude extract (2 and 3), and added purified LPO (4). The mean PC1 score of the control was significantly different from that of unblanched, added crude extract, and added purified LPO samples, which were not significantly different from each other (Table A 5.4). The control was best described by "desirable" aroma descriptors while the other samples were described by off-aroma descriptors (Fig. 5.6a and b). However, the mean PC2 score of the sample with added purified LPO was significantly different from those of unblanched and added crude extract (Table A 5.4). The sample with added purified LPO seemed to be best described by "stale/oxidized" and "painty" compared to the other samples (Fig. 5.6a and b).

The results of univariate ANOVA on each descriptor showed significant treatment differences among the samples for "cooked cabbage", "overall intensity", "painty", "corn", and "sweet" (Table A 5.5). A panelist effect was significant for all aroma descriptors; however, this is not unusual and reflects some panelists' usage of

different portions of the intensity scale (Power, 1988). The replication effect was not significant for any of the aroma descriptors, indicating good reproducibility. Panelist by treatment interaction effects were significant for "painty", "sweet", "corn" and "cobby/husky"; however, most of the panelists responded similarly. The mean values of aroma intensity of most off-aroma descriptors including "painty", "cooked cabbage", and "cobby/husky" of the unblanched sample were significantly higher, while the descriptor "corn" was significantly lower than that of the control. The aroma profile of the unblanched sample incubated at 30°C for 3 hr seemed to be similar to that of the unblanched sample frozen stored at -23.3°C for 1.75 years (Fig. 5.5 and 5.7). One possible cause of the off-aroma formation is the enzymatic action either during incubation at -23.3°C for 1.75 years, or at 30°C for 3 hr.

Off-aroma formation of the samples with added crude enzyme extract and purified LPO with approximately the same total LPO activity as originally present in unblanched sample was investigated. The sample with added crude enzyme extract was significantly higher in intensity of most off-aroma descriptors including "painty", "stale/oxidized", "cooked cabbage", "straw/hay" than that of the control, but the "corn" aroma was significantly lower (Fig. 5.7). The sample with added crude enzyme extract was similar to that of the unblanched sample in that the mean intensity of off-aroma descriptors was higher than the control, while that of "desirable" aroma descriptors was lower than the control. However, the mean intensity of descriptors "overall intensity" and "cooked cabbage" of

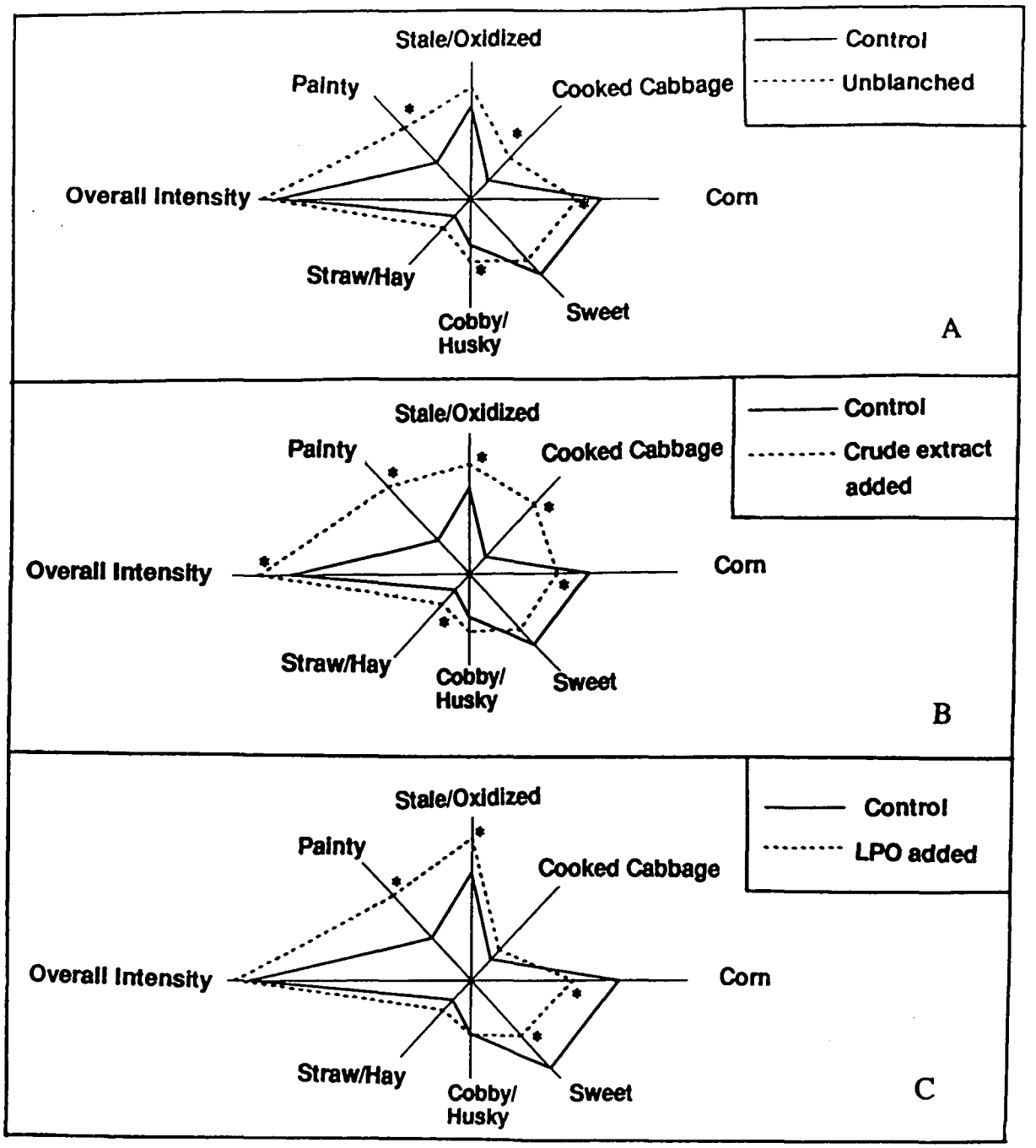

Fig. 5.7- A typical spider-web diagram of descriptive analysis for sweet corn homogenate samples (A=unblanched, B=added crude enzyme extract, and C=added LPO; respectively) compared with the control; the distance from the center is the mean value for that aroma descriptor. The mean designated with "*" are statistically significant difference from those of control ($p \leq 0.05$).

the sample with added crude extract was significantly higher than that of the unblanched and added LPO samples (Table 5.4).

The results indicate that the crude enzyme extract from sweet corn germs causes off-aroma formation and that the increase in intensity of off-aroma descriptors possibly masks the "desirable" aroma descriptors rather than there being a true reduction in the intensity of desirable aroma descriptors. The formation of typical cooked "corn" aroma is generally believed to be "heat activated", while raw sweet corn has very little aroma. Dimethyl sulfide is one of principle low-boiling volatile compounds that contributes to cooked "corn" aroma (Bills and Keenan, 1968; Williams and Nelson, 1973; Flora and Wiley, 1974; Dignan and Wiley, 1976; Wiley, 1985).

Adding purified LPO from sweet corn germs to the sample resulted in a significant increase in the off-aroma descriptors "painty" and "stale/oxidized", and a significant decrease in "desirable" aroma descriptors "sweet" and "corn" (Fig. 5.7). The descriptors "painty" and "stale/oxidized" also significantly increased in the sample with added crude extract. The significant decrease in "sweet" and "corn" aroma might be due to masking by the increase in intensity of those off-aroma descriptors. This result is supported by the work of Wagenknecht (1959) and Lee (1981), who suggested that enzymes, particularly LPO, in the fraction containing the germ, induced off-flavors in unblanched sweet corn. McDaniel et al. (1988) reported that the mean intensity of the descriptor "stale/oxidized" evaluated by a trained descriptive panel was higher in the under-

Table 5.4- Mean ratings[y], standard deviations (SD), and LSD values for aroma descriptors among the treatments for the LPO involvement study.

Descriptors	Treatments				LSD ($p \leq 0.05$)	Sig.[z]
	Control	Unblanched	Crude extract	LPO		
Overall intensity	9.09[b] (1.22)	9.81[b] (1.29)	10.71[a] (1.35)	9.71[b] (1.68)	0.84	**
Painty	2.38[b] (1.99)	4.62[a] (3.54)	6.10[a] (2.81)	4.71[a] (3.20)	2.02	**
Stale/ Oxidized	4.24[b] (1.81)	5.14[ab] (1.35)	5.38[a] (1.60)	5.62[a] (1.53)	1.06	p = .06
Cooked cabbage	1.19[c] (1.97)	2.67[b] (2.39)	4.86[a] (2.95)	1.71[bc] (1.95)	1.41	***
Straw/ Hay	1.10[b] (1.67)	1.86[ab] (1.82)	2.05[a] (2.40)	1.67[ab] (1.83)	0.79	p = .09
Corn	6.24[a] (1.22)	5.14[b] (1.32)	4.62[b] (1.80)	4.33[b] (1.35)	0.95	**
sweet	4.86[a] (1.88)	3.95[ab] (1.75)	3.86[ab] (1.77)	3.10[b] (1.48)	1.15	*

Table 5.4 (continued)

Cobby/ Husky	2.10[b] (1.61)	2.86[a] (1.49)	2.81[ab] (1.94)	2.14[ab] (1.56)	0.75	p = .08

[y] Sixteen point intensity scale (0 = none, 15 = extreme).

[z] *, **, *** refers to significance at $p \leq 0.05$, 0.01 and 0.001, respectively by ANOVA.

[a-c] Means with the same letter, in the same row, are not significant different at the 0.05 significant level by LSD.

blanched frozen stored corn kernel than that in the commercial blanched frozen stored kernel.

Formation of the off-aroma descriptors "painty" and "stale/oxidized" in the sweet corn sample by adding purified LPO or crude extract from the germ tissue could be due to the action of LPO in catalyzing the hydroxyperoxidation of polyunsaturated fatty acids and esters containing a *cis,cis*-1,4-pentadiene system as similar to that occurs in oil seeds such as soybean, peanut. This reaction would initially yield hydroperoxides which would subsequently degrade to form a variety of secondary products, including aldehydes, alcohols, and ketones, which could result in off-aroma formation (Eskin et al., 1977; MacLeod and Ames, 1988). LPO possibly catalyzes the oxidation of sweet corn polyunsaturated fatty acids, mainly in germ tissue, since polyunsaturated fatty acids are high in the germs of sweet corn kernels (Wagenknecht, 1959; Gardner and Inglett, 1971; Flora and Wiley, 1972; Pascual and Wiley, 1974; Puangnak, 1976; Weber, 1978a and 1978b), and this might ultimately result in off-aroma develop. LPO activity per unit weight in the germs of sweet corn, at the stage of maturity selected by the frozen food industry for freezing, is significantly higher than that in the degermed fraction (Theerakulkait and Barrett, a, manuscript in prep.). This suggests that the germs are the important target in inducing off-aroma formation due to enzymatic oxidation by lipoxygenase.

Other previous studies also indicate that LPO plays a significant role in off-flavor and off-aroma formation. Kalbrener et al. (1974) reported that "musty/stale" was one of the predominant flavor

descriptors of the linoleic hydroperoxide produced by soy LPO action on linoleic acid. Ashraf and Synder (1981) reported that the "painty" off-flavor in soy milk was decreased in samples with less residual LPO activity. The terms "painty", "stale/oxidized" and related terms such as "cardboard", "rancid" have been used to describe off-flavor in peanut and vegetable oil (Johnsen et al., 1988; Civille and Dus, 1992). Moreover, the "painty" and "stale/oxidized" descriptors are among flavor descriptors in the American Oil Chemists' Society flavor quality scale used to describe the flavor attributes of oils. "Oxidized" and "painty" are the descriptors used to describe the flavor grade of 6 (fair to poor flavor) and 4 (poor to very poor flavor), respectively (Warner, 1985). One of the mechanisms involved in off-flavor production in edible oil is enzymatic lipid oxidation by LPO (Mistry and Min, 1992). Moreover, LPO was also demonstrated to be the key enzyme in the development of off-aroma in other plants such as English green pea and green bean (Williams et al., 1986).

Although lipoxygenase appears to be a primary culprit, other sweet corn germ enzymes may be involved in the formation of other off-aroma descriptors, particularly "cooked cabbage", which was significantly higher in the sample with added crude extract than that with added purified LPO. Gardner (1970) reported that linoleate hydroperoxide isomerase was present in the germs of mature corn and catalyzed the hydroperoxide products by the action of LPO. Velasco et al. (1989) also believed that LPO was important in off-aroma development in sweet corn, however; they suggested that

other enzymes, such as hydroperoxide isomerase and hydroperoxide lyase, may also be important.

Experiments three- POD involvement

In the PCA model of aroma descriptors for the study of the involvement of POD in off-aroma formation, the first two PC explained most of the total variation, with 78.53 and 13.58 % for PC1 and PC2, respectively (Table A 5.6). Similar to the LPO experiment, PC1 can be defined as a "desirable" and "off-aroma" descriptor axis (Fig. 5.8a). However, mean PC scores among the samples were significantly different only for PC1 (Table A 5.7). The replication effect was not significant in either case indicating good repeatability.

Based on the LSD test of means in PC1, the samples could be classified into 2 groups: the control and added purified POD samples, and unblanched and added crude extract samples. The mean PC1 score of the added purified POD sample was not significantly different from that of the control, but was significantly different from those of unblanched and added crude extract samples. However, the unblanched and added crude extract samples were not significantly different among each others (Table A 5.7). The control and added purified POD samples were similar in aroma profile and could be best described by "desirable" aroma descriptors "sweet" and "corn", while added crude extract and unblanched samples were best described by off-aroma descriptors (Fig. 5.8a and b).

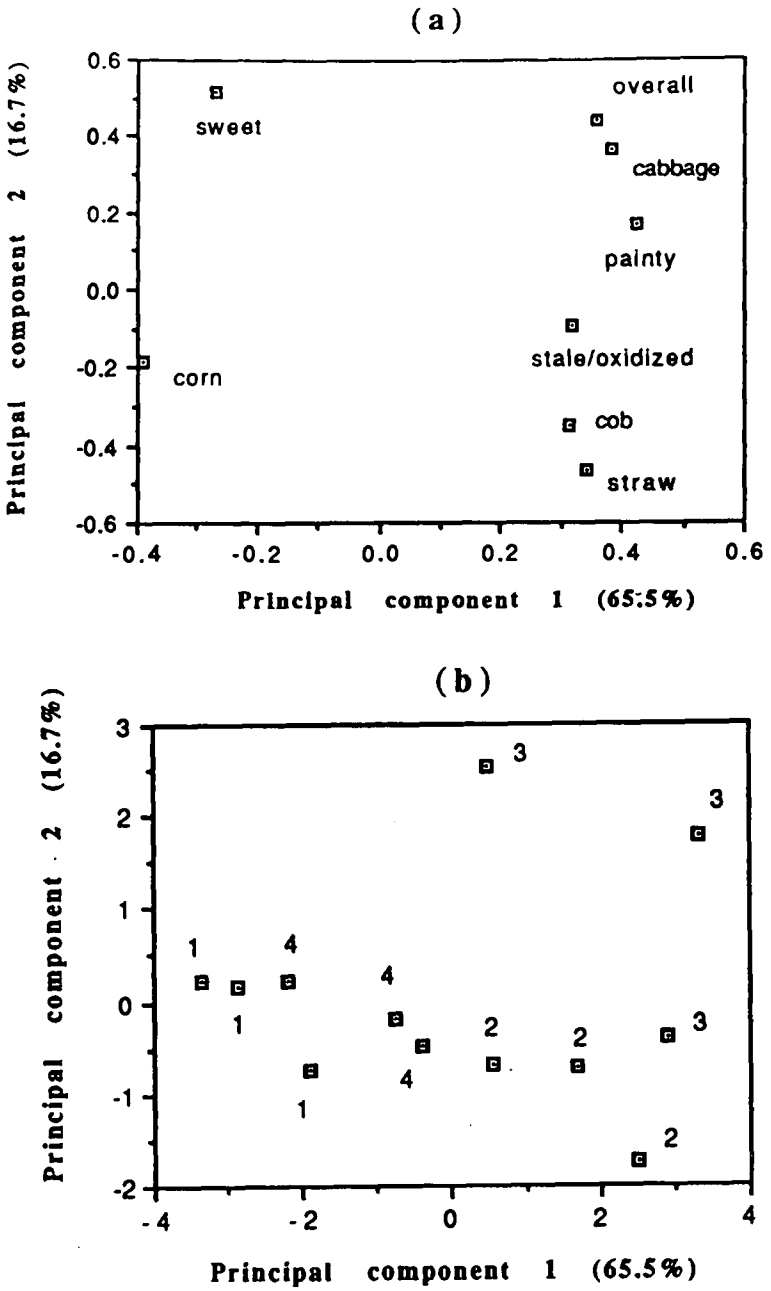

Fig. 5.8- (a) Loading of aroma descriptors used for descriptive analysis, and (b) Principal component analysis plot of aroma intensity rating of aroma descriptors for the samples of sweet corn homogenate (1=control, 2= unblanched, 3= added crude enzyme extract and 4=added POD ; each contains 3 replications) on PC1 vs. PC2.

The results of univariate ANOVA on each descriptor showed significant treatment differences among the samples for "cooked cabbage", "overall intensity", "painty", "straw and hay", and "corn" (Table A 5.8). The experiment had good reproducibility as indicated by the non-significant replication effect. The panelist by treatment interaction was significant for most of the descriptors except "straw/hay". However, it was found that most of the panelists responded similarly. The mean intensity of most off-aroma descriptors of the unblanched sample including "painty", "cooked cabbage", and "straw/hay" were significantly higher than the control, but "corn" aroma was significantly lower than that of the control (Table 5.5). This result is similar to that of the unblanched sample from the LPO study.

The mean values of intensity for the added crude enzyme extract sample were significantly higher than the control in "overall intensity" and the off-aroma descriptors "painty" and "cooked cabbage". The "corn" aroma was significantly lower than that of the control (Fig. 5.9). The aroma profile of the sample with added crude enzyme extract was similar to that of unblanched sample in that the mean intensity of descriptors describing off-aroma was higher than the control while that of descriptors describing "desirable" aroma was lower than that of the control. However, the mean intensity of descriptor "cooked cabbage" was significantly higher than that of the unblanched sample (Table 5.5).

The mean values for intensity of all aroma descriptors for the added POD sample were not significantly different from that of the

Table 5.5- Mean ratings[y], standard deviations (SD), LSD values for aroma descriptors among the treatments for the POD involvement study.

	Treatments					
	Control	Unblanched	Crude extract	POD	LSD ($p \leq 0.05$)	Sig.[z]
Overall intensity	8.67[c] (0.97)	9.76[ab] (1.55)	10.57[a] (1.40)	8.95[bc] (1.32)	0.90	**
Painty	1.57[c] (1.96)	4.43[ab] (3.44)	5.57[a] (2.34)	2.57[bc] (2.50)	1.96	**
Stale/ Oxidized	3.33[a] (2.08)	4.33[a] (1.96)	4.29[a] (2.55)	4.29[a] (2.35)	1.58	ns
Cooked cabbage	1.05[c] (1.66)	2.90[b] (2.66)	4.86[a] (2.65)	1.48[bc] (1.94)	1.69	***
Straw/ Hay	1.05[b] (1.32)	2.00[a] (1.76)	1.52[ab] (1.81)	1.29[b] (1.55)	0.48	**
Corn	6.19[a] (1.36)	4.90[b] (1.64)	3.90[bc] (1.95)	5.29[ab] (1.38)	1.16	**
sweet	4.71[a] (2.39)	3.48[a] (1.63)	4.05[a] (2.80)	4.38[a] (2.18)	1.43	ns

Table 5.5 (continued)

Cobby/ Husky	2.33[a] (2.11)	3.05[a] (1.63)	2.62[a] (1.91)	2.29[a] (1.90)	1.04	ns

[y] Sixteen point intensity scale (0 = none, 15 = extreme).

[z] ns not-significant at $p \leq 0.05$.
*, **, *** refers to significance at $p \leq 0.05$, 0.01 and 0.001, respectively by ANOVA F-test.

[a-c] Means with the same letter, in the same row, are not significantly different at the 0.05 significance level.

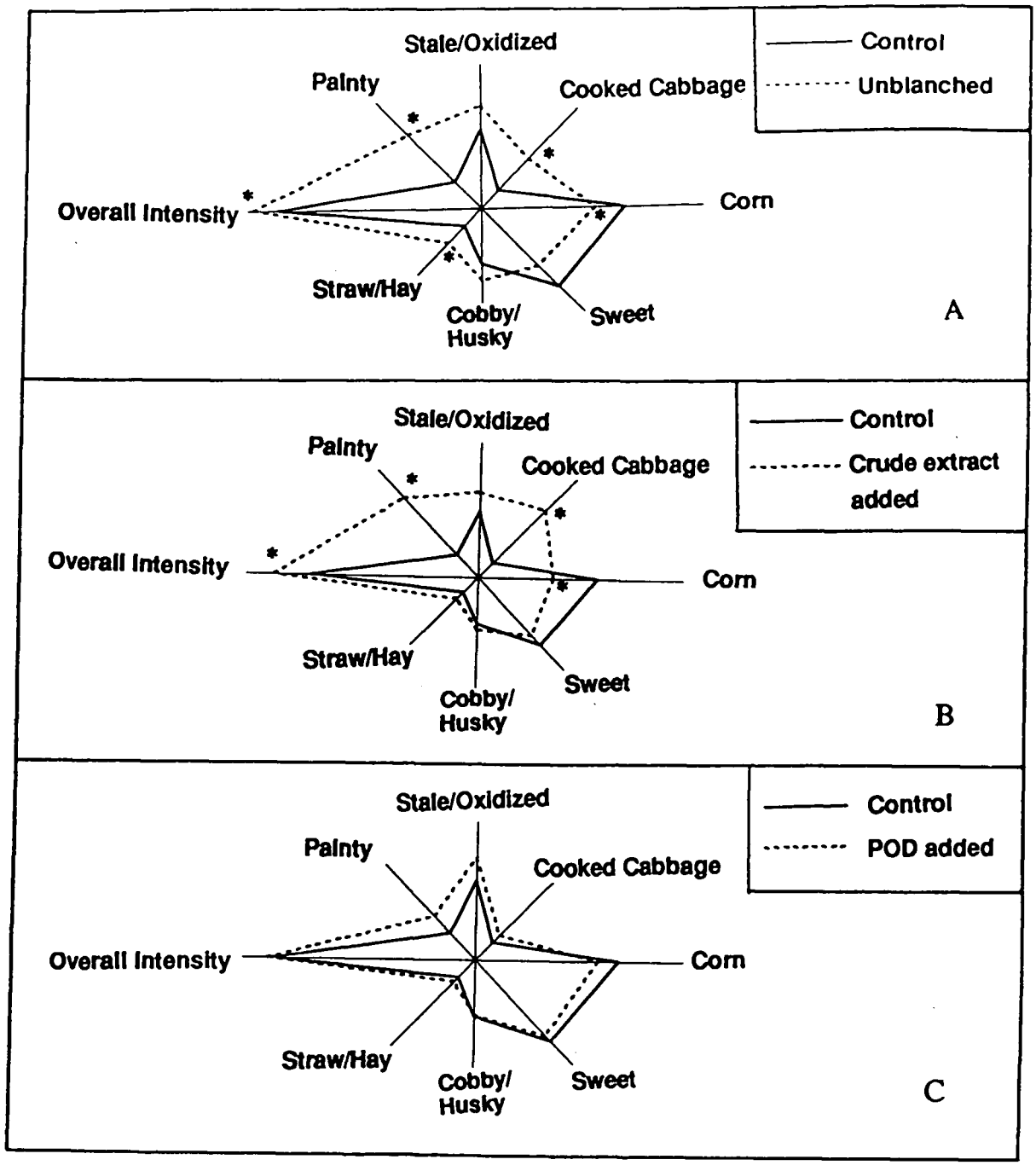

Fig. 5.9- A typical spider-web diagram of descriptive analysis for sweet corn homogenate samples (A=unblanched, B=added crude enzyme extract, and C=added POD; respectively) compared with the control; the distance from the center is the mean value for that aroma descriptor. The mean designated with "*" are statistically significant difference from those of control ($p \leq 0.05$).

control. However, adding crude extract, which contained the same total POD activity, resulted in significant increases in mean intensity of off-aroma descriptors "painty" and "cooked cabbage" and a significant decrease in mean intensity of on-aroma descriptor "corn". These results suggests that POD in sweet corn germs might not be important in off-aroma formation, especially in the formation of "painty" and "cooked cabbage" aroma. Velasco et al. (1989) also suggested that POD was not important in off-aroma in sweet corn. Moreover, there is evidence indicating that POD is not responsible for off-aroma formation in other plants, such as green bean, English pea, and broccoli (Williams et al., 1986; Lim et al., 1989).

CONCLUSIONS

There is significant evidence indicated that LPO in sweet corn germs is important in off-aroma formation; particularly for the aromas described as "painty" and "stale/oxidized". On the other hand, POD in sweet corn germs does not appear to be as important. Other enzymes in the germs may also be involved in off-aroma formation, especially the "cooked cabbage" aroma. Therefore, understanding the roles of other enzymes in sweet corn germs, singly and in combination, would be helpful in clearly identifying the complete enzyme systems involved in off-aroma formation in sweet corn. Moreover, studying the correlation of LPO activity, off-aroma descriptors and chemical off-aroma marker(s) would be also useful. Finally, the results suggest that LPO seems to be more important and appropriate than POD for use as a blanching index.

ACKNOWLEDGMENTS

Author Theerakulkait would like to express sincere gratitude to the International Rotary Foundation for providing him with a Rotary Freedom From Hunger scholarship for 3 years. He also would like to express sincere appreciation to the committee on Grants-in-Aid of Research, Sigma Xi, The Scientific Research Society for award him some grant in supporting the research. Authors would like to thank you very much for contribution and support from National Frozen Food Co., Albany, Oregon, in providing the sweet corn samples for the research, and Agricultural Research Foundation (Oregon Process Commission) for some financial support.

The authors also would like to thank you very much for the contribution, suggestion and/or support from the following persons: Dr. Reg McParland, Central Service Laboratory for Gene Research and Biotechnology, Oregon State University, Corvallis, OR; Dr. Dean Malencik at Dr. Sonia Anderson's laboratory, Dept. of Biochem. & Biophys., Oregon State University, Corvallis, OR; Dr. David Thomas, Dept. of Statistics, Oregon State University, Corvallis, OR; Dr. Sonia Rubico, Dept. of Food Sci. & Tech., Oregon State University, Corvallis, OR; Dr. John Whitaker, Dept. of Food Sci. & Tech., U. of California at Davis, Davis, CA; and Dr. Patricia Velasco. Special thanks to all sweet corn panelists for contribution of their valuable time and efforts.

REFERENCES

Ashraf, H-R.L. and Snyder, H.E. 1981. Influence of ethanolic soaking of soybeans on flavor and lipoxygenase activity of soymilk. J. Food Sci. 46: 1202-1204.

Bills, D.D. and Keenan, T.W. 1968. Dimethyl sulfide and its precursor in sweet corn. J. Agric. Food Chem. 16: 643-645.

Burnette, F.S. 1977. Peroxidase and its relationship to food flavor and quality: a review. J. Food Sci. 42: 1-6.

Civille, G.V. and Dus, C.A. 1992. Sensory evaluation of lipid oxidation in foods. Ch. 15, In *Lipid oxidation in food*, A.J.St. Angelo (Ed.), 279-291. American Chemical Society, Washington, DC.

Dignan, D.M. and Wiley, R.C. 1976. DMS levels in the aroma of cooked frozen sweet corn as affected by cultivar, maturity, blanching and packaging. J. Food Sci. 41: 346-348.

Eskin, N.A.M., Grossman, S. and Pinsky, A. 1977. Biochemistry of lipoxygenase in relation in food quality. CRC Crit. Rev. Food Sci. Nutr. 9: 1-40.

Flora, L.F., and Wiley, R.C. 1972. Effect of various endosperm mutants on oil content and fatty acid composition of whole kernel corn (*Zea mays* L.). J. Am. Soc. Hortic. Sci. 97(5): 604-607.

Flora, L.F. and Wiley, R.C. 1974. Sweet corn aroma, chemical components and relative importance in the overall flavor response. J. Food Sci. 39: 770-773.

Flurkey, W.H. and Jen, J.J. 1978. Peroxidase and polyphenol oxidase activities in developing peaches. J. Food Sci. 43: 1826-1828, 1831.

Gardner, H.W. 1970. Sequential enzymes of linoleic acid oxidation in corn germ: lipoxygenase and linoleate hydroperoxide isomerase. J. Lipid Res. 11: 311-321.

Gardner, H.W. and Inglett, G.E. 1971. Food products from corn germ: enzyme activity and oil stability. J. Food Sci. 36: 645-648.

Gibian, M.J. and Vandenberg, P. 1987. Product yield in oxygenation of linoleate by soybean lipoxygenase: the value of the molar extinction coefficient in spectrophotometric assay. Anal. Biochem. 163: 343-349.

Ganthavorn, G. and Powers, J.R. 1989. Partial purification and characterization of Asparagus Lipoxygenase. J. Food Sci. 52: 371-73.

Johnsen, P.B., Civille, G.V., Vercellotti, J.R., Senders, T.H. and Dus, C. A. 1988. Development of a lexicon for the description of peanut flavor. J. Sensory Studies. 3: 9-17.

Joslyn, M.A. 1949. Enzyme activity in frozen vegetable tissue. Adv. Enzymol. 9: 613-640.

Kalbrener, J.E., Warner, K. and Eldridge, A.C. 1974. Flavors derived from linoleic and linolenic acid hydroperoxides. Cereal Chem. 51: 406-416.

Lee, Y.C. 1981. Lipoxygenase and off-flavor development in some frozen foods. Korean J. Food Sci. Technol. 13: 53-56.

Lim, M.H., Velasco, P.J., Pangborn, R. M. and Whitaker, J. R. 1989. Enzymes involved in off-aroma formation in broccoli. Ch. 7, In *Quality Factors of Fruits and Vegetables*, J.J. Jen (Ed.), 72-83. American Chemical Society, Washington, DC.

Lundahl, D. S. and McDaniel, M.R. 1988. The panelist effect - fixed or random? J. Sensory Studies. 3: 113-121.

MacLeod, G. and Ames, J. 1988. Soy flavor and its improvement. CRC Crit. Rev. Food Sci. Nutr. 27: 219- 400.

McDaniel, M.R., Montgomery, M.W., Lantham, D. and Lundahl, D.S. 1988. Evaluation of high temperature-short time (HTST) blanched frozen sweet corn. J. Food Sci. 53(3): 760-764, 853.

Mistry, B.S. and Min, D.B. 1992. Oxidized flavor compounds in edible oils. In *Off-Flavors in Foods and Beverages*, G. Charalambous (Ed.), 171-209. Elsevier Science Publishers B.V., The Netherlands.

Morris, H.J. 1958. Application of peroxidase test paper in food processing. Food Technol. 12: 265-267.

Pascual, L.R. and Wiley, R.C. 1974. Fatty acids in the major lipid fractions of maturing sweet corn (*Zea mays* L.). J. Am. Soc. Hortic. Sci. 99(6): 573-576.

Power, J.J. 1988. Current practices and applications of descriptive methods. In *Sensory Analysis of Foods*, J.R. Piggot (Ed.), 187-266. Elsevier Applied Science, London.

Puangnak, W. 1976. Effect of hybrid, maturity and kernel structure on lipid content, composition and aroma development of sweet corn. M.S. thesis. University of Maryland, College Park.

SAS Institute, Inc. 1987. SAS/STAT™ Guide for personal computers. Version 6 Edition. SAS Institute Inc., Cary, NC.

Sheu, S.C. and Chen, A.O. 1991. Lipoxygenase as blanching index for frozen vegetable soybeans. J. Food Sci. 56(2): 448-451.

Smittle, D.A., Thornton, R.E. and Dean, B.B. 1972. Sweet corn quality deterioration: A survey of industry practices and possible methods of control. Wash. Agric. Expt. Stat. Circ. 555.

Steele, R.G.D., and Torrie, J.H. 1980. Analysis of variances III: Factorial experiments. Ch. 15, In *Principles and Procedures of Statistics- A Biomedical Approach*, R.G.D. Steele and J.H. Torrie (Eds.), 357-58. McGraw-Hill Book Co., Inc., New York, NY.

Stone, H., Sidel, J., Oliver, S., Woolsey, A., and Singleton, R.C. 1974. Sensory evaluation by quantitative descriptive analysis. Food Technol. 28: 24-34.

Theerakulkait, C. and Barrett, D.M. (a). Lipoxygenase in sweet corn germ: isolation and some physicochemical properties. In preparation for J. Food Sci.

Theerakulkait, C. and Barrett, D.M. (b). Partial purification and characterization of sweet corn germ lipoxygenase. In preparation for J. Food Biochem.

Velasco, P.J., Lim, M.H., Pangborn, R.M. and Whitaker, J.R. 1989. Enzymes responsible for off-flavor and off-aroma in blanched and frozen stored vegetables. Appl. Biochem. Biotechnol. 11: 118-127.

Wagenknecht, A.C. 1959. Lipoxidase activity and off-flavor in underblanched frozen corn-on-the-cob. Food Res. 24: 539-547.

Warner, K. 1985. Sensory Evaluation of Flavor Quality of Oils. Ch. 11, In *Flavor Chemistry of Fats and Oils*, D. Min and T. Smouse (Ed.), 207-221. American Oil Chemists' Society, Champaign, IL.

Weber, E.J. 1978a. Corn lipids. Cereal Chem. 55(5): 572-584.

Weber, E.J. 1978b. The lipids of corn germ and endosperm. J. Am. Oil Chem. Soc. 56: 637-641.

Wiley, R.C. 1985. Sweet corn aroma: studies of its chemical components and influence on flavor. Ch. 13, In *Evaluation of Quality of Fruits and Vegetables*, H.E. Patee (Ed.), 349-366. AVI Publishing Co., Westport, CT.

Williams, D.C., Lim, M.H., Chen, A.O., Pangborn, R.M., and Whitaker, J.R. 1986. Blanching of vegetables for freezing - which indicator enzyme to choose. Food Technol. 40(6): 130-140.

Williams, M.P. and Nelson, P.E. 1973. Effect of hybrids and processing on the dimethyl sulfide potential of sweet corn. J. Food Sci. 37: 408-410.

CHAPTER 6:
CONCLUSIONS

A higher concentration of lipoxygenase was found in the germ as compared to the degermed fraction of sweet corn (var. Jubilee) at the maturity selected for freezing, suggesting that industry should pay more attention to the germ fraction. The germ may be an important site for off-flavor and off-aroma formation in unblanched sweet corn due to enzymatic oxidation. The conditions for extraction and isolation of lipoxygenase from sweet corn germs were optimized for the first time. Determination of physicochemical properties such as pH and temperature optimum for activity, pH and temperature stability were also useful for providing a better understanding of lipoxygenase in germs.

Lipoxygenase was purified from the germs of sweet corn using a combination of conventional column chromatography and FPLC for the first time. This purification method would be useful for purifying lipoxygenase from germs in order to investigate other characteristics such as the role of lipoxygenase in off-aroma formation. Understanding the characteristics of the enzyme would be helpful for food scientists and technologists to explain and control the phenomena related to quality changes of sweet corn caused by the enzyme. Lipoxygenase activity was effectively inhibited by both synthetic and natural phenolic antioxidants, in particular, BHA and quercetin. Therefore, the heat treatment for blanching might be

reduced by combining the heat treatment with addition of a phenolic antioxidant.

There is significant evidence suggesting that lipoxygenase in sweet corn germs is important in off-aroma formation; particularly for the aromas described as "painty" and "stale/oxidized". On the other hand, peroxidase in sweet corn germs does not appear to be as important. Other enzymes in the germs may also be involved in off-aroma formation, especially for production of the "cooked cabbage" aroma. Therefore, understanding the roles of other enzymes in sweet corn germs, singly and in combination, would be helpful in clearly identifying the complete enzyme systems involved in off-aroma formation in sweet corn. Moreover, studying the correlation of lipoxygenase activity, off-aroma descriptors and chemical off-aroma marker(s) would be extremely beneficial. Results indicate that lipoxygenase might be more important and appropriate than peroxidase for use as a blanching index in the frozen sweet corn industry. We strongly recommend that the frozen sweet corn industry consider the use of lipoxygenase as a blanching index and support research activities related to development of a rapid method for lipoxygenase determination.

BIBLIOGRAPHY

Adams, J.B. and Ongley, M.H. 1989. The behavior of green bean lipoxygenase on chromatography and isoelectric focusing. Food Chem. 31: 57-71.

Al-obaidy, H.M. and Siddiqi, A.M. 1981a. Inhibition of broad bean lipoxygenase. J. Food Sci. 46: 597-605.

Al-obaidy, H.M. and Siddiqi, A.M. 1981b. Properties of broad bean lipoxygenase. J. Food Sci. 46: 622-626.

Alen, J.C., Eriksson, C., and Galpin, J.R. 1977. Affinity chromatography of lipoxygenases. Eur. J. Biochem. 73: 171-177.

Allen, J.C. 1968. Soybean lipoxygenase. I. purification and the effect of organic solvents upon the kinetics of the reaction. Eur. J. Biochem. 4: 201-208.

Alsoe, K.O. and Alder-Nissen, J. 1988. Thermal denaturation of lipoxygenase from soya beans in the presence of its natural substrate. Lebensm.-Wiss. u. Technol. 21: 8-12.

Arai, S., Nogushi, M., Kaji, M., Kato, H., and Fujimaki, M. 1970. n-Hexanal and some volatile: alcohols their distribution in raw soybean tissues and formation in crude soy protein concentrate by lipoxygenase. Agric. Biol. Chem. 34: 1420-1423.

Ashraf, H-R.L. and Snyder, H.E. 1981. Influence of ethanolic soaking of soybeans on flavor and lipoxygenase activity of soymilk. J. Food Sci. 46: 1202-1204.

Auerman, L.Y., Popov, M.P., Dubtsov, G.G., and Samsonov, M.M. 1971. Lipoxygenase activity of wheat grain and flour. Prikl. Biokhim. Mikrobiol. 7: 678-681.

Axelrod, B. 1974. Lipoxygenases. In *Food Related Enzymes*, Advances in Chemistry Series 136., J.R. Whitaker (ed.), 324-328. American Chemical Society, Washington, DC.

Belefant, H. and Fong, F. 1991. Lipoxygenases in developing *Zea mays* kernels. Plant Physiol. Biochem. 29(2): 99-104.

Ben Aziz, A., Grossman, S., and Ascarelli, I., and Budowski, P. 1970. Linoleate oxidation induced by lipoxygenase and heme proteins: A direct spectrophotometric assay. Anal. Biochem. 34: 88-100.

Bills, D.D. and Keenan, T.W. 1968. Dimethyl sulfide and its precursor in sweet corn. J. Agric. Food Chem. 16: 643-645.

Bottcher, H. 1975. Enzyme activity and quality of frozen vegetables. I. Remaining residues of peroxidase. Nahrung 19: 193-179.

Boyes, S., Perera, C., and Young, H. 1992. Kiwifruit lipoxygenase: preparation and characteristics. J. Food Sci. 57(6): 1390-1394.

Bradford, M.N. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72: 248-254.

Brandt, M.A.; Skinner, E.; and Coleman, J. 1963. Texture profile method. J. Food Sci. 28: 404-410.

Burnette, F.S. 1977. Peroxidase and its relationship to food flavor and quality: a review. J. Food Sci. 42: 1-6.

Carncross, W.E. and Sjostrom, L.B. 1950. Flavor profile: a new approach to flavor problems. Food Technol. 4: 308-311.

Catsimpoolas, N. 1969. Isolation of soybean lipoxidase by isoelectric focusing. Arch. Biochem. Biophys. 131: 185-190.

Chan, H.W.S. 1973. Soya-bean lipoxygenase: an iron-containing dioxygenase. Biochim. Biophys. Acta. 327: 32-35.

Chang, P.R.Q. and McCurdy, A.R. 1985. Lipoxygenase activity in fourteen legumes. Can. Inst. Food Sci. Technol. J. 18: 94-96.

Chen, A.O. and Whitaker, J.R. 1986. Purification and characterization of a lipoxygenase from immature English peas. J. Agric. Food Chem. 34: 203-211.

Christopher, J.P., Pistorius, E.K., and Axelrod, B. 1970. Isolation of an isozyme of soybean lipoxygenase. Biochim. Biophys. Acta. 198: 12-19.

Christopher, J.P., Pistorius, E.K., and Axelrod, B. 1972. Isolation of a third isozyme of soybean lipoxygenase. Biochim. Biophys. Acta. 284: 54-62.

Civille, G.V. and Dus, C.A. 1992. Sensory evaluation of lipid oxidation in foods. Ch. 15, In *Lipid oxidation in food*, A.J. St. Angelo (Ed.), 279-291. American Chemical Society, Washington, DC.

Cobb, B.G., and Hannah, L.C. 1981. The metabolism of sugars in maize endosperms. (Abstr.) Plant Physiol. 67: 107.

Creech, R.G. 1968. Carbohydrate synthesis in maize. Adv. Agron. 20: 275-322.

De Lumen, B.O. and Kazeniac, S.J. 1976. Staining for lipoxygenase activity in electrophoresis gel. Anal. Biochem. 72: 428-432.

Den, T.V. and Mendoza, E.M.T. 1982. Purification and characterization of two lipoxygenase isozymes from cowpea [*Vigna unguiculata* (L.) Walp.]. J. Agric. Food Chem. 30: 54-60.

Diel, E. and Stan, H-J. 1978. Purification and characterization of two isozymes of lipoxygenase from soybeans. Planta 142: 321-328.

Dignan, D.M. and Wiley, R.C. 1976. DMS levels in the aroma of cooked frozen sweet corn as affected by cultivar, maturity, blanching and packaging. J. Food Sci. 41: 346-348.

Doderer, A., Kokkelink, I.K., van der Veen, S., Valk, B.E., Schram, A. W., and Douma, A.C. 1992. Purification and characterization of two lipoxygenase isozymes from germinating barley. Biochim. Biophys. Acta. 1120: 97-104.

Dohi, T, Anamura, S., Shirakawa, M., Okamoo, H. and Tsujimoto, A. 1991. Inhibition of lipoxygenase by phenolic compounds. Jpn J. Pharmacol. 55: 547-550.

Draheim, J.E., Carroll, R.T., McNemar, T.B., Dunham, W.R., Sands, R. H. and Funk, M.O. J. 1989. Lipoxygenase isozymes: a spectroscopic and structural characterization of soybean seed enzymes. Arch. Biochem. Biophys. 269(1): 208-218.

Dreesen, T.D., Dickens, M. and Koch, R.B. 1982. Partial purification and characterization of a Ca^{2+}- stimulated lipoxygenase from soybean seeds. Lipids. 17(12): 964-969.

Egmond, M.R., Vliegenthart, J.F.G. and Boldingh, J. 1972. Stereospecificity of the hydrogen abstraction at carbon atom n-8 in the oxygenation of linoleic acid by lipoxygenases from corn germs and soya beans. Biochem. Biophys. Res. Commun. 48(5): 1055-1060.

Einstein, M.A. 1991. Descriptive techniques and their hybridization. In *Sensory Science Theory and Applications in Foods*, H.T. Lawless and B.P. Klein (eds.), 317-338. Marcel Dekker, Inc., New York.

Eskin, N.A.M., Grossman, S. and Pinsky, A. 1977. Biochemistry of lipoxygenase in relation in food quality. CRC Crit. Rev. Food Sci. Nutr. 9: 1-40.

Evan, C.D., frankel, E.N., Cooney, P.M., and Moser, H.A. 1960. Effects of autoxidation prior to deodorization on oxidative and flavor stability of soybean oil. J. Am. Oil Chem. Soc. 37: 452-456.

Farkas, D.F. and Goldsmith, S.A. 1962. Studies on the kinetics of lipoxidase inactivation using thermal and ionizing energy. J. Food Sci. 27: 262-276.

Faure, M., Lissi, E., Torres, R., and Videla, L.A. 1990. Antioxidant activities of lignans and flavonoids. Phytochemistry 29(12): 3773-3775.

Finazzi-Agro, A., Avigliano, L., Veldink, G.A., Vliegenthart, J.F.G., and Boldigh, J. 1973. The influence of oxygen on the fluorescence of lipoxygenase. Biochim. Biophys. Acta. 326: 462-470.

Flora, L.F., and Wiley, R.C. 1972. Effect of various endosperm mutants on oil content and fatty acid composition of whole kernel corn (*Zea mays* L.). J. Am. Soc. Hortic. Sci. 97(5): 604-607.

Flora, L.F. and Wiley, R.C. 1974. Sweet corn aroma, chemical components and relative importance in the overall flavor response. J. Food Sci. 39: 770-773.

Flurkey, W.H. and Jen, J.J. 1978. Peroxidase and polyphenol oxidase activities in developing peaches. J. Food Sci. 43: 1826-1828, 1831.

Fong, F., and Smith, J.D., 1985. Rapid isolation and purification of immature zygotic embryos. Maize Gen. Coop. Newslett. 59: 30-31.

Funk, M.O., Andre, J.C., Otsuki, T. 1987. Oxygenation of trans polyunsaturated fatty acids by lipoxygenase reveals steric features of the catalytic mechanism. Biochemistry 26: 6880-6884.

Funk, M.O., Whitney, M.A., Hausknecht, E.C., O'Brien, E.M. 1985. Resolution of the isozymes of soybean lipoxygenase using isoelectric focusing and chromatofocusing. Anal. Biochem. 146: 246-251.

Galliard, T. and Chan, H.W.S. 1980. Lipoxygenases. In *The Biochemistry of Plants.* A Comprehensive Treatise, Vol.4, P.K. Stumpf (ed.), 131-161, Academic Press, Inc., New York.

Galliard, T. 1983. Enzymatic degradation of cereal lipids. Ch. 6. In *Lipids in Cereal Technology*, P.J. Barnes (ed.), 111-147, Academic Press, Inc., New York.

Ganthavorn, C., Nagel, C.W. and Powers, J.R. 1991. Thermal inactivation of asparagus lipoxygenase and peroxidase. J. Food Sci. 56(1): 47-49, and 79.

Ganthavorn, G. and Powers, J.R. 1989. Partial purification and characterization of Asparagus Lipoxygenase. J. Food Sci. 52: 371-73.

Gardner, H.W. 1970. Sequential enzymes of linoleic acid oxidation in corn germ: lipoxygenase and linoleate hydroperoxide isomerase. J. Lipid Res. 11: 311-321.

Gardner, H.W. 1975. Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with nonenzymic. J. Agric. Food Chem. 23(2): 129-136.

Gardner, H.W. 1980. Lipid enzymes: lipases, lipoxygenases and "hydroperoxidases". In *Autoxidation in Food and Biological Systems*, M.G. Simic and M. Karel (eds.), 447-504. Plenum Press, New York.

Gardner, H.W. 1985. Flavors and bitter tastes from oxidation of lipids by enzymes. Ch. 10, In *Flavor Chemistry of Fat and Oils*, D.B. Min and T.H. Smouse (Eds.), 189-206. American Oil Chemists' Society, Champaign, IL.

Gardner, H.W. 1988. Lipoxygenase pathway in cereals. Ch. 6, In *Advances in Cereal Science and Technology*, Vol. 9, Y. Pomeranz (Ed.), 161-215. American Association of Cereal Chemists, Incorporated, St. Paul, Minnesota.

Gardner, H.W. 1991. Recent investigations into the lipoxygenase pathway of plants. Biochim. Biophys. Acta. 1084: 221-239.

Gardner, H.W. and Inglett, G.E. 1971. Food products from corn germ: enzyme activity and oil stability. J. Food Sci. 36: 645-648.

Gardner, H.W., Kleiman, R., Christianson, D.D., and Weisleder, D. 1975. Positional specificity of γ-ketol formation from linoleic acid hydroperoxides by a corn germ enzyme. Lipids. 10(10): 602-608.

Gardner, H.W. and Weisleder, D. 1970. Lipoxygenase from *Zea mays*: 9-D-hydroperoxy-*trans*-10,*cis*-12-octadecadienoic acid from linoleic acid. Lipids. 5: 678-683.

Gegenheimer, P. 1990. Preparation of extracts from plants. Ch. 14, In Guide to Protein Purification. *Meth. Enzymol.*, Vol. 182, M.P. Deutscher (Ed.), 174-193. Academic Press, Inc., San Diego, California.

Gibian, M.J. and Vandenberg, P. 1987. Product yield in oxygenation of linoleate by soybean lipoxygenase: the value of the molar extinction coefficient in spectrophotometric assay. Anal. Biochem. 163: 343-349.

Grosch, W., Laskawy, G., and Weber, F. 1976. Formation of volatile carbonyl compounds and cooxidation of carotene by lipoxygenase from wheat, potato, flax, and beans. J. Agric. Food Chem. 24: 456-459.

Grossman, S., Ben-Aziz, A., Budowski, P., Ascarelli, I., Gertler, A., Birk, Y. and Bondi, A. 1969. Enzymic oxidation of carotene and linoleate by alfalfa: extraction and separation of active fractions. Phytochemistry 8: 2287-2293.

Grossman, S., Trop, M., Avtalion, R. and Pinsky, A. 1972a. Egg plant lipoxygenase: isolation and partial characterization. Lipids. 7: 467-473.

Grossman, S., Trop, M., Yaroni, S., and Wilchek, M. 1972b. Purification of soybean lipoxygenase by affinity chromatography. Biochim. Biophys. Acta. 289: 77-81.

Grossman, S. and Zakut, R. 1979. Determination of activity of lipoxygenase (lipoxidase). In *Methods of Biochemical Analysis,* Vol. 25, D. Glick. (ed.), 303-329. J. Wiley and Sons, New York.

Guss, P.L., Richardson, T., and Stahmann, M.A. 1967. The oxidation-reduction enzymes of wheat. III. Isozymes of lipoxidase in wheat fractions and soybean. Cereal Chem. 44: 607-610.

Haigh, R. 1986. Safety and necessity of antioxidants: EEC approach. Food Chem. Toxicol. 24: 1031-1034.

Halpin, B.E. and Lee, C.Y. 1987. Effect of blanching on enzyme activity and quality changes in green peas. J. Food Sci. 52: 1002-1005.

Hamberg, M. and Samuelsson, B. 1967. On the specificity of the oxygenation of unsaturated fatty acids catalyzed by soybean lipoxidase. J. Biol. Chem. 242: 5329-5335.

Hayase, F. and Kato, H. 1984. Antioxidative components of sweet potatoes. J. Nutr. Sci. Vitaminol. 30: 37-46.

Haydar, M. and Hadziyev, D. 1973. A study of lipoxidase in pea seeds and seedlings. J. Sci. Food. Agric. 24: 1039-1053.

Hidaka, T., Katsuki, S. and Nagata, Y. 1986. Partial purification and properties of pumpkin lipoxygenase with carotene-bleaching activity. J. Food Biochem. 10: 55-73.

Hildebrand, D.F. 1989. Lipoxygenases. Physiol. Plant. 76: 249-253.

Hildebrand, D.F., Hamilton-Kemp, T.R., Legg, C.S. and Bookjans, G. 1988. Plant lipoxygenase: occurrence, properties and possible functions. Curr. Top. Plant Biochem. Physiol. 7: 201-219.

Holman, R.T. 1955. Measurement of lipoxidase activity. In *Methods of Biochemical Analysis*, Vol. 2, D. Glick. (ed.), 113-119. J. Wiley and Sons, New York.

Holman, R.T., Egwim, P.O., and Christie, W.W. 1969. Substrate specificity of soybean lipoxidase. J. Biol. Chem. 244: 1149-1151.

Husain, R.S., Cillard, J. and Cillard, P. 1987. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 26(9): 2489-2491.

Ida, S., Masaki, Y., and Morita, Y. 1983. The isolation of multiple forms and product specificity of rice lipoxygenase. Agric. Biol. Chem. 47: 637-641.

Imida, K., Fukushima, S., Shivaiet, T., Ohtani, M., Nakanishi, K., and Ito, N. 1983. Promoting activities of butylated hydroxyanide and butylated hydroxytoluene on 2-stages urinary bladder carcinogenesis and inhibition of γ-glutamyl transpeptidase-positive foci development in the liver of rats. Carcinogenesis. 4: 895.

Johnsen, P.B., Civille, G.V., Vercellotti, J.R., Senders, T.H. and Dus, C. A. 1988. Development of a lexicon for the description of peanut flavor. J. Sensory Studies 3: 9-17.

Joslyn, M.A. 1949. Enzyme activity in frozen vegetable tissue. Adv. Enzymol. 9: 613-640.

Kalbrener, J.E., Warner, K. and Eldridge, A.C. 1974. Flavors derived from linoleic and linolenic acid hydroperoxides. Cereal Chem. 51: 406-416.

Kato, T., Ohta, H., Tanaka, K. and Shibata, D. 1992. Appearance of new lipoxygenases in soybean cotyledons after germination and evidence for expression of a major new lipoxygenase gene. Plant Physiol. 98: 324-330.

Kazeniac, S. and Hall, R.M. 1970. Flavor chemistry of tomato volatiles. J. Food Sci. 35: 519-530.

Khalil, T. and Kramer, A. 1971. Histological and histochemical studies of sweet corn (*Zea mays* L.) pericarp as influenced by maturity and processing. J. Food Sci. 36: 1064-1069.

King, D.L. and Klein, B.P. 1987. Effect of flavonoids and related compounds on soybean lipoxygenase-1 activity. J. Food Sci. 52(1): 220-221.

Kinsella, J.E. and Damodaran, S. 1980. Flavor problems in soy proteins: origin, nature, control and binding phenomena. In *The analysis and Control of Less desirable Flavors in Foods and Beverages*, 95-131. Charalambous, G. (ed.), 95-131. Academic Press, Inc., New York.

Klein, B.P. 1976. Isolation of lipoxygenase from split pea seeds, snap beans, and peas. J. Agric. Food Chem. 24(5): 938-942.

Koch, R.B., Brumfiel, B.L., and Brumfiel, M.N. 1971. Calcium requirement for lipoxygenase catalyzed linoleate oxidation. J. Am. Oil Chem. Soc. 48: 532-538.

Koch, R.B., Stein, B. and Ferrari, C.G. 1958. Linoleic acid and trilinolein as substrates for soybean lipoxidase(s). Arch. Biochem. Biophys. 78: 165-179.

Kon, S., Wagner, J.R., Guadagni, D.G., and Horvat, R.J. 1970. pH adjustment control of oxidative off-flavors during grinding of rax legume seeds. J. Food Sci. 35: 343-345.

Kuhn, H., Schewe, T. and Rapoport, S. 1982. The stereochemistry of the reactions of lipoxygenases and their metabolites. proposed nomenclature of lipoxygenases and related enzymes. In *Free radicals in Biology*, Vol. V, W.A. Pryor (ed.), 274-311. Academic Press, Inc., New York.

Labuza, T.P. 1971. Kinetics of lipid oxidation in foods. CRC Crit. Rev. Food Technol. 2: 355-405.

Larson, R.A. 1988. The antioxidants of higher plants. Phytochemistry 27(4): 969-978.

Lee, C.Y., Smith, N.L. and Hawbecker, D.E. 1989. An improved blanching technique for frozen sweet corn on-the-cob. Tech. innov. in freezing and refrigeration. UC Davis July 9-12: 85-90.

Lee, C.Y., Smith, N.L., and Hawbecker, D.E. 1988. Enzyme activity and quality of frozen green beans and affected by blanching and storage. J. Food Quality 11: 279-287.

Lee, F.A. 1954. Chemical changes taking place in the crude lipids during the storage of frozen raw vegetables. Food Res. 19: 515-520.

Lee, F.A. and Wagenknecht, A.C. 1951. On the development of off-flavor during the storage of frozen raw peas. Food Res. 16: 239-244.

Lee, F.A. and Wagenknecht, A.C. 1958. Enzyme action and off-flavor in frozen peas II. the use of enzymes prepared from garden peas. Food Res. 23: 584-590.

Lee, F.A. and Wagenknecht, A.C., and Hening, J.C. 1955. A chemical study of the progressive development of off-flavor in frozen raw vegetables. Food Res. 20: 298-297.

Lee, Y.C. 1981. Lipoxygenase and off-flavor Development in some frozen foods. Korean J. Food Sci. Technol. 13: 53-56.

Leighton, T., Ginther, C., Nortario, V., Harter, W. 1991. Quercetin and quercetin glycosides: genetic regulation, metabolic processing, and *in vitro* effects on oncogenic transformation. Presented at the 202nd National Meeting of the American Chemical Society, New York, NY, Symposium on Phenolic Compounds in Foods and Health, AGFD Abstract No. 116.

Leoni, O., Iori, R. and Palmieri, S. 1985. Purification and properties of lipoxygenase in germinating sunflower seeds. J. Food Sci. 50: 88-92.

Letan, A. 1966. The relation of structure to antioxidant activity of quercetin and some of its derivatives. I. primary activity. J. Food Sci. 31: 518-523.

Lilius, E.M. and Laasko, S. 1982. A sensitive lipoxygenase assay based on chemiluminescence. Anal. Biochem. 119: 135-141.

Lim, M.H., Velasco, P.J., Pangborn, R.M. and Whitaker, J.R. 1989. Enzymes involved in off-aroma formation in broccoli. Ch. 7, In *Quality Factors of Fruits and Vegetables*. J. J. Jen (Ed.), 72-83. American Chemical Society, Washington, DC.

Lu, A.T. and Whitaker, J.R. 1974. Some factors affecting rates of heat inactivation and reactivation of horseradish peroxidase. J. Food Sci. 36: 1173-1178.

Lulai, E.C. and Baker, C.W. 1976. Physicochemical characterization of barley lipoxygenase. Cereal Chem. 53: 777-786.

Lulai, E.C. and Baker, C.W. and Zimmerman, D.C. 1981. Metabolism of linoleic acid by berley lipoxygenase and hydroperoxide isomerase. Plant Physiol. 68: 950-955.

Lundahl, D.S. and McDaniel, M.R. 1988. The panelist effect - fixed or random? J. Sensory Studies 3: 113-121.

Mack, A.J., Peterman, T.K. and Siedow, J.N. 1987. In *Isozymes: Current Topics in Biological and Medical Research,* Vol. 13, M. C. Rattazzi, Scandalios and G.S. Whitt (eds.), 127-154. Alan R. Liss, Inc., New York.

MacLeod, G. and Ames, J. 1988. Soy flavor and its improvement. CRC Crit. Rev. Food Sci. Nutr. 27: 323-327.

Maeura, Y., Weisburger, J.H., and Williams, G. 1984. Dose-dependent reduction of N-2-fluorenylacetamide-induced liver cancer and enhancement of bladder cancer in rats by butylated hydroxytoluene. Cancer Res. 44: 1604-1610.

Marcus, L., Prusky, D. and Jacoby, B. 1988. Purification and characterization of avocado lipoxygenase. Phytochemistry 27(2): 323-327.

Marshall, S.W. 1987. Sweet corn. Ch. 14, In *Corn Chemistry and Technology*, S.A. Watson and P.E. Ramstad (eds.), 431-445. Am. Assoc. of Cereal Chemists, Inc., St. Paul, Minnesota.

Matoba, T., Hidaka, H., Narita, H., Kitamura, K., Kaizuma, N., and Kato, M. 1985. Lipoxygenase-2 isozyme is responsible for generation of n-hexanal in soybean homogenate. J. Agric. Food Chem. 33: 852-855.

Matsui K., Irie, M., Kajiwara, T., Kakuno, T. and Hatanaka, A. 1993. Rapid degradation of cucumber cotyledon lipoxygenase. Phytochemistry 32(6): 1387-1391.

Mattick, L.R. and Hand, D.R. 1969. Identification of a volatile component in soybeans that contributes to raw bean flavor. J. Agric. Food Chem. 17: 15-17.

McConnell, J.E.W. 1956. Enzyme-heat resistance and regeneration: review of literature on thermal inactivation of enzymes in foods. National Canners Association. 1-12.

McDaniel, M.R., Montgomery, M.W., Lantham, D. and Lundahl, D.S. 1988. Evaluation of high temperature-short time (HTST) blanched frozen sweet corn. J. Food Sci. 53(3): 760-764, 853.

Meilgaard, M., Civille, G.V., and Carr, B.T. 1987. Descriptive analysis techniques. In *Sensory Evaluation Techniques*. 119-127. CRC Press, Boca Raton, FL.

Mistry, B.S. and Min, D.B. 1992. Oxidized flavor compounds in edible oils. In *Off-Flavors in Foods and Beverages*, G. Charalambous (Ed.), 171-209. Elsevier Science Publishers B.V., The Netherlands.

Mitsuda, H., Yasumoto, K., Yamamoto, A., and Kusano, T. 1967. Study on lipoxygenase part I. preparation of crystalline enzyme and assay by polarographic method. Agric. Biol. Chem. 31(1): 115-118.

Morris, H.J. 1958. Application of peroxidase test paper in food processing. Food Technol. 12: 265-267.

Naim, M., Gestetner, B., Bondi, A. and Birk, Y. 1976. Antioxidative and antihemolytic activities of soybean isoflavones. J. Agric. Food Chem. 24: 1174-1176.

Naito, S., Yamaguchi, N. and Yokoo, Y. 1982. Fractionation of antioxidants from cacao bean husk (studies on natural antioxidant. part IV). J. Jpn. Soc. Food. Sci. Technol. 29: 529-533.

Navaratnam, S., Feiters, M.C., Al-Hakim, M., Allen, J.C., Veldink, G.A., and Vliegenthart, J.F.G. 1988. Iron environment in soybean lipoxygenase-1. Biochim. Biophys. Acta. 956: 70-76.

Nelson, M.J. 1988. Catecholate complexes of ferric soybean lipoxygenase 1. Biochemistry 27: 4273-4278.

Nelson, M.J., Batt, D.G., Thompson, J.S., and Wright, S.W. 1991. Reduction of the active site iron by potent inhibitors. J. Biol. Chem. 266(13): 8225-8229.

Nicholas, J., Autran, M., and Drapron, R. 1982. Purification and some properties of wheat germ lipoxygenase. J. Sci. Food Agric. 33: 53-63.

Nicholas, J. and Drapron, R. 1977. Some physicochemical properties of horse bean (*Vicia faba* L.). Ann. Technol. Agric. 26: 119-132.

Ohta, H, Ida, S., Mikami, B. and Morita, Y. 1986. Purification and characterization of rice lipoxygenase component 3 from embryo. Agric. Biol. Chem. 50(12): 3165-3171.

Oszmianski, J. and Lee, C.Y. 1990. Inhibitory effect of phenolics on carotene bleaching in vegetables. J. Agric. Food Chem. 38: 688-690.

Park, K-H., Kim, Y-M. and Lee, C-W. 1988. Thermal inactivation kinetics of potato tuber lipoxygenase. J. Agric. Food Chem. 36(5): 1012-1015.

Pascual, L.R. and Wiley, R.C. 1974. Fatty acids in the major lipid fractions of maturing sweet corn (*Zea mays* L.). J. Am. Soc. Hortic. Sci. 99(6): 573-576.

Penefsky, H.S., and Tzagoloff, A. 1971. Extraction of water-soluble enzymes and protein from membrane. Ch. 19, In *Meth. Enzymol.*, Vol. 22, W.B. Jakoby (Ed.), 204-219. Academic Press, Inc., New York.

Pinsky, A., Grossman, S. and Trop, M. 1971. Lipoxygenase content and antioxidant activity of some fruits and vegetables. J. Food Sci. 36: 571-572.

Pistorius, E.K. and Axelrod, B. 1974. Iron, an essential component of lipoxygenase. J. Biol. Chem. 249: 3183-3186.

Poca, E., Rabinovitch-Chable, H., Cook-Moreau, J., Montserrat, P., and Rigaud, M. 1990. Lipoxygenases from *Zea mays* L. purification and physicochemical characteristics. Biochim. Biophys. Acta. 1045: 107-114.

Power, J.J. 1988. Current practices and applications of descriptive methods. In *Sensory Analysis of Foods*, J.R. Piggot (Ed.), 187-266. Elsevier Applied Science, London.

Pratt, D.E. 1965. Lipid antioxidants in plant tissue. J. Food Sci. 30: 737-741.

Puangnak, W. 1976. Effect of hybrid, maturity and kernel structure on lipid content, composition and aroma development of sweet corn. M.S. thesis. University of Maryland, College Park.

Rabinovitch-Chable, H., Cook-Moreau, J. Breton, J-C. and Rigaud, M. 1992. Purification of lipoxygenase and hydroperoxide dehydrase in flaxseeds: interaction between these enzymatic activities. Biochem. Biophys. Res. Commun. 188(2): 858-864.

Rackis, J.J., Honig, D.H., Sessa, D.J. and Moser, H.A. 1972. Lipoxygenase and peroxidase activities of soybeans as related to the flavor profile during maturation. Cereal Chem. 49: 586-597.

Rackis, J.J., Sessa, D.J. and Honig, D.H. 1979. Flavor problems of vegetable food proteins. J. Am. Oil Chem. Soc. 56: 262-271.

Rainey, B.A. 1986. Importance of reference standards in training panelists. J. Sensory Studies 1: 149-154.

Ramadoss, C.S., Pistorius, E.K., Axelrod, B. 1982. High performance liquid chromatographic separation of lipoxygenase isozymes in crude soybean extracts. Anal. Biochem. 127: 25-31.

Reid, D.S. 1990. Optimizing the quality of frozen foods. Food Technol. 44(7): 78-82.

Reynolds P.A. and Klein, B.P. 1982. Purification and characterization of a type-1 lipoxygenase from pea seeds. J. Agric. Food Chem. 30: 1157-1163.

Rhee, K.S. and Watts, B.M. 1966. Effect of antioxidants on lipoxygenase activity in model systems and pea (*Pisum sativum*) slurries. J. Food Sci. 31: 699-674.

Roza, M. and Francke, A. 1973. Soyabean lipoxygenase: An iron-containing enzyme. Biochim. Biophys. Acta. 327: 24-31.

Sanders, T.H., Pattee, H.E. and Singleton, J.A. 1975. Lipoxygenase isozymes of peanut. Lipids. 10(11): 681-685.

Sanz, L.C., Perez, A.G. and Olias, J.M. 1992a. Purification and catalytic properties of chickpea lipoxygenases. Phytochemistry 31(9): 2967-2972.

Sanz, L.C., Perez, A.G., Rios, J.J. and Olias, J.M. 1992b. Physico-chemical properties of chickpea lipoxygenase. Phytochemistry 31(10): 3381-3384.

Sanz, L.C., Perez, A.G., Rios, J.J. and Olias, J.M. 1993. Positional specificity of ketodienes from linoleic acid aerobically formed by lipoxygenase isozymes from kidney bean and pea. J. Agric. Food Chem. 41(5): 696-699.

SAS Institute, Inc. 1987. SAS/STAT™ Guide for personal computers. Version 6 Edition. SAS Institute Inc., Cary, NC.

Schewe, T., Rapoport, S. and Kuhn, H. 1986. Enzymology and physiology of reticulocyte lipoxygenase: comparison with other lipoxygenases. In *Adv. in Enzymology and Related Areas of Molecular Biology*, Vol. 58, A. Meister (ed.), 191-272. An Interscience Publication, John Wiley & Sons, New York.

Schilstra, M.J., Veldink, G.A., and Vliegenthart, F.G. 1993. Kinetic analysis of the induction period in lipoxygenase catalysis. Biochemistry 32: 7686-7691.

Schwimmer, S. 1981. Source Book of Food Enzymology. AVI Publishing Co., Inc., Westport, CT.

Sessa, D.J. 1979. Biochemical aspects of lipid-derived flavors in legumes. J Agric. Food Chem. 27(2): 234-239.

Sheu, S.C. and Chen, A.O. 1991. Lipoxygenase as blanching index for frozen vegetable soybeans. J. Food Sci. 56(2): 448-451.

Shibata, D., Steczko, J., Dixon, J.E., Andrews, P.C., Hermodson, M., and Axelrod, B. 1988. Primary structure of soybean lipoxygenase L-2. J. Biol. Chem. 263: 6816-6821.

Shibata, D., Steczko, J., Dixon, J.E., Hermodson, M., Yazdanparast, R. and Axelrod, B. 1987. Primary structure of soybean lipoxygenase L-1. J. Biol. Chem. 262: 10080-10085.

Shiiba, K., Negishi, Y., Okada, K. and Nagao, S. 1991. Purification and characterization of lipoxygenase isozymes from wheat germ. Cereal Chem. 68(2): 115-122.

Shkarina, T.N., Kuhn, H. and Schewe, T. 1992. Specificity of soybean lipoxygenase-1 in hydrated reverse micelles of sodium bis (2-ethylhexyl) sulfosuccinate (areosol OT). Lipids. 27(9): 690-693.

Siedow, J.N. 1991. Plant lipoxygenase: structure and function. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 145-188.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fugimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150: 76-85.

Smittle, D.A., Thornton, R.E. and Dean, B.B. 1972. Sweet corn quality deterioration: A survey of industry practices and possible methods of control. Wash. Agric. Expt. Stat. Circ. 555.

Song, Y., Love, M.H. and Muphy, P. 1990. Subcellular localization of lipoxygenase-1 and -2 in germinating soybean seeds and seedlings. J. Amer. Oil Chem. Soc. 67(12): 961-965.

Steele, R.G.D., and Torrie, J.H. 1980. Analysis of variances III: Factorial experiments. Ch. 15, In *Principles and Procedures of Statistics- A Biomedical Approach*, R.G.D Steele and J.H. Torrie (Eds.), 357-58. McGraw-Hill Book Co., Inc., New York.

Stevens, F.C., Brown, D.M., and Smith, E.L. 1970. Some properties of soybean lipoxygenase. Arch. Biochem. Biophys. 136: 413-421.

Stone, H., Sidel, J., 1985. Descriptive analysis. In *Sensory Evaluation Practices*, 194-225, Academic Press, Inc., New York.

Stone, H., Sidel, J., Oliver, S., Woolsey, A., and Singleton, R.C. 1974. Sensory evaluation by quantitative descriptive analysis. Food Technol. 28(11): 24-34.

Surrey, K. 1964. Spectrophotometric method for determination of lipoxidase activity. Plant Physiol. 39: 65-70.

Svensson, S.G. and Eriksson, C.E. 1972. Thermal inactivation of lipoxygenase from peas (*Pisum sativum* L.) II. influence of substrate and reaction products. Lebensm.-Wiss. u. Technol. 5(4): 124-127.

Svensson, S.G. and Eriksson, C.E. 1974. Thermal inactivation of lipoxygenase from peas (*Pisum sativum* L.) III. activation energy obtained from single heat treatment experiments. Lebensm.-Wiss. u. Technol. 7(3): 142-144.

Szczesniak, A.S. 1963. Classification of textural characteristics. J. Food Sci. 28: 385-389.

Szczesniak, A.S., Brandt, M.A., and Friedman, H.H. 1963. Development of standard rating scales for mechanical parameters of texture and correlation between the objective and the sensory methods of texture evaluation. J. Food Sci. 28: 397-403.

Takahama, U. 1985. Inhibition of lipoxygenase-dependent lipid peroxidation by quercetin: mechanism of antioxidative function. Phytochemistry 24(7): 1443-1446.

Tappel, A.L. 1963. Lipoxidase. In *The Enzymes*, Vol. 8, P.D. Boyer, H. Lardy and K. Myrback (eds.), 275-283, Academic Press, Inc., New York.

Tappel, A.L., Lundberg, W.O. and Boyer, P.D. 1953. Effect of temperature and antioxidants upon the lipoxidase-catalyzed oxidation of sodium linoleate. Arch. Biochem. Biophys. 42: 293-304.

Theerakulkait, C. and Barrett, D.M. (a). Lipoxygenase in sweet corn germ: isolation and some physicochemical properties. In preparation for J. Food Sci.

Theerakulkait, C. and Barrett, D.M. (b). Partial purification and characterization of sweet corn germ lipoxygenase. In preparation for J. Food Biochem.

Theorell, H., Holman, R.T., and Akeson, A. 1947a. Crystalline lipoxidase. Acta Chem. Scand. 1: 571-76.

Theorell, H., Holman, R. T., and Akeson, A. 1947b. A note on the preparation of crystalline soybean lipoxidase. Arch. Biochem. 14: 250-252.

Torel, J., Cillard, J. and Cillard, P. 1986. Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry 25(2): 383-385.

USDA. 1988. Agricultural Statistics. United States Government Printing Office, Washington, DC.

van Aarle, P.G.M., de Barse, M.M.J., Veldink, G.A. and Vliegenthart, F.G. 1991. Purification of lipoxygenase from ungerminated barley: characterization and product formation. FEBS Lett. 280 (1): 159-162.

Velasco, P.J., Lim, M.H., Pangborn, R.M. and Whitaker, J.R. 1989. Enzymes responsible for off-flavor and off-aroma in blanched and frozen stored vegetables. Appl. Biochem. Biotechnol. 11: 118-127.

Veldink, G.A., Garssen, G.J., Vliegenthart, J.F.G., and Boldingh, J. 1972. Positional specificity of corn germ lipoxygenase as a function of pH. Biochem. Biophys. Res. Commun. 47(1): 22-26.

Veldink, G.A., Vliegenthart, J.F.G. and Boldingh, J. 1977. Plant lipoxygenase. Prog. Chem. Fats Other Lipids. 15: 131-166.

Verma, A.K. 1991. Anticarcinogenicity of the flavonol quercetin. Presented at the 202nd National Meeting of the American Chemical Society, New York, NY, Symposium on Phenolic Compounds in Foods and Health, AGFD Abstract No. 113.

Vernooy-Gerritsen, M., Veldink, G.A., Vliegenthart, J.F.G. 1982. Specificities of antisera directed against soybean lipoxygenase-I and -II and purification of lipoxygenase-II by affinity chromatography. Biochim. Biophys. Acta. 708: 330-334.

Vick, B.A. and Zimmerman, D.C. 1987. Oxidative systems for modification of fatty acids: the lipoxygenase pathway. In *The Biochemistry of Plants*, Vol. 9 (Lipids: structure and function). Stumpf, P.K. (ed)., 53-90. Academic Press, Inc., New York.

Vliegenthart, J.F.G. and Veldink, G.A. 1982. Lipoxygenases. Ch. 2, In *Free Radicals in Biology*, Vol. V, W.A. Pryor (ed.), 29-64. Academic Press, Inc., New York.

Vliegenthart, J.F.G., Veldink, G.A., Verhagen, J., and Slappendel, S. 1983. Lipoxygenases from plant and animal origin. In *Biological Oxidations*, H. Sund and V. Ullrich (eds.) 203-223. Springer-Verlag, New York.

Von Ceumern, S. and Hartfiel, W. 1984. Activity of lipoxygenase in cereals and possibilities of enzyme inhibition. Fette Seifen Anstrichm. 86: 204-208.

Wagenknecht, A.C. 1959. Lipoxidase activity and off-flavor in underblanched frozen corn-on-the-cob. Food Res. 24: 539-547.

Wagenknecht, A.C. and Lee, F.A. 1956. The action of lipoxidase in frozen raw peas. Food Res. 21: 605-610.

Wagenknecht, A.C. and Lee, F.A. 1958. Enzyme action and off-flavor in frozen peas. Food Res. 23: 25-31.

Wallace, J.M. and Wheeler, E.L. 1979. Two lipoxygenase isozymes and an activator in wheat germ. Phytochemistry 18: 389-393.

Wardale, D.A. and Galliard, T. 1975. Subcellular localization of lipoxygenase and lipolytic acyl hydrolase enzymes in plants. Phytochemistry 14: 2323-2329.

Wardale, D.A. and Lambert, E.A. 1980. Lipoxygenase from cucumber fruit: localization and properties. Phytochemistry 19: 1013-1016.

Warner, K. 1985. Sensory Evaluation of Flavor Quality of Oils. Ch. 11, In *Flavor Chemistry of Fats and Oils*, D. Min and T. Smouse (Eds.), 207-221. American Oil Chemists' Society, Champaign, IL.

Weber, E.J. 1978a. Corn lipids. Cereal Chem. 55(5): 572-584.

Weber, E.J. 1978b. The lipids of corn germ and endosperm. J. Am. Oil Chem. Soc. 56: 637-641.

Weisburger, J.H. 1991. Mechanisms underlying the mutagenic, carcinogenic, and chemopreventive effects of phenols and catechols. Presented at the 202nd National Meeting of the American Chemical Society, New York, NY, Symposium on Phenolic Compounds in Foods and Health, AGFD Abstract No. 86.

Whitaker, J.R. 1963. Determination of molecular weights of proteins by gel filtration on Sephadex. Anal. Chem. 35: 1950-1953.

Whitaker, J.R. 1972. Effect of temperature on enzyme-catalyzed reaction. Ch. 11, In *Principles of enzymology for the food sciences*, J.R. Whitaker (ed.), 319-349, Marcel Dekker, New York.

Wiley, R.C. 1985. Sweet corn aroma: studies of its chemical components and influence on flavor. Ch. 13, In *Evaluation of Quality of Fruits and Vegetables*, H.E. Patee (Ed.), 349-366. AVI Publishing Co., Westport, CT.

Wiley, R.C., Schales, F.D. and Corey, K.A. 1989. Sweet Corn. Ch. 4, In *Quality and Preservation of Vegetables*, N.A.M. Eskin (Ed.), 121-157. CRC Press, Inc., Boca Raton, FL.

Williams, D.C., Lim, M.H., Chen, A.O., Pangborn, R.M., and Whitaker, J.R. 1986. Blanching of vegetables for freezing - which indicator enzyme to choose. Food Technol. 40(6): 130-140.

Williams, M.P. and Nelson, P.E. 1973. Effect of hybrids and processing on the dimethyl sulfide potential of sweet corn. J. Food Sci. 37: 408-410.

Winter, E. 1969. Behavior of peroxidase during blanching of vegetables. Z. Lebensm. Unters. Forsch. 141: 201-208.

Wolf, W.J. 1975. Lipoxygenase and flavor of soybean products. J. Agric. Food Chem. 23: 136-141.

Yabuuchi, S. 1976. Occurrence of a new lipoxygenase isozyme in germinating barley embryos. Agric. Biol. Chem. 40: 1987-1992.

Yabuuchi, S., Liser, R.M., Axelrod, B., Wilcox, J.R., and Nielsen, N.C. 1982. Enzyme-linked immunosorbent assay for the determination of lipoxygenase isozymes in soy bean. Crop Sci. 22: 333-337.

Yamamoto, A., Fujii, Y., Yasumoto, K. and Mitsuda, H. 1980. Partial purification and study of some properties of rice germ lipoxygenase. Agric. Biol. Chem. 44(2): 443-445.

Yasumoto, K., Yamamoto, A. and Mitsuda, H. 1970. Effect of phenolic antioxidants on lipoxygenase reaction. Agric. Biol. Chem. 34: 1162-1168.

Yenofsky, R.L., Fine, M., and Lui, C. 1988. Isolation and characterization of a soybean (*Glycine max*) lipoxygenase-3 gene. Mol. Gen. Genet. 211: 215-222.

Yoshida, M., Sakai, T., Hosokawa, N., Marui, N., Matsumoto, K., Fujioka, A., Nishino, H. and Aoike, A. 1990. The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. FEBS Lett. 260(1): 10-13.

Zhang, Q., Cavalieri, R.P., Powers, J.R., and Wu, J. 1991. Measurement of lipoxygenase activity in homogenized green bean tissue. J. Food Sci. 56(3): 719-721, 742.

APPENDIX

APPENDIX A

Table A 5.1- Significance level[a] for the main and interaction effects for descriptors of the study of aroma profile of homogenate prepared from unblanched frozen stored corn on the cob and blanched control samples.

	Aroma descriptors							
Sources of Variation	Overall intensity	painty	Stale/ Oxidized	cooked cabbage	straw/ Hay	corn	sweet	cob/ husk
Treatment	*	*	*	*	ns	ns	ns	*
Replication	ns	*	ns	ns	ns	ns	ns	ns
Panelist	*	*	ns	ns	ns	ns	ns	**
Pan*trt	ns	ns	*	ns	**	***	*	ns
Pan*rep	ns	ns	ns	*	ns	ns	ns	*

[a] ns not-significant at $p \leq 0.05$.
*, **, *** refers to significance at $p \leq 0.05$, 0.01 and 0.001, respectively.

Table A 5.2- Mean ratings[y], standard deviations (SD), and LSD values for aroma descriptors indicating a significant difference among the treatments for the study of aroma profile of homogenate prepared from unblanched frozen stored corn on the cob and blanched control samples.

	Aroma descriptors							
Treatment	Overall intensity	painty	Stale/ Oxidized	cooked cabbage	straw/ Hay	corn	sweet	cob/ husk
Control	8.71[b] (1.62)	2.43[b] (2.46)	2.95[b] (1.83)	1.14[b] (1.88)	1.00[a] (1.41)	5.43[a] (1.57)	4.43[a] (1.99)	1.90[b] (1.95)
Unblanched	9.62[a] (1.56)	4.67[a] (3.28)	5.00[a] (1.73)	2.48[a] (2.96)	1.76[a] (1.48)	4.76[a] (1.45)	3.33[a] (1.56)	2.90[a] (1.95)
LSD ($p \leq 0.05$)	0.68	2.17	1.91	1.19	1.24	1.85	1.46	0.76

[y] Sixteen point intensity scale (0 = none, 15 = extreme).
[a,b] Means with the same letter, in the same column, are not significant different at the 0.05 significant level.

TABLE A 5.3- Eigenvectors (loadings), correlation coefficients (in parenthese) and % proportion of variance explained by PC1 and PC2 for each aroma descriptor in the LPO involvement study.

Aroma descriptor	Principal Component	
	1	2
Overall Intensity	0.392 (0.894)	0.269 (0.330)
Painty	0.423 (0.966)	0.088 (0.107)
Stale/Oxidized	0.340 (0.776)	-0.406 (0.498)
Cooked Cabbage	0.321 (0.732)	0.457 (0.561)
Straw/Hay	0.368 (0.840)	0.212 (0.259)
Corn	-0.365 (-0.832)	0.333 (0.408)
Sweet	-0.295 (-0.673)	0.586 (0.719)
Cobby/Husky	0.383 (0.691)	0.216 (0.265)
Proportion (%)	64.99	18.81

Table A 5.4- Mean scores, significance levels, and LSD values for PC1 and PC2 indicating a significant difference among the treatments for the LPO involvement study.

Principal Component	Treatments				LSD ($p \leq 0.05$)	Sig.[y]
	Control	Unblanched	Crude extract	LPO		
PC1	-3.17[b]	0.41[a]	2.23[a]	0.53[a]	2.38	**
PC2	0.50[a]	0.28[a]	0.97[a]	-1.74[b]	1.48	*

[y] *, ** refers to significance at $p \leq 0.05$, and 0.01, respectively by ANOVA F-test on PC1 and PC2 scores.

[a,b] Means with the same letter, in the same row, are not significantly different at the 0.05 significant level.

Table A 5.5- Significance levels[a] for the main and interaction effects for descriptors of the LPO involvement study.

	Aroma Descriptors							
Sources of Variation	Overall intensity	painty	Stale/ Oxidized	cooked cabbage	straw/ Hay	corn	sweet	cob/ husk
Treatment	**	**	(*)[b]	***	(*)[c]	**	*	(*)[d]
Replication	ns	ns	ns	ns	ns	ns	ns	ns
Panelist	*	**	*	**	***	*	**	***
Pan*trt	ns	*	ns	ns	ns	*	*	*
Pan*rep	ns	ns	ns	ns	ns	ns	**	**

[a] ns not-significant at $p \leq 0.05$.
*, **, *** refers to significance at $p \leq 0.05$, 0.01 and 0.001, respectively.
(*)[b], (*)[c], (*)[d] refers to non-significant by ANOVA (with p= 0.06, 0.09 and 0.08, respectively), but statistical significance ($p \leq 0.05$) by Fisher's LSD test.

TABLE A 5.6- Eigenvectors (loadings), correlation coefficients (in parenthese) and % proportion of variance explained by PC1 and PC2 for each aroma descriptor in the POD involvement study.

Aroma descriptor	Principal Component	
	1	2
Overall Intensity	0.360 (0.823)	0.438 (0.506)
Painty	0.422 (0.965)	0.165 (0.190)
Stale/Oxidized	0.319 (0.731)	-0.094 (-0.109)
Cooked Cabbage	0.384 (0.879)	0.362 (0.418)
Straw/Hay	0.341 (0.780)	-0.468 (-0.540)
Corn	-0.393 (-0.900)	-0.184 (-0.212)
Sweet	-0.270 (-0.618)	0.516 (0.596)
Cobby/Husky	0.315 (0.721)	-0.348 (-0.401)
Proportion (%)	65.46	16.66

Table A 5.7- Mean scores, significance levels, and LSD values for PC1 and PC2 among the treatments for the POD involvement study.

Principal Component	Treatments				LSD ($p \leq 0.05$)	Sig.[y]
	Control	Unblanched	Crude extract	POD		
PC1	-2.70[b]	1.58[a]	2.23[a]	-1.11[b]	2.36	**
PC2	-0.11[ab]	-1.04[b]	1.31[a]	-0.15[ab]	1.60	ns

[y] ns not-significant at $p \leq 0.05$.
** refers to significance at $p \leq 0.01$ by ANOVA on PC1 and PC2 scores.

[a,b] Means with the same letter, in the same row, are not significantly different at the 0.05 significant level by LSD.

Table A 5.8- Significance level[a] for the main and interaction effects for descriptors of the POD involvement study.

Sources of Variation	Aroma descriptors							
	Overall intensity	painty	Stale/ Oxidized	cooked cabbage	straw/ Hay	corn	sweet	cob/ husk
Treatment	**	**	ns	***	**	**	ns	ns
Replication	ns	ns	ns	ns	ns	ns	ns	ns
Panelist	ns	*	*	*	*	*	**	**
Pan*trt	**	***	**	**	ns	***	**	**
Pan*Rep	***	ns	ns	ns	ns	*	*	**

[a] ns not-significant at $p \leq 0.05$.
*, **, *** refers to significance at $p \leq 0.05$, 0.01 and 0.001, respectively.