AN ABSTRACT OF THE THESIS OF

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Title: Peroxidase and Lipoxygenase Activities and Their Effect on the Stability of Polyunsaturated Fatty Acids in Two Different Varieties of Sweet Corn (Zea mays L.), Jubilee and GH 2684, During Frozen Storage.

Abstract approved : _____
Daniel P. Selivonchick

The effect of different blanching treatments and packaging materials on the enzymatic (lipoxygenase and peroxidase) activity and fatty acid stability of two different varieties of sweet corn on the cob (Jubilee and GH 2684) was evaluated during nine months of frozen storage at -23.3°C.

The initial moisture content in the kernels of the two sweet corn varieties averaged 72.5 %. After nine months of frozen storage the moisture content in the kernels of corn depended greatly on the packaging material used. The ears stored in Cryovac B and E bags showed the best moisture retention (72.2 % final moisture content), followed by the polyethylene bags (71.4 %) while the ears stored without packaging material showed severe dehydration (70.1 %).

The peroxidase and lipoxygenase activities were determined using spectrophotometric assays on a crude extract obtained from liquid nitrogen powdered corn. Both unblanched varieties of sweet corn showed similar initial peroxidase specific activity and general behavior during the nine months of frozen storage. The presence of lipoxygenase isozymes with different thermal stabilities in both varieties was suggested by the higher lipoxygenase specific activity found in Jubilee after freezing and nine months of frozen storage (0.135 units/mg protein) compared with the GH 2684 variety (0.115 units/mg protein).

Complete inactivation of lipoxygenase was obtained after 9 minutes steam blanching at 100°C. Peroxidase was more heat resistant showing some remaining specific activity after 9 minutes steam blanching with a complete inactivation after 15 minutes steam blanching. No regeneration of either enzyme was observed during the nine months of frozen storage suggesting a permanent disruption of the active site of both enzymes.

Relative fatty acid content was determined by gas chromatographic analysis of fatty acids methyl esters. The major fatty acids present in both varieties were palmitic (14.93 %), stearic (2.79 %), oleic (31.54 %), linoleic (46.87 %) and linolenic (1.89 %) acids. Good stability of the polyunsaturated fatty acids was observed during the nine months storage at -23.3°C, with autoxidation as the main mechanism responsible for the decrease in the relative

percent of polyunsaturated fatty acids. Some enzymatic oxidation also occurred, decreasing the linolenic acid content.

The control of the degradation of polyunsaturated fatty acids depended mostly on the frozen storage temperature (-23.3°C) and not on the oxygen permeability of the different packaging materials.

The results obtained in our study suggested that blanching of the ears of sweet corn had an important effect on reducing the enzyme activity but little effect on the polyunsaturated fatty acid degradation after 9 months of storage at -23.3°C.

Peroxidase and Lipoxygenase Activities and Their Effect on the Stability of Polyunsaturated Fatty Acids in Two Different Varieties of Sweet Corn (Zea mays L.), Jubilee and GH 2684, During Frozen Storage.

by

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DEDICATION

This thesis is dedicated to my wife, Mónica, that with her wisdom, dedication and love gave me the confidence to continue working toward my Master's degree.

Mónica TE AMO.

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PEROXIDASE AND LIPOXYGENASE ACTIVITIES AND THEIR EFFECT ON THE STABILITY OF POLYUNSATURATED FATTY ACIDS IN TWO DIFFERENT VARIETIES OF SWEET CORN (ZEA MAYS L.), JUBILEE AND GH 2684, DURING FROZEN STORAGE.

INTRODUCTION

Sweet corn (Zea mays L.), which is one of the most popular vegetables grown in the United States (Boyer and Shannon, 1982); it ranks second in farm value for processing, and fourth in fresh market among vegetable crops (Marshall, 1987).

Sweet corn is very perishable in its fresh state due to its high respiration rate. To provide a nutritious, palatable and high quality product over long storage periods, several preservation technologies have been developed. Freezing maintains more of the product's fresh quality than any of the other economically feasible approaches. Lower temperature results in a slower rate for most deteriorative reactions such as senescence, enzymatic decay, chemical decay and microbial growth (Labuza, 1982).

Since freezing alone does not completely prevent off flavor development or color and texture deterioration, blanching, a pre-freezing step, is used to inactivate the enzymes responsible for deteriorative reactions. It is well established that these enzyme systems remain active even at sub-zero temperatures (Katsaboxakis, 1984). A significant correlation has been reported between off flavor development

and peroxidase activity in frozen sweet corn on the cob (Lee and Hammes, 1979).

In fact, peroxidase is widely used as adequate blanching indicator because its high resistant to heat inactivation, presence in most vegetables and fruits, and sensitive and simple colorimetric tests available to measure its activity (Richardson and Hyslop, 1985). However, Williams et al. (1986) reported that peroxidase is not directly involved in quality deterioration of frozen unblanched vegetables and its complete inactivation results in overblanching. In order to optimize the quality of frozen foods, the use of just the sufficient heat treatment to inactivate those enzymes responsible for deleterious changes is recommended (Reid, 1990; Williams et al., 1986).

Several studies have suggested the use of lipoxygenase activity as indicator of adequate blanching for leguminous vegetables (Sheu and Chen, 1991; Chen and Hwang, 1988; Williams et al., 1986; Chen and Whitaker, 1986 and Wagenknecht and Lee, 1958), corn (Garrote et al., 1985; Wagenknecht, 1959) and potato (Park et at., 1988) because this enzyme is closely related to the destruction of essential fatty acids, development of off flavors and pigment degradation.

The objective of the present study was to evaluate the relationship between lipoxygenase and peroxidase activities with the stability of polyunsaturated fatty acids in two

different varieties of sweet corn on the cob (Jubilee and GH 2684) subjected to different blanching treatments and stored using different packaging materials for 9 months at -23.3°C.

LITERATURE REVIEW

Corn

Corn (Zea mays L.) is a tall annual plant belonging to the grass family (Graminacea). Corn apparently originated in Mexico and spread northward to Canada and southward to Argentina. Although secondary centers of origin in South America are possible, the oldest archaeological corn (7000 years) was found in Mexico's valley of Tehuacan. Following European discovery of the Americas, corn moved quickly to Europe, Africa and Asia and due to its environmental adaptability, is now grown on every continent except Antarctica (Benson and Pearce, 1987).

Whereas most of the modern races of corn are derived from phototypes developed by early native agriculturists of Mexico, Central and South America, one outstanding exception is the product of postcolonial North America, a yellow dent corn that dominates the U.S. Corn Belt, Canada and much of Europe today (Benson and Pearce, 1987).

Sweet corn is believed to have originated from a mutation in the Peruvian race, Chullpi (Marshall, 1987). It differs from other corn genotypes in terms of its genetic makeup rather than in its systematic or taxonomic characterization. It has a mutation at the sugary locus on chromosome 4, affecting the endosperm composition causing it to accumulate twice as much sugar and eight to ten times more water soluble

polysaccharides than normal field corn (Marshall, 1987). Table 1 shows the U.S. sweet corn production in different regions, representing one of the more popular vegetables grown in the United States.

Although corn is an important source of calories, only a fraction is directed to human consumption. Because it is inexpensive and convenient for farmers, more than half the U.S. crop is used for animal feeding (Rhoades, 1993).

Sweet corn hybrid cultivars

Many cultivars developed have genetic characteristics that increase the sugar content of fresh sweet corn. Wiley et al. (1989) have classified the sweet corn hybrids, according to its sugar content, into five major groups:

Sugary Hybrids (su): Contain the sugary (su) gene which blocks the conversion of sugar (mostly sucrose) to starch after moving from the leaves to the kernel. All types of sweet corn disrupt this synthesis of sugar to starch.

se Hybrids: The primary effect of the se gene is to make sweet corn taste sweeter and extend the time sweet corn stays tender and edible. This gene also increases the maltose content of kernels, contributing to a distinctive taste which many find desirable.

Sweet Gene Hybrids: These hybrids have a sweetness level 50% higher than the sugary hybrids. The ear contains kernels that are 25% shrunken (sh2) and 75% normal sugary. The sh2

Table 1: U.S. sweet corn production (1000 tons in husk) from 1982 to 1991

	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991
New York	129.6	132.9	157.9	121.5	138.8	193.1	95.0	110.8	166.3	118.2
Pennsylvania	16.3	9.2	16.8	16.3	11.5	10.5	10.4	9.8	16.1	11.6
Delaware	20.6	18.3	28.6	26.4	23.4	25.5	26.4	34.4	35.2	35.7
Maryland	39.7	32.0	48.6	54.4	41.5	32.5	34.3	24.1	43.6	42.5
EAST	206.1	192.4	251.9	218.5	215.1	261.6	166.1	179.1	261.2	207.9
Illinois	187.4	144.6	194.2	206.7	151.8	149.3	120.0	202.5	178.4	215.6
Wisconsin	562.1	568.3	638.6	668.8	656.2	770.0	609.0	810.2	809.1	998.5
Minnesota	683.6	498.6	669.3	682.6	734.8	729.3	544.0	650.9	752.7	786.8
MID-WEST	1433.1	1211.5	1502.0	1558.1	1542.9	1648.6	1273.0	1663.6	1740.2	2001.0
Idaho	187.3	117.3	142.2	147.6	148.7	166.4	163.8	182.0	166.4	180.6
Washington	469.4	349.3	245.8	326.8	295.5	402.3	430.5	470.5	471.8	498.6
Oregon	410.0	331.7	365.4	354.7	322.5	343.9	353.4	394.9	396.5	400.0
WEST	1066.7	798.4	753.3	829.1	766.6	912.6	947.7	1047.5	1034.7	1079.1
OTHER STAT	34.6	24.9	44.9	34.4	34.8	43.8	33.7	59.2	84.6	92.8
For freezing	1126.9	900.7	969.7	1110.7	1083.5	1228.3	1092.0	1261.3	1443.8	1459.9
For canning	1613.5	1326.3	1582.5	1529.3	1475.9	1638.3	1328.4	1688.1	1676.8	1920.8
PROCESSING	2740.5	2227.1	2552.2	2640.0	2559.4	2866.6	2420.4	2949.3	3120.6	3380.7
FRESH MARK	721.8	743.4	779.5	778.7	752.5	783.2	715.6	806.8	837.0	738.8
TOTAL U.S.	3462.2	2970.5	3331.6	3418.7	3311.9	3649.8	3136.0	3756.1	3957.7	4119.5

Source : The Almanac of canning freezing and preserving industries (1992)

gene results in much greater accumulation of sugar than su genes.

Supersweet Hybrids: These hybrids rely primarily on the sh2 gene instead of the su gene for sweetness. Kernels are sweeter and more watery due to the absence of water soluble polysaccharides.

Improved Supersweet Hybrids: A genetic combination of su and sh2 result in corn that is 20% sweeter than the Supersweets and 125% sweeter than normally sugary hybrids.

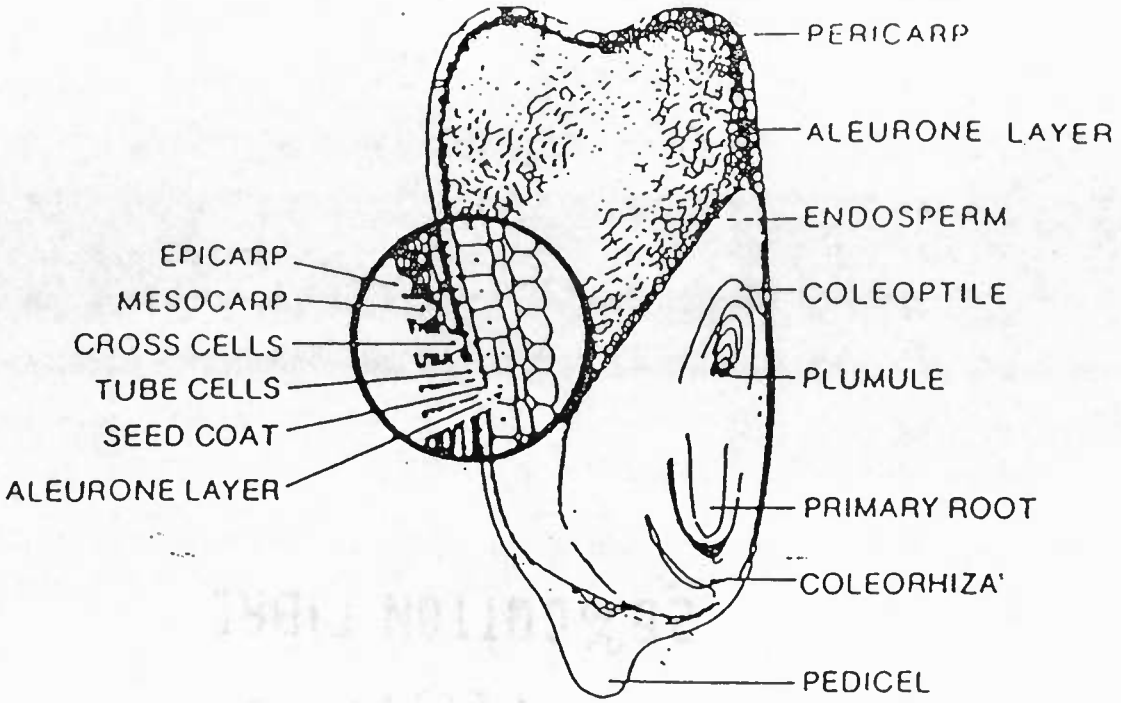
Sweet Corn Structure and Composition

Structure: The structures of sweet corn kernel are shown in Figure 1. The major parts are the pedicel (tip cap), pericarp (epicarp and mesocarp), germ (coleoptile, plumule, primary root) and endosperm. The kernel composition, structure, shape and size are important factors influenced to a great extent by gene modification (Wiley et al., 1989).

Composition: The average composition of sweet corn is shown in Table 2.

Carbohydrates: Carbohydrates are the major constituents of corn, with sucrose as the predominant sugar. Small quantities of reducing sugars mainly glucose and fructose are also found. The main polysaccharides are starch, water soluble polysaccharides (phytoglycogen), hemicellulose, pectin and cellulose (Wiley et al., 1989).

Figure 1: Schematic diagram of a longitudinal seccion of a corn kernel.



Source: Khalil, T. and Kramer, A. J. (1971)

Table 2: Average composition of whole corn and hand-dissected fractions (moisture free basis)

Fraction	Kernel (%)	Starch (%)	Protein (%)	Lipid (%)	Sugar (%)	Ash (%)
Whole grain	100.0	71.5	10.3	4.8	2.0	1.4
Endosperm	82.3	86.4	9.4	0.8	0.6	0.3
Germ	11.5	8.2	18.8	34.5	10.8	10.1
Pericarp	5.3	7.3	3.7	1.0	0.3	0.8
Tip cap	0.8	5.3	9.1	3.8	1.6	1.6

Source: Gardner and Inglett, 1971

Lipids: Lipids are present mainly in the germ in the form of triacylglycerols that constitute 85% of the total lipid (Weber, 1987). Lipids are of interest in corn processing because of possible oxidation involving flavor and color changes that may develop in kernels with high oil content (Wiley et al., 1989). The oil in corn is high in polyunsaturated fatty acids and a good source of linoleic acid (2.9% of the whole corn, dry basis) which is an essential fatty acid in the diet and also aids in the absorbability of fat-soluble nutrients (Wright, 1987).

Proteins: The proteins in corn have relatively high percentage of the sulfur-bearing amino acids, methionine and cysteine, but are deficient in the essential amino acids lysine and tryptophan (Wright, 1987). The combination of corn protein and protein from soybean or other legumes produces a diet that is nutritionally balanced, in terms of amino acids composition, than protein from either source alone (Wilson, 1987).

Moisture: The moisture content of corn kernels has a significant influence on quality changes, processing properties and earnings (Watson, 1987). As the sweet corn plant grows and develops, the percent dry matter of the kernels increases. The kernel moisture content is used as an index of maturity for the determination of harvest of sweet corn for processing. In general, flavor, texture, color and endosperm quality decline as kernel moisture drops below 75%

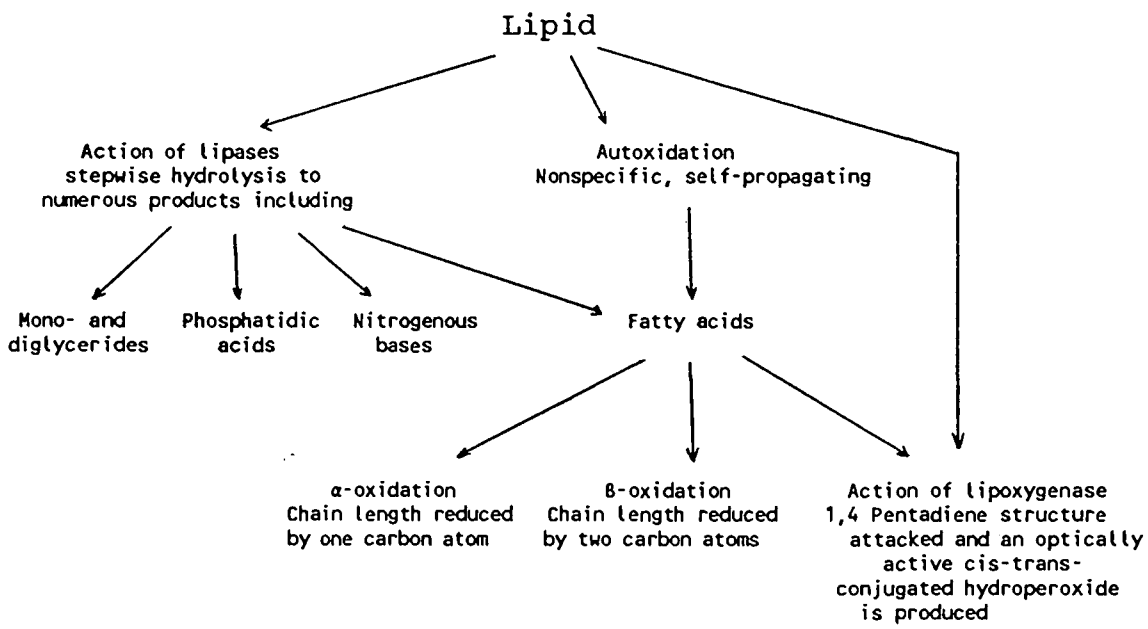
(Wiley et al.,1989).

The Oxidative Deterioration of Food Lipids

Lipid oxidation is one of the major causes of food spoilage. It is of economical concern to the food industry because it leads to the development of various off-flavors and off-odors in edible oils and fat containing foods. These foods become rancid and unacceptable with a reduction in shelf-life. In addition, oxidative reactions can decrease the nutritional quality of food, and certain oxidation products are potentially toxic (Nawar, 1985).

Changes in lipids can be brought about by two different mechanisms: 1) partial or complete hydrolysis of the lipid to fatty acids and other components, followed by oxidation of unsaturated fatty acids and 2) direct oxidation of the unsaturated acids in the intact lipids, followed by hydrolytic degradations. Figure 2 shows the lipid degradation pathways. The hydrolytic processes are brought about by enzymes normally classified as lipases, the oxidation processes may be catalyzed by enzymes (particularly by lipoxygenases) or by metals, their salts or organic complexes (Aylward and Haisman, 1969).

Figure 2: Mechanisms of lipid degradation



Source: Aylward and Haisman, 1969.

Autoxidation

The oxidative deterioration of food lipids involves, primarily, autoxidation reactions which are defined as the reaction of any material with molecular oxygen (Lundberg, 1962).

Oxygen reacts with many organic substrates to yield hydroperoxides and other oxygenated compounds. This oxidation is, in most cases, a free radical chain reaction that can be described in terms of initiation, propagation and termination processes (Frankel, 1980).

Initiation:

In the presence of initiators, unsaturated lipids (RH) form carbon centered alkyl radicals ($R^{\cdot}$) which then propagate in the presence of oxygen by a free radical chain mechanism to form hydroperoxides as primary products:

$$RH \xrightarrow{\text{initiator}} R^{\cdot}$$

Since the formation of free radicals is thermodynamically difficult (activation energy of about 35 Kcal/mol), it normally must occur by some catalytic means (Nawar, 1985). The production of free radicals may take place by direct thermal dissociation (thermolysis), by hydroperoxide decomposition, by metal catalysis and exposure to light (photolysis) with or without intervention of photosensitizers (Frankel, 1980).

Singlet oxygen ($^1O_2$) is the active species involved in the

photooxidative deterioration and responsible for initiation; singlet oxygen is very electrophillic and reacts rapidly with moieties of high electron density, such as carbon double bonds, resulting in hydroperoxides that can then cleave to initiate a conventional free radical chain reaction (Nawar, 1985). There are many ways that singlet oxygen can be generated but the most important is by exposure to light in the presence of photosensitizer such as chlorophylls or heme compounds (Frankel, 1980).

Propagation:

Upon the formation of sufficient free radicals, the chain reaction is propagated by the abstraction of hydrogen atoms at positions α to double bonds. Oxygen addition then occurs at these locations, resulting in the production of peroxy radicals ROO·, and these in turn abstract hydrogen from α-methylenic groups RH of other molecules to yield hydroperoxides ROOH and R· groups (Nawar, 1985):

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot$$

The susceptibility of organic substrates to autoxidation depends on their relative ease to donate hydrogen by reaction. With unsaturated fats, susceptibility to autoxidation is dependent on the availability of allylic hydrogens for reactions with peroxy radicals (Frankel, 1980).

Termination:

The self propagating chain reaction can be stopped by

termination reactions, where two radicals combine to give stable non-radical end products which do not feed the propagating reactions (Hamilton, 1989 and Lundberg, 1962):

$$ROO\cdot + ROO\cdot \longrightarrow \text{nonradical products}$$

Formation of hydroperoxides

Unsaturated fatty acids are more susceptible to oxidation than their saturated analogs (Henderson et al., 1980). Their susceptibility to autoxidation varies according to the lability of their allylic hydrogens (Frankel, 1980).

Hydroperoxides can form by free radical mechanism or by singlet oxygen oxidation with the formation of different hydroperoxide isomers (Table 3). The formation of hydroperoxides by singlet oxygen proceeds via mechanisms different than that for free radical autoxidation. The most important of these is the "ene' reaction, which involves the formation of a six-membered ring transition state (Nawar, 1985). Oxygen is inserted at either end carbon of a double bond, which is shifted to yield an allylic hydroperoxide in the trans configuration (Frankel, 1980).

Lipids can be oxidized by enzymatic pathways to form hydroperoxides by the action of lipoxygenase. Plant and animal lipoxygenases are both regiospecific (catalyze oxygenation of specific carbons) and stereospecific (produce enantiomeric hydroperoxides) (Nawar, 1985).

Table 3: Isomeric hydroperoxides from Oleate, Linoleate and Linolenate esters

Hydroperoxide esters	Oxidation	Relative percent of hydroperoxides					
Oleate	Free radical	8-OH 27	9-OH 23	10-OH 23	11-OH 27		
	Photosensitized		50	50			
Linoleate	Free radical	9-OH 50	10-OH	12-OH	13-OH 50		
	Photosensitized	32	17	17	34		
Linolenate	Free radical	9-OH 32	10-OH	12-OH 11	13-OH 11	15-OH	16-OH 46
	Photosensitized	23	13	12	14	13	25

Free radical oxidation in air and photosensitized oxidation in O_2. Source: Frankel et al. (1981).

Hydroperoxides, the primary initial products of lipid autoxidation, are relatively unstable. They enter into numerous and complex breakdown and interaction mechanisms responsible for the production of myriad compounds of various molecular weights, flavor thresholds and biological significance (Nawar, 1985). In heat-processed foods, autoxidation becomes the primary cause of hydroperoxides which accumulate and initiate further lipid oxidation and other free radical reactions (Gardner, 1975).

Decomposition of hydroperoxides

Lipid hydroperoxides are readily decomposed into a wide range of carbonyl compounds, hydrocarbons, aldehydes, ketones and other materials causing rancidity in foods (Frankel, 1991; Frankel, 1980). Different volatile decomposition products are formed according to the relative thermal stabilities of the lipid oxidation precursors and resulting carbonyl products (Frankel, 1991).

A variety of volatile and nonvolatile secondary products are formed from hydroperoxides. Each hydroperoxide produces a set of initial breakdown products that are typical of the specific hydroperoxide and depend on its position in the parent molecule. Such molecules can themselves undergo further oxidation and decomposition contributing to a large and varied free radical pool (Nawar, 1985).

In biological tissue, linoleic and linolenic acid are

decomposed primarily by the following enzymatic pathways: 1) reduction or nucleophilic reactions, 2) isomerization by linoleic acid hydroperoxide isomerase, 3) epoxidation, 4) vinyl ether formation, 5) anaerobic lipoxygenase reactions and 6) production of volatile aldehydes (Gardner, 1975).

Nonenzymatic decomposition of lipid hydroperoxides could be of much greater significance than enzymatic decomposition during food storage (Gardner, 1975).

The most common route of lipid hydroperoxide decomposition by a free radical mechanism is believed to involve homolytic cleavage of the hydroperoxy group. Among the conditions that promote homolytic decomposition of hydroperoxides are heat, photolysis, metal ions or their complexes, metalloproteins and many other agents that promote free radicals (Gardner, 1975). The products are similar to those of enzymatic transformations or are either homologues or isomers of the enzymatically produced compounds (Galliard and Chan, 1980).

Hydroperoxides decompose homolytically mainly through the formation of peroxy or alkoxy radicals. The major reaction pathways are dimer formation from termination reactions, loss or gain of a hydrogen radical, cyclization of the alkoxy radical to the α unsaturation and 1,4 addition to the conjugated diene (Gardner, 1975).

Heterolytic reactions also may significantly affect lipid hydroperoxide decomposition because nucleophiles can

efficiently attack the hydroperoxy group (Gardner, 1975). Under acid conditions, heterolysis produces ether carbocation intermediates which cleave selectively to form both hexanal and 9-oxononanoate (Frankel, 1991).

Carbon-carbon bond cleavage on either side of the alkoxy group is the second step in decomposition of hydroperoxides. Cleavage on the acid side (the carboxyl or ester side) results in the formation of an aldehyde and an acid (or ester); scission on the hydrocarbon (or methyl) side produces a hydrocarbon and an oxoacid (or oxoester) (Nawar, 1985).

In biological systems or foodstuffs, there are usually ample homolytic catalysts present and free radical reactions are most significant; however, heterolytic reactions cannot be minimized (Gardner, 1975). The major volatile decomposition products of pure hydroperoxides from autooxidized and photosensitized methyl oleate, linoleate and linolenate involves carbon-carbon scission (Frankel et al. 1981).

Hydroperoxides and their secondary products are potentially reactive substances that can cause deterioration of food proteins and amino acids. Protein exposure to peroxidized lipids form lipid-protein complexes that are bound through purely physical forces. The chemical changes caused by the interaction of lipid hydroperoxides and proteins are protein-protein cross links, protein scission, protein-lipids adducts and amino acid damage. The hydroperoxide secondary products readily damage protein and amino acids through

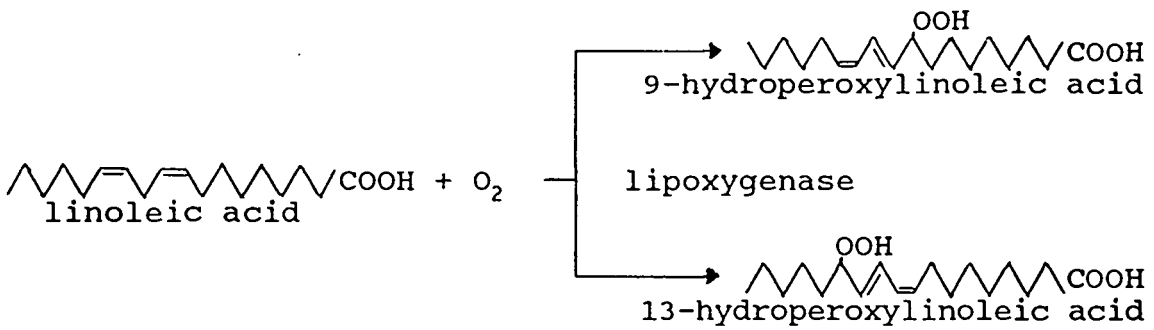
formation of covalent bonds. Aldehydes are specially important because they can form Schiff base adducts with amino groups (Gardner, 1979).

Lipoxygenase (linoleate:oxygen oxidoreductase, E.C.1.13.11.12)

Plant lipoxygenases are members of a class of nonheme iron-containing dioxygenases that catalyze the addition of molecular oxygen to a fatty acid containing a cis,cis-1,4-pentadiene system, to give an unsaturated fatty acid hydroperoxides (Siedow, 1991).

The resulting hydroperoxide product contains a set of cis, trans double bonds that are conjugated as a result of double bond migration during the catalytic cycle. The oxygen can be added to either end of the pentadiene system leading, as in the case of linoleic acid, to the formation of 9- and 13- hydroperoxy linoleic acids (Figure 3) (Siedow, 1991). Most lipoxygenases have positional specificity (formation of one particular positional isomer) and enantiospecificity with respect to the new chiral center generated (Galliard and Chan, 1980). Recently it has been shown that both the cis, trans and trans, cis isomers of the naturally occurring linoleic acid (cis, cis-9,12- octadecadienoic acid) can serve as substrates for soybean lipoxygenase, suggesting that the active site is relatively flexible in its ability to accommodate substrate, the substrate can apparently bind to the active site in either of two possible orientations (Funk

Figure 3: Primary reaction catalyzed by lipoxygenase using linoleic acid as substrate (Siedow, 1991)



Source: Siedow (1991).

et al., 1987).

As the degree of unsaturation in natural free fatty acid substrates increases, the oxygen consumption increases. Monounsaturated fatty acid (18:1) do not act as a substrate for soybean lipoxygenase (Zhuang et al., 1991) on the contrary, they have been shown to inhibit soybean and peanut lipoxygenase (St. Angelo and Ory, 1984). Within a given plant, the level of lipoxygenase activity present in any tissue can vary markedly not only among different organs of the plant but also between different developmental stages of a particular tissue type.

Lipoxygenase Isozymes

Lipoxygenase has been isolated from a wide variety of vegetables including English peas (Chen and Whitaker, 1986), green peas (Halpin and Lee, 1987), green beans (Zhang et al., 1991 and Adams and Ongley, 1989), corn (Poca et al., 1990), asparagus (Ganthavorn and Powers, 1989), green leaves (Hatanaka, 1987), soybeans (Hildebrand et al., 1990, Axelrod et al., 1981), split pea seeds, snap beans and peas (Klein, 1976), wheat (Hakansson and Jagerstad, 1990) and potato tuber (Park et al, 1988).

Soybeans are the richest known source of lipoxygenase and much of the characterization of lipoxygenase has been associated with the enzyme isolated from soybean cotyledons. Four isozymes of lipoxygenase (LOX) have been isolated and

designated as LOX1, LOX2, LOX3a and LOX3b. Lipoxygenase 3a and 3b are very similar in their properties and they may be considered identical (Axelrod et al., 1981).

The isozymes are globular, water-soluble proteins that consist of a single polypeptide having a molecular weight of roughly 96,000. The isozymes differ with respect to their isoelectric points showing values of 5.68, 6.25 and 6.15 for LOX1, LOX2 and LOX3 respectively. The large charge difference between LOX1 and LOX2 and LOX3 allows for relatively easy separation from the other two isozymes using anion exchange chromatography. For separation and purification of LOX2 and LOX3, high performance liquid chromatography and chromatofocusing is used (Siedow, 1991).

The major differences among the three soybean cotyledon lipoxygenase isozymes can be seen at the level of their reactivities. Lipoxygenase 1 has a pH optimum for activity centered around 9.0, while LOX2 shows a sharp pH maximum around pH 6.5 and LOX3 displays a broad optimum around pH 7. Lipoxygenase 1 shows a marked preference for charged fatty acids and shows a little reactivity with fatty acids that are esterified. Lipoxygenases 2 and 3 are more reactive toward neutral fatty acids but will react with free fatty acids, particularly at pHs below 7.0 (Siedow, 1991). Lipoxygenase 1 is most effective in the hydroperoxidation of free fatty acids, whereas LOX2 and LOX3 are relatively more effective in the hydroperoxidation of esterified or neutral fatty acids

(Zhuang et al., 1991).

The product of the reaction of linoleic acid with soybean cotyledon LOX1 is almost exclusively 13-hydroperoxy linoleic acid presumably due to steric constraints within the enzyme that direct the stereospecific addition of oxygen. With LOX2 and LOX3, roughly equal amounts of the 9- and 13- hydroperoxy products are obtained (Siedow, 1991).

Poca et al. (1990) isolated and characterized two lipoxygenase isozymes (L1 and L2) from germinating corn seeds. L2 is active in a pH range from 6 to 9, the products are 9-hydroperoxides with C_{18} fatty acids and show a very low affinity for arachidonic acid. L1 isozyme has an optimal activity at pH of 7 and from 8 to 9 there is a dramatic decrease of its activity. Reactions catalyzed with L1 leads to 13-hydroperoxides with C_{18} fatty acids showing a high relative initial velocity with arachidonic acid producing 15-hydroperoxyeicosatetraenoic acid.

Reaction Mechanism

Inactive LOX1, has a single atom of nonheme iron at the active site exits in a high-spin Fe(II) state; the enzyme is essentially colorless and catalytically not active. The active form of LOX1 can be generated by reaction with one equivalent of hydroperoxide product which oxidizes the active site iron to a high-spin Fe(III) state. This form of the enzyme has a characteristic pale yellow color. A second form

of oxidized LOX1 can be generated in the presence of a stoichiometric excess of product, this form has a distinctly purple color (Siedow, 1991). Lipoxygenases 2 and 3 show qualitatively similar behavior patterns than LOX1 (Galliard and Chan 1980).

In the inactive form all 3 soybean cotyledon isozymes exist in the Fe(II) state and it is activated in the presence of hydroperoxide by conversion to the Fe(III) state. The catalytic significance of the purple form of the enzyme is, at the present, unknown (Siedow, 1991). Addition of linoleic acid converts the yellow or purple enzyme (with excess linoleic acid) to the native enzyme (Galliard and Chan, 1980).

Once resting LOX1 has been oxidized to the active yellow form, it can carry out the enzymatic reaction. Neither the resting state nor the oxidized activated LOX1 binds molecular oxygen directly; catalysis is thought to be initiated by the binding of an appropriate unsaturated substrate at the active site (Siedow, 1991).

The catalytic mechanism was originally thought to involve the stereospecific abstraction of a hydrogen atom from the methylene group at the center of the pentadiene system (▲11 carbon in linoleic acid). Recently, studies using mechanism-based lipoxygenase inhibitors have led to a revision of this scheme; the initial event in the catalysis is the extraction of a methylene proton by an unspecific basic group in the active site. This proton extraction facilitates an

electrophillic attack by the Fe(III) atom on one of the double bonds in the pentadiene system, which leads to the formation of Fe(II) and an organic radical whose unpaired electron is delocalized over the entire 1,4 pentadiene system (Siedow, 1991).

Corey and Nagata (1987) have suggested an alternative mechanism involving the covalent bonding of Fe(III) with a terminal carbon after proton extraction, resulting in a pentadienyl carbanion which does not formally undergo reduction.

The redox-based mechanism continues with the oxygen reacting with the pentadienyl radical which is subsequently reduced to hydroperoxide product by the Fe(II), restoring the enzyme to its initial Fe(III) state (Siedow, 1991).

The formation of secondary products, often referred as the "anaerobic reaction" of LOX1 occurs under conditions of low oxygen tension (Siedow, 1991) with the release of fatty acid radicals from the enzyme (Hildebrand, 1989). This reaction occurs with the linoleic and presumably with the linolenic acid radical leading to the lipohydroperoxidase reactions of lipoxygenases yielding fatty acids dimers and oxodienoic acids (Hildebrand, 1989).

At atmospheric oxygen concentrations, the normal catalytic cycle of LOX1 is apparently rapid and efficient enough that dissociation of the intermediate radical species ($R^{\cdot}$ and $ROO^{\cdot}$) rarely takes place. Lipoxygenase 3 is the most

active isozyme with respect to secondary reactions and can catalyze the formation of secondary products under both aerobic and anaerobic conditions suggesting that the enzyme-bound radical intermediates formed during its reaction sequence are more readily able to dissociate from the enzyme, even at high oxygen concentrations. Lipoxygenase 2 shows little secondary product formation under any condition (Siedow, 1991).

Singlet oxygen could also be generated during lipoxygenase reaction, but it requires the presence of 13-hydroperoxylinoleic acid. Lipoxygenase 3 formed singlet oxygen under these conditions, lipoxygenase 2 was essentially inactive, and lipoxygenase 1 only showed appreciable singlet oxygen formation at low oxygen concentrations (Siedow, 1991).

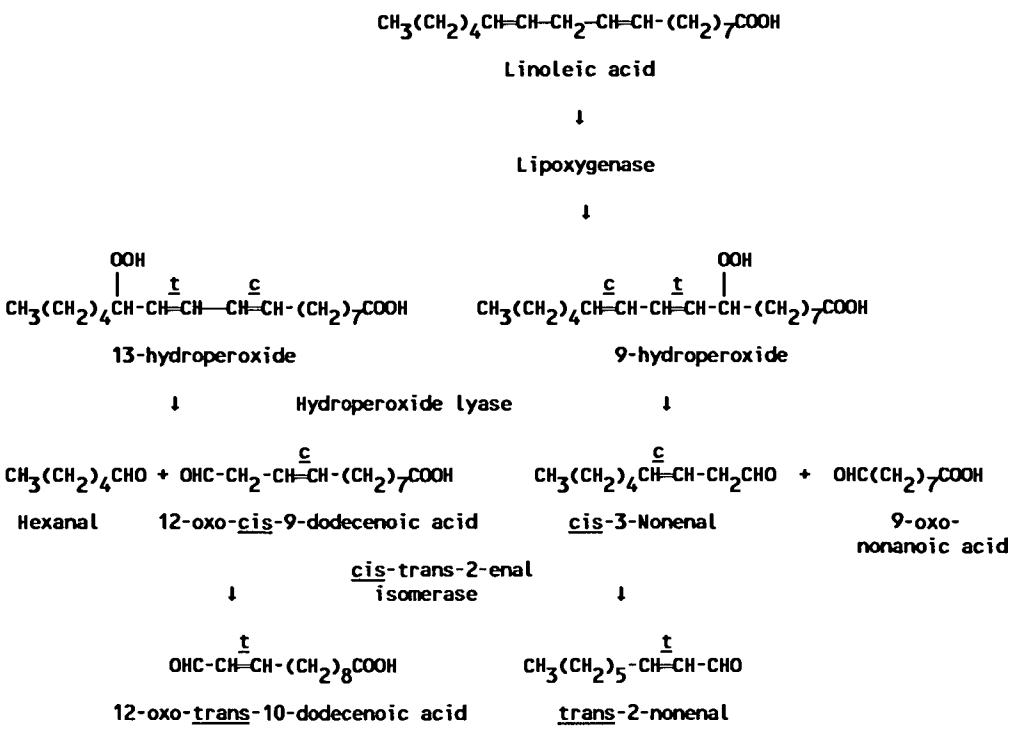
The peroxy radicals, lipoxygenase secondary reactions, are thought to be responsible for the co-oxidation of carotenoids and chlorophylls. Hydrogen abstraction is the rate-limiting step of the overall reaction (Hildebrand, 1989).

Lipoxygenase and Food deterioration

The distinctive feature of all lipoxygenase-catalyzed reactions in both plants and animals is the homolytic cleavage of a sigma bond with the formation of intermediate radicals (Hildebrand, 1989).

Figure 4 shows the enzymatic formation of aldehydic fragments from linoleic acid. The fatty acid hydroperoxide

Figure 4: Enzymatic formation of aldehydic fragments from linoleic acid



Source: Galliard and Chan (1980).

formed by lipoxygenase action are metabolized mainly by hydroperoxide lyase in higher plant tissues (Hildebrand, 1989) leading to the formation of aldehydes and alcohols which are major contributors to off flavors in foods (Sessa, 1979) . The presence of lipoxygenase activity in many foodstuffs can affect their properties, particularly during long-term storage, in both desirable and undesirable ways. For many seeds with high levels of lipoxygenase, the production of hexanal represents a particular problem. Hexanal, even when present in foods in very low concentrations (5 ppb), has a highly undesirable odor creating great difficulties in the production of acceptable food products (Hildebrand, 1989). Lipoxygenase has a very important role in enhancing food quality as well as creating deleterious effects (Rackis, 1979). These changes in food quality are summarized in Table 4.

Lipoxygenase 1 and LOX2 generate mainly C_6 aldehydes from linoleic acid but the C_6 aldehyde formation is lowered by LOX3. It appears that LOX3 competes with hydroperoxide lyase for the fatty acid hydroperoxides produced in the primary lipoxygenase reactions resulting in products unavailable for C_6 aldehyde generation. Lipoxygenase 3 may also reduce C_6 aldehyde production by converting a proportion of the fatty acid substrates into 9- rather than 13- hydroperoxides (Zhuang et al, 1991).

Table 4: Food quality changes mediated directly or indirectly by lipoxygenase

A. Color changes
Bleaching of hard wheat flour via carotene destruction (desirable)
Bleaching of pasta products via carotenoid destruction (undesirable)
Participation in loss of green color due to chlorophyll destruction in processed green vegetables (undesirable)
Destruction of xanthophyll and other carotenoid pigments (undesirable)
Destruction of added food colorants (undesirable)
Destruction of skin pigmentation in some food fishes (undesirablle)
Superficial scald in stored apples

B. Flavor changes
Production of volatiles responsible for desirable aroma in fruits and fresh vegetables
Production of off-flavors in frozen vegetables and stored cereals
Production of off-flavors in high protein foods (legume seeds)
Participation in rancidity of meats

C. Texture changes
Production of favorable effects on the rheological properties of wheat flour doughs and eventually on the texture of baked goods via SS-HH balance and hydrophobic bonding of lipids to glutens

D. Nutritional quality changes
Destruction of vitamin A and provitamin A
Destruction of nutritionally essential polyunsaturated fatty acids
Interaction of enzymatic product with some essential amino acids of proteins to lower the protein nutritional quality and functionality

E. In vivo functions
Participation on the biogenesis of ethylene by creating active oxygen needed to convert methionine to ethylene
Conversion of carotenoids to plant growth affectors, such as abscissions and growth inhibitors
Conversion of the unsaturated mammalian fatty acid, arachidonic, to hydroxy fatty acids
Destruction, anaerobic, of peroxides arising from wounded tissue.

Source: Richardson and Hyslop, 1985.

Peroxidases (EC 1.11.1.7, donor: hydrogenperoxide oxidoreductase)

Peroxidases are a ubiquitous group of enzymes occurring in higher plants and leukocytes (Richardson and Hyslop, 1985). There are 3 classes of peroxidases: peroxidases that contain ferriprotoporphyrin III as the prosthetic group (higher plants and microorganisms), verdoperoxidases which has an iron porphyrin nucleus other than ferriprotoporphyrin III (lactoperoxidase) and flavoprotein peroxidases with FAD as prosthetic group (streptococci and several animal tissues) (Scott, 1975).

Peroxidases are defined as enzymes catalyzing the following reaction:

$$ROOH + AH_2 \longrightarrow H_2O + ROH + A$$

The catalytic process appears to result in the transient oxidation of ferric ion (Fe^{3+}) to higher valence (Fe^{5+} or Fe^{4+}) states. The peroxide ROOH, from hydrogen peroxide or an organic peroxide, is reduced while an electron donor (AH_2) is oxidized. The electron donor may be ascorbate, phenols, amines or other organic compounds (Richardson and Hyslop, 1985).

Peroxidase appears to be important from the standpoint of nutrition, color and flavor. Peroxidase activity can lead to the oxidative destruction of vitamin C, also catalyze the bleaching of carotenoids in the absence of unsaturated fatty acids and the decoloration of anthocyanins. Peroxidase, like

most heme pigments, catalyzes the nonenzymatic peroxidative degradation of unsaturated fatty acids yielding volatile and flavorful carbonyl compounds that contribute to oxidized flavor (Richardson and Hyslop, 1985).

Because of its high resistant to thermal inactivation, and its extensive distribution, peroxidase has been widely used as an index of enzyme activity in plant tissues. It is generally accepted that if peroxidase is destroyed by a given heat treatment it is unlikely that any other enzyme system will have survived (Aylward and Haisman, 1969).

Effect of Temperature on food quality

The basic principle of food preservation is to process the products in a form that will ensure high enough quality, safety and nutritional value. There are several methods used to prevent food deterioration, in this review we are going to focus on the processing methods that either, lower the temperature to slow down the reactions, or raise the temperature to denature enzymes (Labuza, 1982).

During the processing of foods tissue damage occurs that can cause the release of various food chemical constituents into the cellular fluid environment. These chemicals can then react with each other or with external factors to lead to deterioration of food and result in a shortening of the shelf life (Labuza, 1982).

Enzymatic reactions are usually very rapid at room

temperature. In general, most enzymes show optimal activity in the 30-40°C range and begin to denature above 45°C. Enzymes function very slowly at subfreezing temperatures and their activities increases as the temperature is increased (Labuza, 1982).

Heat treatment: blanching

Steam or hot water blanching of vegetables is a prefreezing process of primary importance because heat inactivates the naturally occurring enzymes which are responsible for quality losses during frozen storage (Katsaboxakis, 1984). Williams et al (1986) classified four groups of enzymes primarily responsible for quality deterioration of unblanched vegetables. Lipoxygenases, lipases and proteases that can cause off flavor development; pectic enzymes and cellulases that can cause textural changes; polyphenol oxidase, chlorophyllase and peroxidase that can cause color changes and ascorbic acid oxidase and thiaminase that can reduce nutritional value.

Although the most important effect of blanching is the inactivation of the naturally occurring enzymes in the vegetable tissue, it also reduces the level of infection by microorganisms, and improves the visual color of vegetables as a result of the removal of gases from the vegetable surface and from intercellular spaces and improves flavor by expelling gases and other volatile degradation products formed during

the post harvest interval (Katsaboxakis, 1984).

Blanching will also increase the digestibility of foods by partially denaturing proteins, gelatinizing starch and softening the cell walls allowing digestive enzymes better access to the food components. Increase bioavailability of vitamins such as B_6, niacin, folacin and certain carotenoids by releasing them from poorly digested complexes, facilitating intestinal adsorption, and finally reduces the antinutritional factors (Dietz and Erdman, 1989).

The adverse effects of blanching are mainly the permanent modification of the cellular structure in the vegetable tissue, the solubilization and/or destruction of some nutrients and vitamins in the blanching media and the conversion of green chlorophylls to yellow green pheophytins (Katsaboxakis, 1984). The blanching process destroys the semipermeable properties of vegetables essential to maintain turgor, and bulk water is able to diffuse along gradients within the tissues, modifying and thickening the cell walls (Bald, 1991). The immersion of food directly into boiling water during blanching results in significant leaching of water soluble vitamins and other nutrients, these losses can be reduced with the use of steam (Dietz and Erdman, 1989).

To minimize thermally induced textural changes or nutrient leaching, it has been suggested that blanching be optimized for each product by determining the heat treatment just sufficient to inactivate those enzymes responsible for

deleterious changes during freezing and frozen storage (Reid, 1990). It has been suggested that heating vegetables to complete destruction of peroxidase is more than adequate to destroy the enzymes directly involved in quality loss resulting in overblanching of the product (Williams, 1986). Kozlowski (1974) reported that the amount of loss of essential substances during blanching depends more on the blanching time than on the temperature. The size of the vegetable, maturity, surface area, etc. are also important factors. At the completion of blanching the product is at a high temperature. In fact, the range of temperature between 10°C and 50°C is critical to the final quality of the product. The product is susceptible to reinfection by microorganisms, color modification, loss of nutrients and development of off-flavors (Gruda and Postolski, 1986).

Freezing and frozen storage

Freezing is a method of maintaining the quality, nutritional value and sensory properties of foods for extended periods. Freezing is the process that changes a product's water content to ice and reduces the temperature from ambient to storage level temperature (ASHRAE, 1990).

The goal for a frozen product is to retain as much of its turgor as possible, and to approach the living characteristics of the product such that the consumer will make a favorable comparison with the fresh material. A successful freezing

process is one that maintains the osmotic integrity of a maximum number of cellular compartments of the vegetable. This should be achieved without compromising any other required properties and efficiently retaining the turgor of the cells (Bald, 1991).

Richardson and Hyslop (1985) have pointed out that freezing of tissues results in damage to the membranes of suborganelles and delocalization of enzymes because of changes in osmotic pressure, pH and salt concentration. These cellular modifications favor the release of a wide array of hydrolytic enzymes that cause extensive tissue alterations.

During freezing, the tissues are exposed to a sequence of potentially damaging stresses. Ice crystals cause disruption and physical lesions in hydrated materials as they invade space previously occupied by disperse solutions (Bald, 1991). The solutes are concentrated in the unfrozen pools of water resulting in a concentration of electrolytes and pH changes which are a major cause of biochemical damage including effects on enzymatic activities in frozen systems. Whether the effect is on the activation, stabilization or inhibition of the enzymatic activity depends on the enzyme, the nature and concentration of salts, pH, temperature and the types of other substances present (Richardson and Hyslop, 1985).

The texture of frozen products can be affected by small gas bubbles that result from changing solubilities at low temperatures. These changes can cause significant mechanical

injury and also hypertonic stress due to the composition of the residual solution. A compromise in the osmotic integrity of viable cells tissues can lead to a loss of turgor and texture in the product (Bald, 1991).

At storage temperatures of -20°C, injuries will still occur due to the growth of ice crystals and the effects of any remaining residual solution. These effects limit the practical storage period at such temperature. Deterioration of product quality due to continued freezing stress is an inevitable consequence of extended storage, but it can be reduced by minimizing the level of stress imposed during processing (Bald, 1991).

The rate of freezing has little or no practical influence on the quality of frozen product unless very slow rates are used. In some cases very rapid freezing can be detrimental to the appearance or texture of the product (Jul, 1984). Most tests have failed to show any significant difference between moderate and fast freezing rates. As far as organoleptic quality is concerned there is no advantage in using freezing rates higher than the ones generally used in industry (0.2-0.5 cm/h). Fast freezing is advantageous for fruits and vegetables in that their structural form is better maintained after quick rather than slow freezing. The advantage of fast freezing and a somewhat improved texture for fruits and vegetables seems by and large to be observed for products which are eaten uncooked, simply because blanching results in

extensive denaturation of much the same type that is caused by freezing and masks any difference between a slowly and a quickly frozen product (Jul, 1984).

The difference in crystal size and distribution has little effect on the final quality of the thawed product. However, the freezing rate is important with regard to microbiological considerations, dehydration and volume of production (Bald, 1991).

Quality problems in frozen products are not found, except in cases where fluctuating temperatures warmer than -12°C are encountered. In such cases, one may observe mass movement of water within the product to such a degree that direct damage occurs. The main changes in frozen product occur not during the freezing process but during freezer storage (Jul, 1984).

Dehydration of frozen food occurs during the freezing process and during frozen storage. It is the result of moisture transfer between the food and its environment because of a difference in the water pressure between the product and the air in the storage room. Water loss by evaporation or sublimation during freezing and storage may change overall product quality and is of economical importance to food processors. Changes in color, flavor and attractiveness reduce the advantages for freezing preservation (Norwig and Thompson, 1984). Dehydration causes deteriorative reactions to occur more rapidly in localized areas of a food product. Sublimation of ice effects chemical reactions like fat

oxidation, protein denaturation and discoloration and important textural changes may also occur in frozen products due to desiccation (Norwig and Thompson, 1984). Dehydration can be decreased or controlled by vapor impermeable packing, high relative humidity in cold storage and a constant storage temperature (Norwig and Thompson, 1984). Besides its function in protecting dehydration, packages are used to protect against oxidation and permeability of aromatic volatiles (Gruda and Postolski, 1986). If unpacked products are frozen in line immediately after heat treatment and thereafter packed, there will be almost no increase in the number of bacteria present (Bald, 1991).

MATERIALS AND METHODS

Processing of the sweet corn

Fresh sweet corn (Zea mays L.) of Jubilee and GH 2684 (Rogers NK seed Co, Research Center) varieties were obtained from Oregon State University Vegetable Experimental Station during the 1992 season. The Jubilee and GH 2684 varieties were harvested on the same day, using the percent moisture content of the kernel as an index of maturity.

Figure 5 shows the processing steps in the preparation of the frozen sweet corn on the cob samples. Approximately 1000 ears of sweet corn of each variety were manually harvested. The ears appeared to be a homogeneous sample and were harvested with minimal damage. The ears were immediately transported to the Oregon State University Food Science and Technology Pilot Plant where they were randomly divided into three lots (replications). Each lot was husked and randomly divided into three groups of 39 and were processed in two batches. One group served as a control and the other two were steam blanched at 100°C for 9 and 15 minutes, respectively. After blanching, the sweet corn ears were cooled immediately in water (18°C) for 25 minutes, drained and frozen on trays in a blast freezer at -34.4°C (-30°F) for two hours.

The frozen ears from each of the treatment groups were cut at each end to obtain ears of 5½ inches in length. The ears were divided into four subgroups of twelve ears each and

were packed using polyethylene, Cryovac E-Bag (E bags), Cryovac Barrier Bag (B bags)(Cryovac Division, W.R. Grace & Co., Duncan, SC) and no packaging material as control. Cryovac E and Cryovac B bags had a moisture permeability of 0.1 g/100 cm^2/24 hr at 100% relative humidity (RH) and 38°C. They differed in oxygen permeability, which was 40 mL/100 cm^2/24 hr at 23°C and 1 atm for E-bags and 0.3-0.4 mL/100 cm^2/24 hr at 23°C and 1 atm for B-bags (Deak et al, 1987). Ears were placed three to a pack for each packing material. The polyethylene bags were hand sealed and the Cryovac B and E bags were heat sealed using a Verwachting vacuum sealer. Note: The ears to be packed in the Cryovac bags were packed first in polyethylene bags for 20 days because the Cryovac bags didn't arrived by the time the experiment started.

The material was placed into cardboard cases, stored at -23.3°C (-10°F) and analyzed at 0, 3, 6 and 9 months.

Moisture Analysis

The moisture content was determined using the microwave method of Becwar et al. (1977). Analysis were performed before processing and after 9 months of frozen storage at -23.3°C (-10°F). Each cultivar sample was a composite of kernels from six ears for the determination of the initial moisture content, while for the nine months analysis of the sample was a composite of kernels from 3 ears. One hundred grams were blended for 2 minutes, stored in covered jars and

placed in ice to cool down. Blended corn samples (ten grams per sample) were placed on a pre-weighed glass petri dish. Care was exercised to obtain uniform sample thickness in all dishes. Two samples at a time were placed in an Amana "Radarange" microwave oven (Model No. RR-3, 1600 watt, operating frequency 2450 megahertz) and heated for 3 minutes. Samples were then removed and temporally held in a covered glass desiccator until they were again weighed. Replicates were performed on each of the samples for each cultivar.

Sample Preparation

Whole kernels of sweet corn (100 grams) were frozen in liquid nitrogen and blended using a Waring blender at 100 rpm for 1 minute. In order to keep the sample frozen for efficient blending, liquid nitrogen was added every 15 seconds. A fine powder was obtained which was stored in a cold container at -23.3°C (-10°F) until analyzed.

Enzyme Assays

Enzyme Extraction:

The nitrogen powdered corn (2 grams) was placed in a centrifuge tube and 20 ml of 0.1M Tris-HCl buffer pH 8.0 was added. The mixture was homogenized using a tissuemizer at a speed of 50 rpm for 3 minutes. When homogenization was finished, the probe was rinsed with 1 ml of 0.1M Tris-HCl

buffer pH 8.0. The homogenate was centrifuged using a Sorval RC5 superspeed refrigerated centrifuge in S34 rotor at 12,000 rpm for 1 hour at 4°C. The supernatant was removed avoiding the floating top layer and used for activity measurements.

Lipoxygenase Activity:

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12) was assayed by the method of Chen and Whitaker (1986). The substrate solution was prepared by mixing 157.2 μL linoleic acid, 157.2 μL Tween 20 and 10 mL distilled water, clarified by adding 1 mL of 1.0N NaOH and diluted with 0.1M sodium phosphate buffer pH 7.0 to a final volume of 200 ml. Prior to performing the assay, the substrate solution was transferred to an amber container and aerated with oxygen for 10 minutes and allowed to stand in a shaking water bath (American YB-521) at 25°C for 10 minutes.

For the assay, 150 μL of the enzyme extract was added to 2.85 mL of linoleic acid substrate solution in a quartz cuvette, mixed and lipoxygenase activity measured from the change in absorbance at 234 nm over time using a Shimadzu 160U UV-VIS spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that produced a change of 1.0 absorbance unit per minute.

Peroxidase Activity:

Peroxidase was assayed by the method of Sheu and Chen (1991). The substrate solution was prepared by mixing 558 μL guaiacol and 194.4 μL of 30% hydrogen peroxide; the solution was then diluted to 100 mL with 0.2M sodium phosphate buffer pH 6.0 to give a concentration of 0.05M guaiacol and 0.2M hydrogen peroxide. Peroxidase activity was measured from initial increase in absorbance at 420 nm over time with a Shimadzu 160U UV-VIS recording spectrophotometer. One unit of enzyme was defined as the amount of enzyme that produced a change of 1.0 absorbance unit per minute.

Protein:

Protein was assayed using the method of Lowry et al. (1951) by measuring absorbance at 700 nm. Bovine serum albumin was used as a standard.

Fatty acids analysis

The corn lipids were extracted following the procedures proposed by Bligh and Dyer (1958). Approximately 3 grams of the nitrogen powdered corn were placed in a screw capped tube and 2.5 ml of deionized distilled water, 5 ml of methanol and 2.5 chloroform were added, mixed and stored at 5°C. After 24 hours 2 ml of deionized distilled water and 2.5 ml of chloroform were added to the mixture. The samples were

centrifuged to separate the aqueous from the non polar phase. Lipids were extracted, taken to dryness under nitrogen and stored at -80°C for further analysis.

Fatty acids were analyzed by the fatty acid methyl esters (FAME) method (Selivonchick, 1977). The lipid extracted was transesterified by heating in 4% methanolic H_2SO_4 solution at 80-90°C for 90 minutes in Teflon lined screw capped test tubes. In order to solubilize the lipid, benzene was added. To extract the methyl esters, 1 mL of millipore distilled water and 2 mL of hexane were added. The solution was mixed using a Vorter mixer, allowed to separate into two phases and the top phase collected into a Teflon lined screw capped test tube. The hexane step was repeated to ensure complete extraction. The hexane was evaporated under nitrogen and the dried sample was taken up into 0.5 ml iso-octane, flushed with nitrogen and stored at -80°C until analyzed by gas chromatography (Selivonchick et al., 1977).

Analysis of methyl esters was performed on a Hewlet-Packard 5890 Gas Chromatograph equipped with FID, 3393A Integrator and 9122 Dual Disc Drive. A 30m, 0.25mm ID fused silica Supelco 2330 capillary column was used for analysis of all nitrogen powdered corn fatty acid methyl esters. The chromatographic conditions were as follows: injection port and detector were held at 220°C, the column was run isothermally at 175°C for 10 minutes and rise to 210°C at 5°C/min with a final hold time of 10 minutes. The carrier gas was helium.

Fatty acid methyl esters were identified by comparing to authentic standards Nu Check 20A (Nu Check Prep) and corn oil fatty acids.

Determination of peroxide value

The peroxide value was determined using the method of Schmedes and Holmer (1989). The method is based on the oxidation of Fe(II) to Fe(III) by peroxides; Fe(III) forms a violet complex with thiocyanate and this complex is quantified spectrophotometrically.

A ferrous chloride solution was prepared by mixing equal volumes of barium chloride solution (8 mg $BaCl_2.2H_2O$/ml) and ferrous sulfate solution (10 mg $FeSO_4.7H_2O$/ml). To the mixture was added 20 μl/ml concentrated HCl and the precipitated $BaSO_4$ was allowed to settle for one hour at 4°C to obtain a clear supernatant. An ammonium thiocyanate solution was prepared at a concentration of 300 mg NH_4SCN/ml.

A stock solution was prepared by weighing 240 mg $FeCl_3.(6H_2O)$ into a flask with the addition of 3 ml of concentrated HCl and 200 μl 30% H_2O_2. This was done to oxidize Fe(II) that was present. After 10 minutes the remaining H_2O_2 was removed by heating the flask in boiling water for 10 min. After cooling, the solution was transferred into a 50 ml volumetric flask and made to volume with deionized distilled water. The final concentration of the stock solution was 4.8 mg $FeCl_3.(6H_2O)$/ml which corresponds to 1 mg Fe(III)/ml. Six

different standard solutions containing 0.05 mg - 0.35 mg Fe(III) were prepared by diluting the stock solution with water and concentrated HCl making the final concentration of HCl 0.25 M in the standards.

The standard curve was prepared by adding 20 μl of each standard solution and mixing with 4.955 ml of chloroform:methanol (3:5 ratio) and 25 μl thiocyanate solution. After 10 minutes the absorbance was determined at 505 nm.

To determine the peroxide value in the samples, the extracted corn lipids were weighed, diluted with 2 ml of chloroform, mixed using a vortex mixer and the solution was divided into halves. One ml was mixed with 3.975 ml of chloroform:methanol (3:5 ratio), 25 μl of thiocyanate solution and 25 μl of ferrous chloride solution. After 10 minutes the absorbance was determined at 505 nm against a reagent blank prepared omitting the lipid addition. The other ml was handled in precisely the same manner except that no ferrous chloride reagent was added (lipid blank).

For calculation of the peroxide value, the absorbance of the sample and lipid blank were converted to mg Fe(III) per 5 ml solution using standard curve values and the net value in terms of mg of Fe(III) per 5 ml of solvent was calculated by the difference between of the values obtained for the sample and the lipid blank.

$$\text{Peroxide value} = \frac{\text{Net mg Fe(III) per 5 ml}}{\text{g of lipid used x 55.84}}$$

The peroxide value was expressed in terms of mili-equivalents of oxygen per kilogram of lipid (Stine et al., 1954).

Volatile Headspace Analysis

Hexanal was determined using a headspace gas chromatography assay described by Robards et al. (1988) with some modifications. Nitrogen powdered sweet corn (2 grams) was placed into a 5 mL screw cap vial (Supelco Inc, Bellefonte, PA); 25 µl of a 500 ppm solution of 2-heptanone in iso-octane was added as an internal standard and hermetically sealed using a teflon mininert valve (Supelco Inc, Bellefonte, PA). Each sample was heated on a heating block at 90°C for 1 hour to establish equilibrium. A headspace volume of 250 µl was withdrawn from the vial with a 1000 µl gas tight syringe, preheated to 90°C, and injected into a Hewlett-Packard 5890A gas chromatograph equipped with FID, 3393A Integrator and 9122 Dual Disc Drive. The capillary column used was a 30 m, 0.25 mm ID fused silica Supelco 2330. Helium was used as the carrier gas. The injection and detector temperatures was 220°C and the column was programmed as follows: 50°C isothermal for 3 minutes, then increased at 6°C/min to 185°C and held for 5 minutes. Hexanal peaks were identified and quantified by means of their retention times

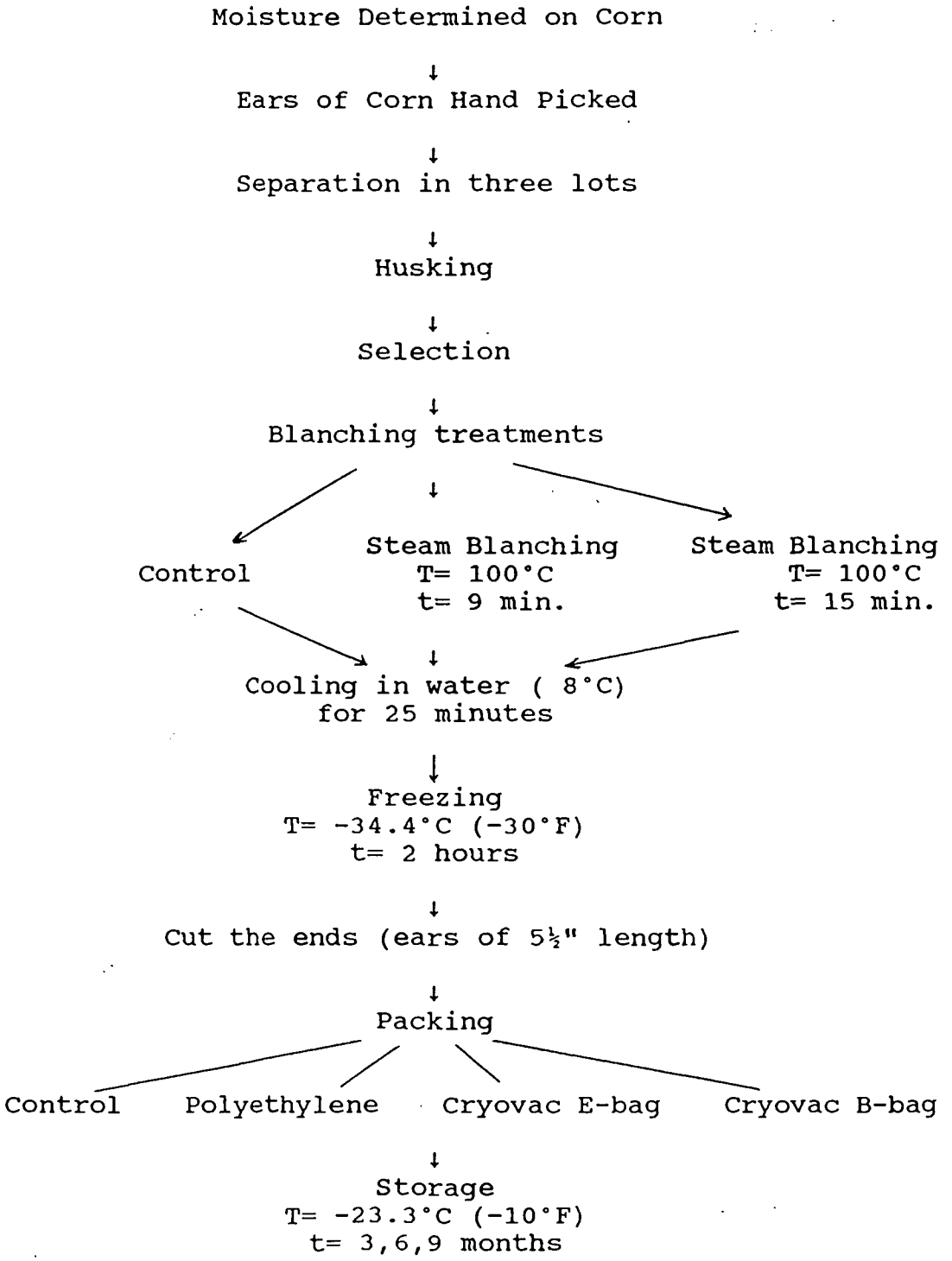
and areas based on a hexanal pure standard (Supelco Inc, Bellefonte, PA). Hexanal standard curve was prepared by adding known amounts of pure hexanal (1-100 ppm) into the vials, proceeding as indicated above, and injecting 250 μl of the completely vaporized compound into the GC system.

Statistical Analysis

The experiment was conducted using a split-plot design. The split occurred at the blanching stage, with variety and blanching as whole plot and packaging material and storage time as sub-plot. The split-plot design results from a specialized randomization scheme for a factorial experiment. The principle is that whole plots, to which levels of one or more factors are applied, are divided into subplots or subunits to which levels of one or more additional factors are applied. Thus each whole plot becomes a block for the subunit treatments (Steel and Torrie, 1980). The data were evaluated using Statistical Analysis System (SAS).

The data were further evaluated by a regression analysis using the stepwise procedure (Ramsey and Schafer, 1992). The regression analysis were performed using the Statistical Graphics System.

Figure 5: Flow Diagram of Sweet Corn Processing



RESULTS AND DISCUSSION

Raw material and moisture content

The percent moisture content is commonly used to determine the proper harvest date of sweet corn for processing (Becwar et al, 1977). The initial moisture content in the kernels, used as an index of maturity, was determined for both sweet corn varieties studied. The Jubilee variety had a moisture content of 72.6 ± 0.7 % while the GH 2684 variety had a moisture content of 72.4 ± 0.6 % at the harvest date. Wiley et al. (1989) defined standard sweet corn varieties for processing according to their moisture content as Grade A Fancy with 73 - 75 % moisture, Grade B Extra Standard with 71 - 72 % moisture, Grade C Standard with 69 - 70 % moisture and Grade D Substandard with 66 - 68 % moisture. According to this grading system, the corn used in the present study could be considered as Grade A Fancy sweet corn, since the moisture content was very close to 73 %.

After nine months of frozen storage, only the packaging material had a significant effect on the moisture content of the sweet corn samples.

Figure 6 shows the average moisture content in the kernels of the sweet corn after 9 months of frozen storage using different packaging materials, the values reported represent averages for both varieties of sweet corn since there was no statistical difference among them. The kernels

of the sweet corn ears stored in Cryovac B and Cryovac E bags showed similar moisture losses, as expected because of their same moisture permeability (0.1 g/100 cm^2/24 hr at 100 % relative humidity and 38°C). Considering an initial moisture content of 72.5 % (average of both sweet corn varieties), the kernels decreased only an average of 0.3 % and 0.4 % respectively.

The kernels of the ears of sweet corn packed in polyethylene bags showed a greater decrease in moisture content (1.1 %) but the most critical moisture loss was observed in the kernels of the control ears that were stored without packaging material with an average moisture loss of 2.4 %. The kernels of the control ears of corn showed severe dehydration manifested by kernel shrinking (Figure 7). With a final moisture content in the kernels of 70.1 %, the control ears of sweet corn would have changed from a Fancy A grade to a Standard C grade.

Enzymatic activities during frozen storage

Lipoxygenase activity

Table 5 shows the results of lipoxygenase specific activity for the different blanching treatments and packaging materials used for Jubilee and GH 2684 varieties during the 9 months of frozen storage.

Figure 8 shows the mean lipoxygenase specific activity

during the 9 months of frozen storage for both sweet corn varieties and different blanching treatments. The values are average comparisons of the different packaging materials; average values are presented since no significant statistical differences were observed.

There was substantial evidence that the initial level of lipoxygenase specific activity in the unblanched whole kernels varied between the varieties evaluated (2 sided p-value < 0.0001, step backward regression analysis). The Jubilee variety showed an initial mean lipoxygenase specific activity of 0.143 ± 0.032 units/mg protein, 32.4 % higher than the mean lipoxygenase specific activity showed by the GH 2684 variety, with a level of 0.108 ± 0.017 units/mg protein. Differences in enzyme activity among varieties have been reported in several studies, and it is known that lipoxygenase activity not only varies among varieties but also among different organs of the plant (Garrote et al., 1985; Pinsky et al., 1971; Vick and Zimmerman, 1976) and between different developmental stages (Kermasha and Metche, 1987). Wagenknecht (1958), working with several varieties of sweet corn, found that varietal differences in residual lipoxidase activity can not be attributed to differences in relative abundance of the enzyme in the raw corn, but more likely to different resistance to thermal destruction of the enzyme.

There was also substantial evidence (2 sided p-value < 0.0001) that the lipoxygenase specific activity decreased

during the nine months of frozen storage in both unblanched sweet corn varieties.

Although some differences in specific activity can be observed from Figure 8, the Jubilee variety (Figure 8) showed the greatest decrease in specific activity between 0 and 3 months with a tendency for further decrease in the following months. The GH 2684 variety showed the greatest reduction in lipoxygenase specific activity between 6 and 9 months. The regression analysis gave convincing evidence that the lipoxygenase specific activity is associated with the frozen storage period even after accounting for the effect of the different varieties (2 sided p-value < 0.0001); decreasing at the same mean rate for both varieties (rate of 0.0052 units/mg protein per month), with an average final specific lipoxygenase activity of 0.087 ± 0.037 and 0.062 ± 0.019 units/mg protein for Jubilee and GH 2684 respectively.

The determination of lipoxygenase activity was based on the absorption at 234 nm of the conjugated dienes formed when the linoleic acid (used as substrate) was oxidized in the presence of lipoxygenase. Figure 9 shows an example of the differences in lipoxygenase activity found between the unblanched and blanched corn crude extracts assayed and the substrate (linoleic acid). All the blanched samples showed similar small increments in absorbance during the assay and they did not behave as a straight line. We assume that these absorbance increments were assay noise which can be explained

on the basis of absorption characteristics of other components present in our crude extract. Berkley and Galliard (1976) reported some limitations of the spectrophotometric method due to interfering absorptions and secondary reactions of the unstable hydroperoxide products when crude extracts are used. Kristie and Thompson (1989) also reported the interfering absorbances of several inhibitors of lipoxygenase such as EDTA, azide, n-propylgallate, etc., when the spectrophotometric assay is used. No autoxidation of the substrate was observed during the lipoxygenase assays (Figure 9).

Garrote et al. (1985) reported the complete inactivation of lipoxygenase in the kernel of sweet corn (Jubilee variety) when blanched for 12 minutes at 100°C. From the results of the present study, it can be concluded that steam blanching at 100°C for 9 minutes was enough to completely inactivate the lipoxygenase specific activity in both varieties of sweet corn on the cob. Blanching for 15 minutes had no further effect on the lipoxygenase specific activity.

Peroxidase activity

Table 6 shows the peroxidase specific activity for the different blanching treatments and packaging materials used for Jubilee and GH 2684 varieties respectively during frozen storage.

Figure 10 shows the mean lipoxygenase specific activity during the 9 months of frozen storage for both sweet corn

varieties and different blanching treatments. The values presented are averages over the different packaging materials, and no statistical differences were observed between samples.

In addition, no significant difference in the initial peroxidase specific activities were found in the kernels of any of the unblanched varieties of sweet corn evaluated. An average specific activity of 1.082 ± 0.039 and 1.075 ± 0.051 units/mg protein was observed for the Jubilee and GH 2684 variety respectively.

After 9 minutes of blanching treatment, some residual peroxidase specific activity was still present in the kernels of both sweet corn varieties (Figure 10), with an initial peroxidase specific activity of 0.048 ± 0.031 and 0.081 ± 0.054 units/mg protein for the Jubilee and GH 2684 varieties respectively. Compared to the unblanched samples, the 9 minute blanching treatment resulted in 95.4 % and 92.8 % reduction of the peroxidase activity for the Jubilee and GH 2684 varieties respectively, suggesting the presence of different heat resistant peroxidase isozymes in the varieties of sweet corn evaluated. Chenchin and Yamamoto (1973) isolated a total of eight peroxidase isozymes from sweet corn and found that peroxidase isozymes in corn consist of heat labile and heat resistant groups along with some of intermediate heat resistance groups. They also found that peroxidase heat stability depended on the variety of sweet corn.

Complete inactivation of peroxidase activity in the kernel was obtained when the ears of sweet corn were blanched for 15 minutes (Figure 10).

A regression analysis showed substantial evidence (2 sided p-value < 0.0001) that the peroxidase specific activity of the unblanched samples decreased as a quadratic function of the frozen storage time. The peroxidase specific activity decreased drastically in the first 3 months with a tendency to stabilize during the following months of frozen storage. The Jubilee and GH 2684 varieties of sweet corn (Figure 10) showed similar behavior during the nine months of frozen storage.

A regression analysis for the 9 minute blanched samples showed that the peroxidase specific activity had a tendency to decrease faster in the GH 2684 than in the Jubilee variety (2 sided p-value < 0.0001), suggesting the presence of peroxidase isozymes that are more stable to low temperatures in the Jubilee variety than in the GH 2684 variety.

In the unblanched sweet corn samples, both enzymes analyzed, lipoxygenase and peroxidase, showed significant specific activity reduction during the 9 months of frozen storage. This reduction might be due to decreased accessibility of enzyme to substrate because of phase changes in the substrate, increased hydrogen bonding between water and either the substrate or the enzyme active site, formation of enzyme polymers, change in mechanism, decreased ionization of enzymes,substrate and buffers, shifts in pH and increased

viscosity of the system (Richardson and Hyslop, 1985). Vetter et al. (1958) defined enzyme inactivation by heat as the result of thermal denaturation of protein involving primarily a change in geometric configuration. The enzyme molecule looses its ability to form enzyme-substrate complexes and, consequently, its catalytic activity. These investigators (Vetter et al., 1958) reported that peroxidase exhibits the ability to regain activity after mild heat inactivation but somewhat longer heating can change the configuration so drastically that return to the original form is prohibited. No regeneration of lipoxygenase or peroxidase specific activity was found in any of the blanched samples during frozen storage (Figures 8 and 10), showing that the blanching treatments (steam at 100°C for 9 and 15 minutes) and the low temperature of storage (-23.3°C) were enough to permanently disrupt the active site of both enzymes.

Fatty acid composition of sweet corn and its stability during frozen storage

Initial fatty acid composition

In Figure 11 is shown a chromatogram of the initial fatty acid methyl esters composition of the unblanched Jubilee variety. The major fatty acids present in the whole kernels were palmitic acid (14.93 ± 1.94 %), stearic acid (2.79 ± 1.00 %), oleic acid (31.54 ± 2.82 %), linoleic acid (46.87 ± 5.88

%) and linolenic acid (1.89 ± 0.36 %) with no statistical difference between the two varieties. The initial fatty acid composition was not affected by the different blanching treatments used. Other minor peaks, each one representing less than 1 % of the total fatty acid composition, were not identified.

Corn genotype has a greater influence on the fatty acid composition than any environmental factor and the fatty acid composition among known corn genotypes covers a wide range (Weber, 1987). Jellum (1970) studied the fatty acid composition of 788 corn strains and found a range from 6 to 22 % for palmitic acid, 1 to 15 % for stearic acid, 14 to 64 % for oleic acid, 19 to 71 % for linoleic acid and 0.5 to 2.0 % for linolenic acid. Although the two different varieties of sweet corn studied had different genotypes, Jubilee a sugary hybrid and GH 2684 a sweet enhanced (se) hybrid, no significant difference in the fatty acid composition was determined between them (2 sided p-value = 0.86).

Changes in the fatty acid composition during storage

The changes on the average relative percent fatty acid composition during the frozen storage period for the different blanching treatments and the packaging materials used were monitored. All the chromatograms followed the same pattern and the results are presented in the Tables 7, 8, 9 and 10. The relative percent composition of the saturated fatty acids

showed no change, while, the relative percentage of oleic acid showed the tendency to increase during the storage time.

The formation of a number of aroma compounds can be traced to lipid degradation at various stages of food processing (Eriksson, 1975). The rate of oxidation is affected by the number, position, and geometry of the double bonds. Relative rates of oxidation for arachidonic, linolenic, linoleic and oleic acids are approximately 40:20:10:1, respectively (Nawar, 1985). Linoleic and linolenic acid are the major polyunsaturated and, therefore, the most susceptible to oxidation fatty acids present in both varieties of sweet corn.

Figure 12 shows the changes in the mean relative percent linoleic acid during the nine months of frozen storage for both sweet corn varieties and different blanching treatments. The values are averages over the different packaging materials since they did not showed statistical differences. During storage, both varieties of sweet corn showed no statistical difference in their relative percent linoleic acid content. The different blanching treatments had no effect on the relative percent linoleic acid content, this suggested that enzymatic oxidation of linoleic acid does not occur during frozen storage. After 3 months of frozen storage a small decrease in the relative percent content of linoleic acid, which tended to stabilize during the following months, was observed. This fact suggested the possible autoxidation of

the fatty acid due to a self-catalytic free radical mechanism.

Figure 13 shows the changes in the mean relative percent linolenic acid content during frozen storage for both sweet corn varieties and different blanching treatments. The relative percent linolenic acid content in the whole kernels of both varieties of sweet corn decreased during frozen storage.

The regression analysis gave convincing evidence that the mean percent linolenic acid content was dependant also on the blanching treatment used (2 sided p-value < 0.0001). The unblanched samples decreased at a faster rate (0.07 % linolenic acid/month of storage) than the blanched samples (0.061 % and 0.056 % linolenic acid/month of storage for 9 and 15 minutes blanching respectively) in both sweet corn varieties suggesting enzymatic oxidation of linolenic acid during storage. Poca et al. (1990) characterized a corn lipoxygenase isozymes (L1) that had high affinity for α-linolenic acid leading to the formation of 13-hydroperoxides.

Autoxidation by free radical mechanisms was suggested by the decrease in relative percent linolenic acid of the blanched samples during frozen storage, especially at 15 minutes where both enzymes evaluated were inactivated.

The packaging materials Cryovac B and E, with low oxygen permeability, were used to reduce the oxygen concentration in the packaging atmosphere, but none of the packaging materials studied showed any effect in the control of polyunsaturated

fatty acid degradation. Galliard (1989) reported that both, enzymic and non enzymic oxidation of lipids require only low levels of oxygen and there are technical and economic problems in achieving and maintaining oxygen level below 1 % in food packaging. However, as the temperature is lowered the viscosity of the product is increased and the water activity reduced impeding the access of oxygen to the food (Nawar, 1985). This suggests that the low rates of lipid degradation observed in all our samples during the 9 months of frozen storage at -23°C mainly depended on the storage temperature that lowered the oxygen diffusion into the corn.

Aldehyde formation

The headspace analysis showed no formation of hexanal, the major secondary product from the final stages of the decomposition of fatty acids hydroperoxides, with a sensitivity of 50 ppm. Robards et al. (1988) reported hexanal levels of 250 ppm in corn chips samples stored at 60°C for 20 days, but they concluded that concentration of 5 ppm of hexanal correspond to an unacceptable flavor. Although we did not find highly oxidized samples, we could not make conclusions regarding the initial steps of decomposition of fatty acids. For this reason we decided to evaluate the formation of hydroperoxides, primary initial products of lipid oxidation, using the peroxide value. The use of an aldehyde specific column and purification steps of the corn volatiles

may have improved the sensitivity of the method.

Peroxide value

Figure 14 shows the changes in peroxide value during frozen storage for both varieties of sweet corn and the different blanching treatments. The values reported are averages over the different packaging materials since no significant difference was observed among them (split-plot analysis). The complete results of the peroxide value analysis are shown in Table 11.

The peroxide value appeared dependant on the variety (2 sided p-value = 0.007) and blanching treatment (2 sided p-value = 0.003). There was statistical evidence that the changes in the peroxide value during the storage time were different in the two varieties (2 sided p-value = 0.015). In Figure 14 the data show that the peroxide value of the unblanched Jubilee variety increased on an average of 50 % from the sixth to the ninth month of storage. The Jubilee variety samples blanched for 9 minutes showed some increments in peroxide value, but none were observed for the Jubilee samples blanched for 15 minutes. The same was true for all the GH 2684 samples tested.

The differences observed in the peroxide value between the two varieties of unblanched sweet corn can be attributed to the higher lipoxygenase activity found in the Jubilee variety. The change in peroxide value in the Jubilee samples

blanched for 9 minutes could be attributed to nonenzymatic lipid oxidation. Eriksson (1975) reported that heat treated peroxidase increased nonenzymatic lipid oxidation considerably with a corresponding decrease in their enzymatic activity. The highest peroxide value obtained was 0.5 meq O_2/kg lipid corresponding to the unblanched Jubilee sample after 9 months of storage. The peroxide value is a good guide for quality of a lipid. For example, freshly refined fats should have peroxide values of less than 1 meq O_2/kg (Rossell, 1989). All the peroxide values obtained in the present study on extracted corn lipids were very low, far below 1 meq O_2/kg lipid. These results along with the good stability of the fatty acid composition during storage confirmed the low level of lipid degradation on the varieties of sweet corn studied stored. The rate of a chemical reaction diminishes with falling temperature but in the case of peroxidation of lipids in the range of 0 to -10°C the rate is accelerated because an increasing proportion of pure ice is formed while the remainder of the water forms a solution of increasing strength and lowered freezing point (Ranken, 1989). The data obtained from the present study showed that at a temperature as low as -23.3°C the rate of lipid oxidation was significantly reduced. This can be explained by the fact that at -20°C most of the water is already frozen (98.2 %) (Ranken, 1989) and the reactants are not in solution.

Color deterioration during frozen storage

After 9 months of storage, visual differences in the color of the corn packaged in the different materials was observed (Figure 15). The corn packaged in the cryovac B and E maintained most of the corn fresh color, while marked fading was observed in the corn stored in polyethylene bags. Severe lost of color was observed in the control ears of corn that were stored without packaging materials. The color deterioration during storage suggested the oxidation of carotenoid pigments due to autoxidation or possibly bleaching by the reaction of lipoxygenase secondary products in unblanched samples. This is possible because of the exposure of the kernel surface to oxygen. These results suggest that color may be a good indicator of the quality of sweet corn during storage.

Figure 6: Average moisture content of the unblanched and blanched (9 and 15 minutes) sweet corn kernels of Jubilee and GH 2684 varieties packaged using three different materials and a control (no packaging material) after 9 months of storage at -23.3°C. The data presented are an average of two replications.

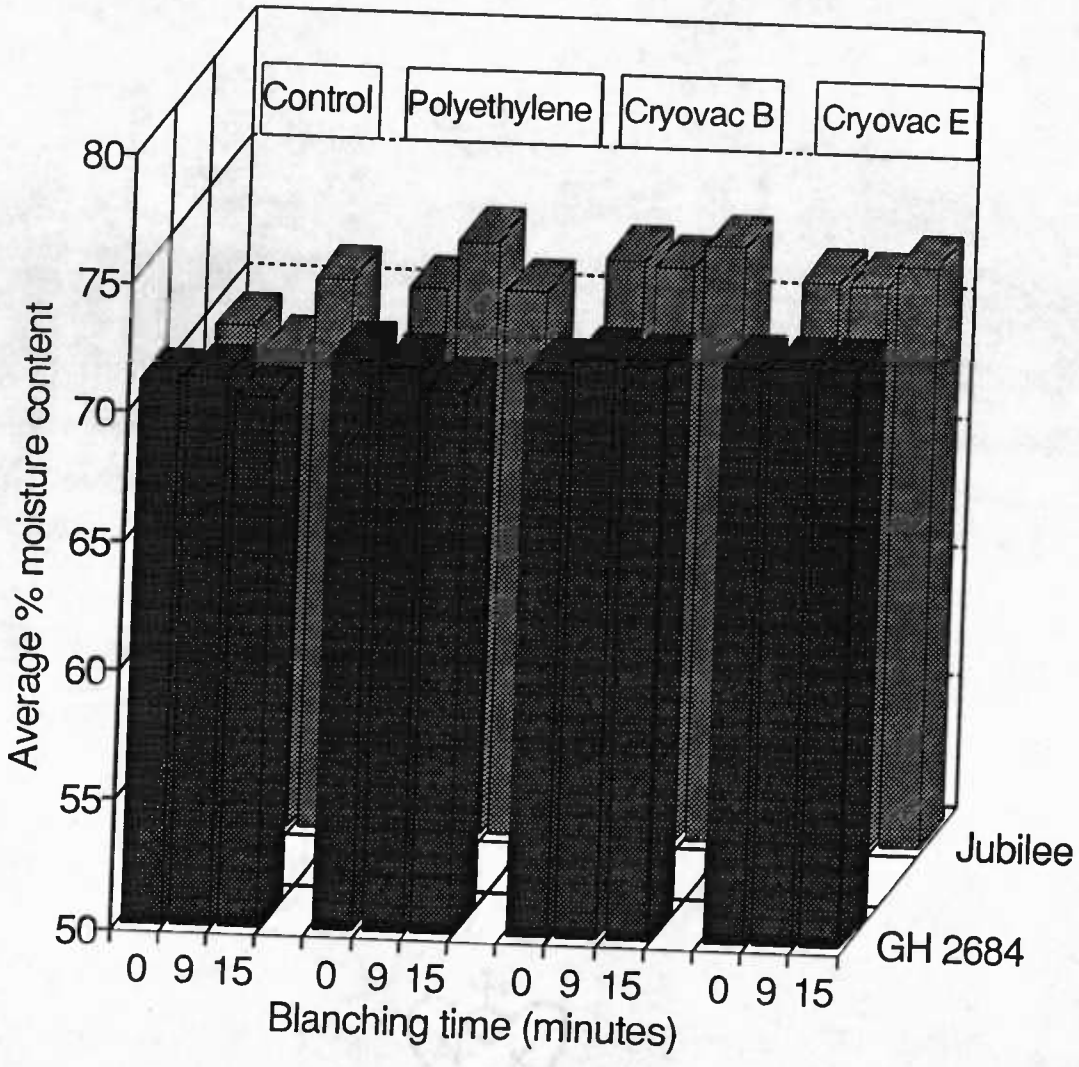

Control
Polyethylene
Cryovac B
Cryovac E
80
75
70
65
60
55
50
Average % moisture content
0 9 15
0 9 15
0 9 15
0 9 15
Blanching time (minutes)
Jubilee
GH 2684

Figure 7: Photograph of corn stored without packaging material (control). Note shrinking of the kernels (→).


Control

Figure 8: Lipoxygenase (LOX) specific activity in the whole kernels of the Jubilee and GH 2684 varieties for the unblanched and blanched (9 and 15 minutes) samples during 9 months of storage at -23.3°C. The data presented correspond to averages over the packaging materials.

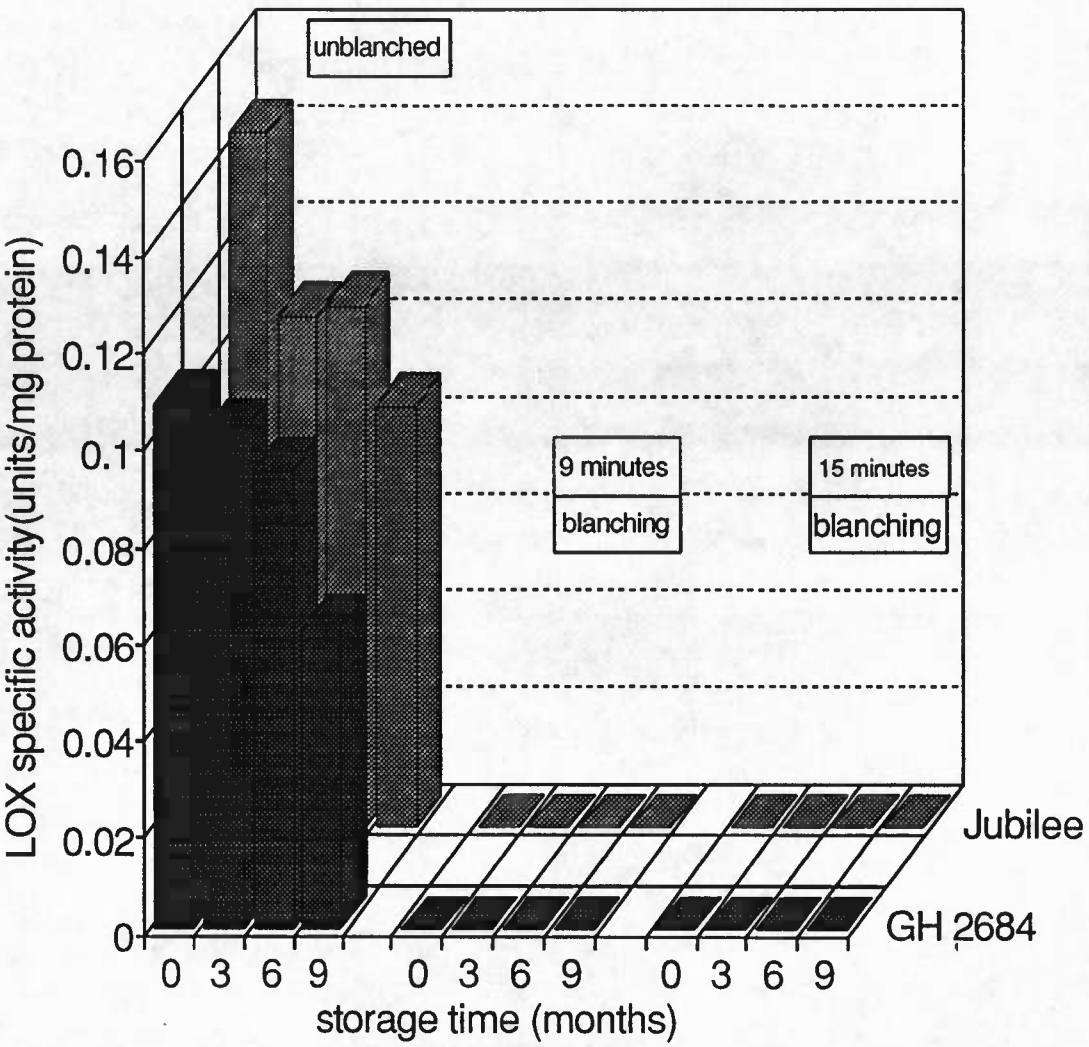

unblanched
9 minutes
blanching
15 minutes
blanching
LOX specific activity(units/mg protein)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0 3 6 9 0 3 6 9 0 3 6 9
storage time (months)
Jubilee
GH 2684

Figure 9: Changes in absorbance at 234 nm of the substrate (linoleic acid) with the addition of corn kernel crude extracts of unblanched samples, 9 minutes blanched sample and control (substrate only) during 200 seconds of assay at 25°C.

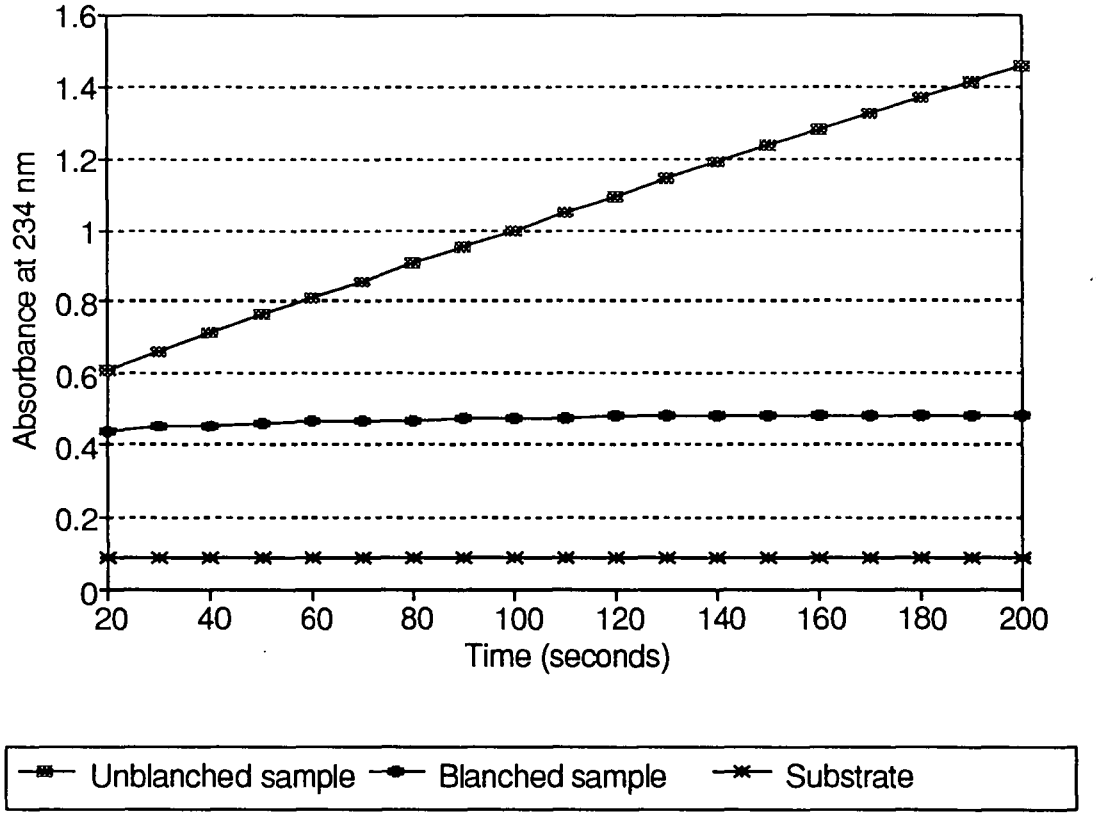

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbance at 234 nm
20
40
60
80
100
120
140
160
180
200
Time (seconds)
Unblanched sample
Blanched sample
Substrate

Figure 10: Peroxidase (POD) specific activity in the whole kernels of the Jubilee and GH 2684 varieties for the unblanched and blanched (9 and 15 minutes) samples during 9 months of storage at -23.3°C. The data presented correspond to averages over the packaging materials.

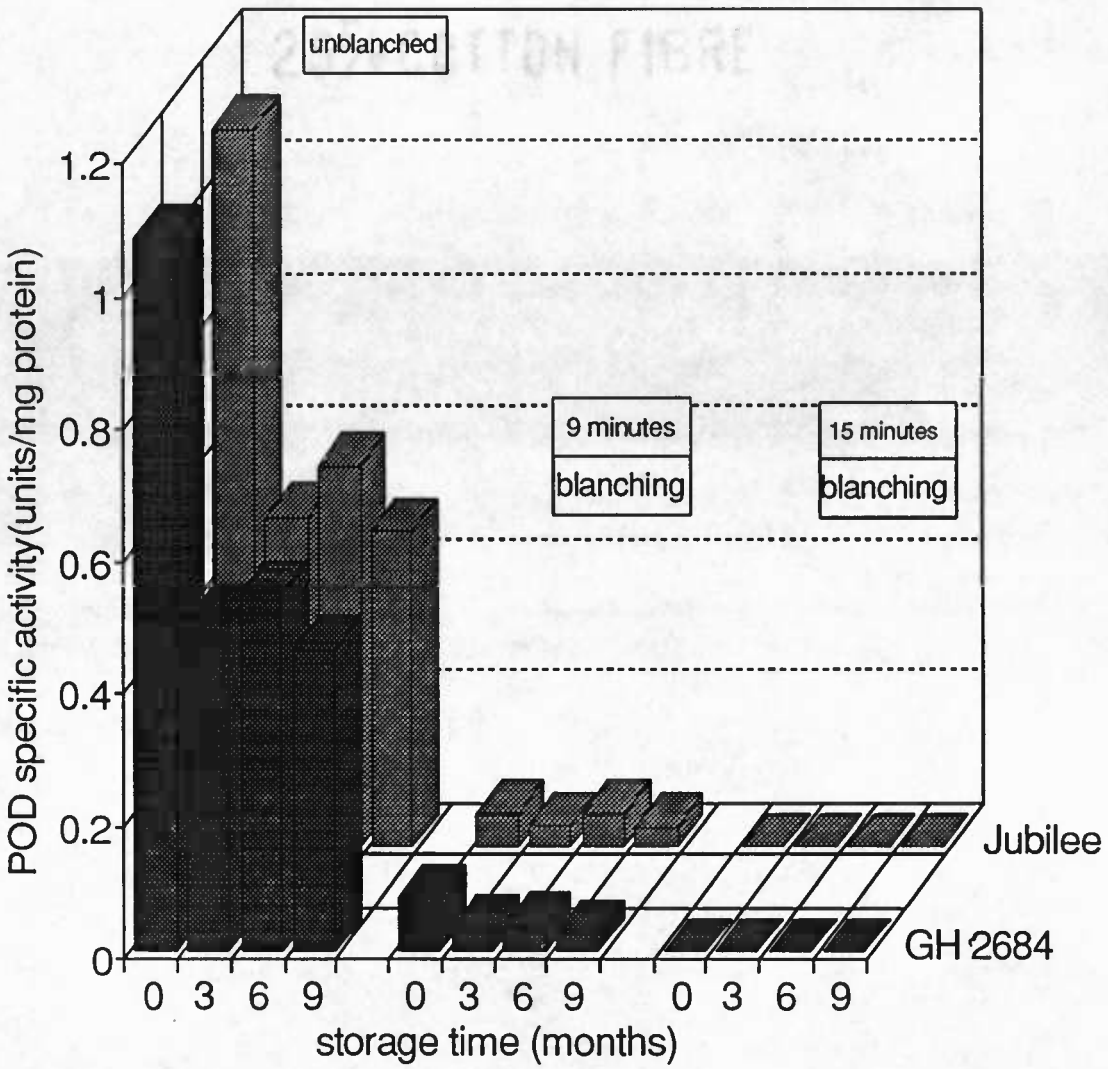

unblanched
9 minutes
blanching
15 minutes
blanching
POD specific activity(units/mg protein)
1.2
1
0.8
0.6
0.4
0.2
0
0 3 6 9
0 3 6 9
0 3 6 9
storage time (months)
Jubilee
GH 2684

Figure 11: Typical gas chromatogram of the fatty acid methyl esters obtained from the two different varieties of sweet corn kernels.

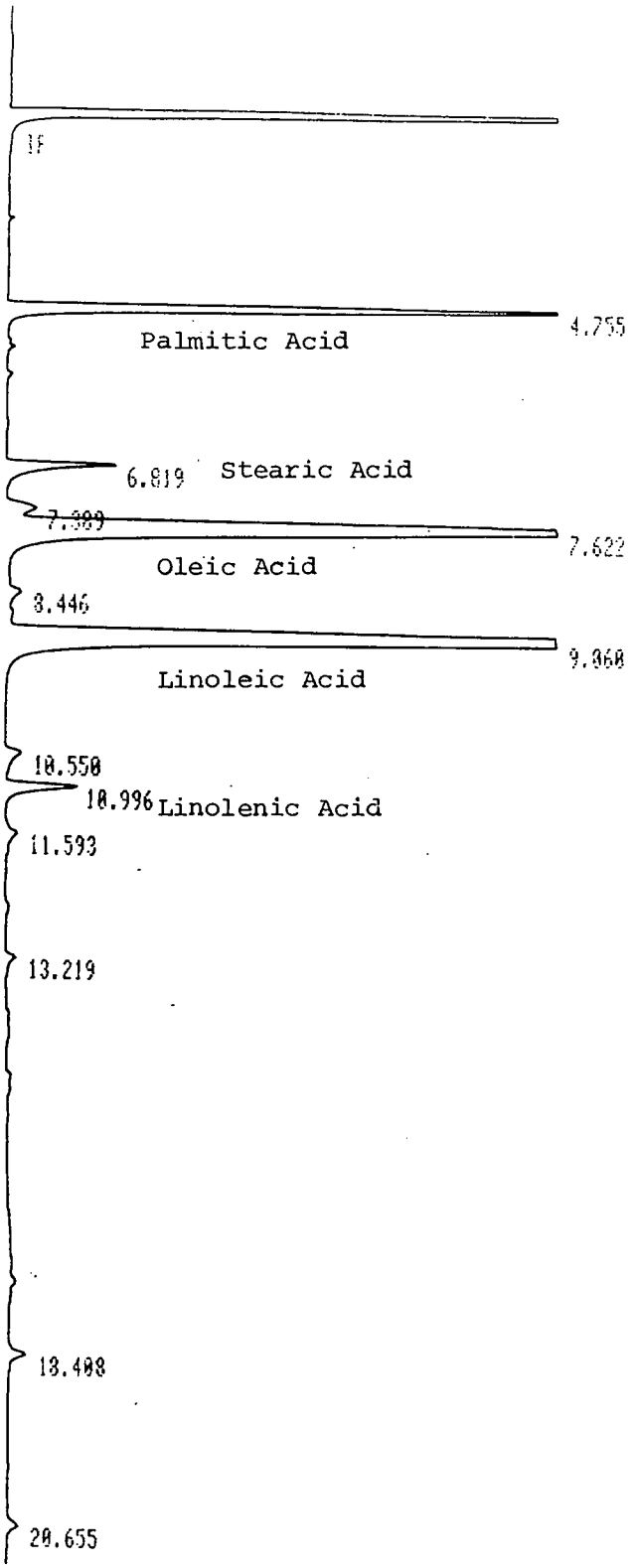

4.755
Palmitic Acid
6.819
Stearic Acid
7.622
Oleic Acid
8.446
9.060
Linoleic Acid
10.550
10.996
Linolenic Acid
11.593
13.219
18.408
20.655

Figure 12: Relative percent linoleic acid content in the whole kernels of the Jubilee and GH 2684 varieties for the unblanched and blanched (9 and 15 minutes) samples during 9 months of storage at -23.3°C. The data presented correspond to averages over the packaging materials.

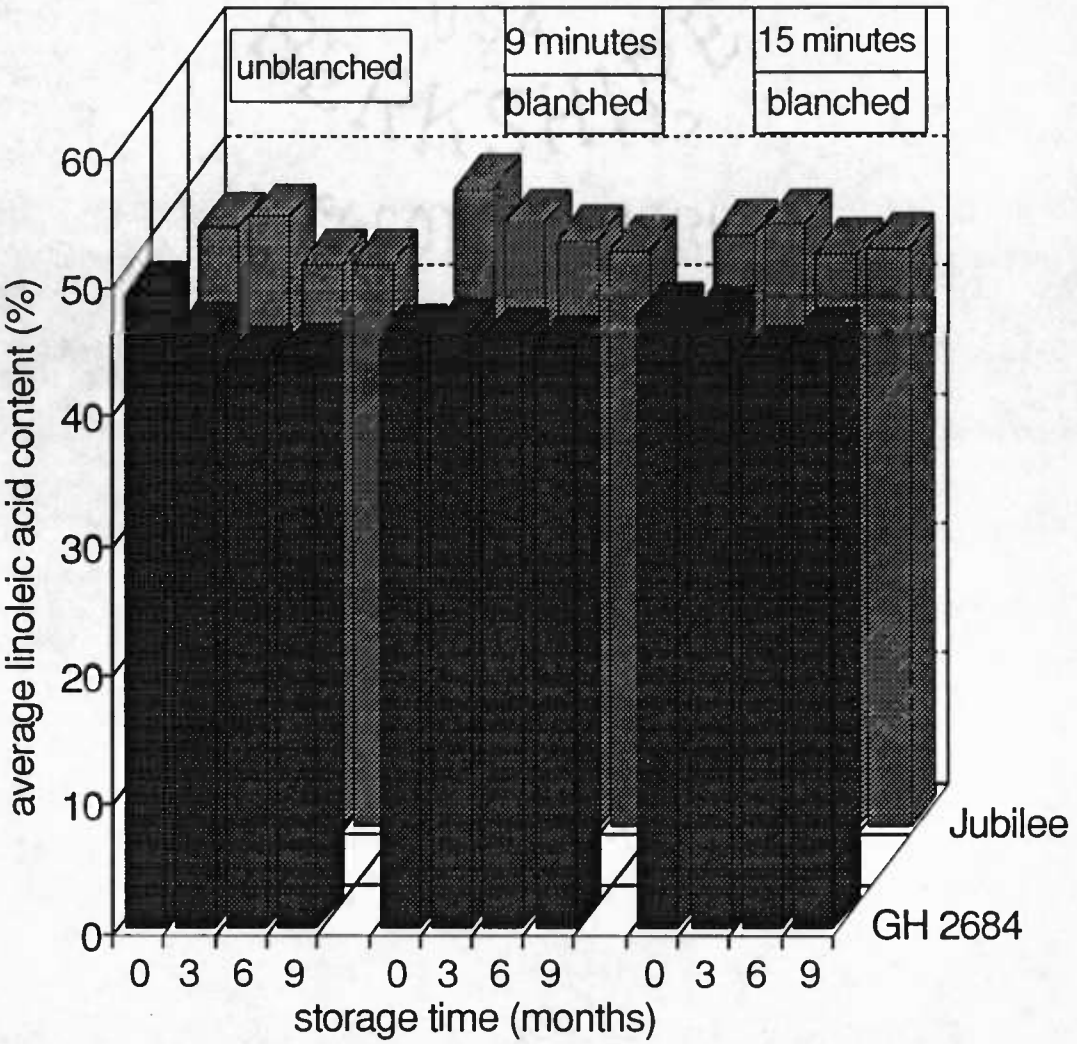

unblanched
9 minutes
blanched
15 minutes
blanched
60
50
40
30
20
10
0
average linoleic acid content (%)
0 3 6 9
0 3 6 9
0 3 6 9
storage time (months)
Jubilee
GH 2684

Figure 13: Relative percent linolenic acid content in the whole kernels of the Jubilee and GH 2684 varieties for the unblanched and blanched (9 and 15 minutes) samples during 9 months of storage at -23.3°C. The data presented correspond to averages over the packaging materials.

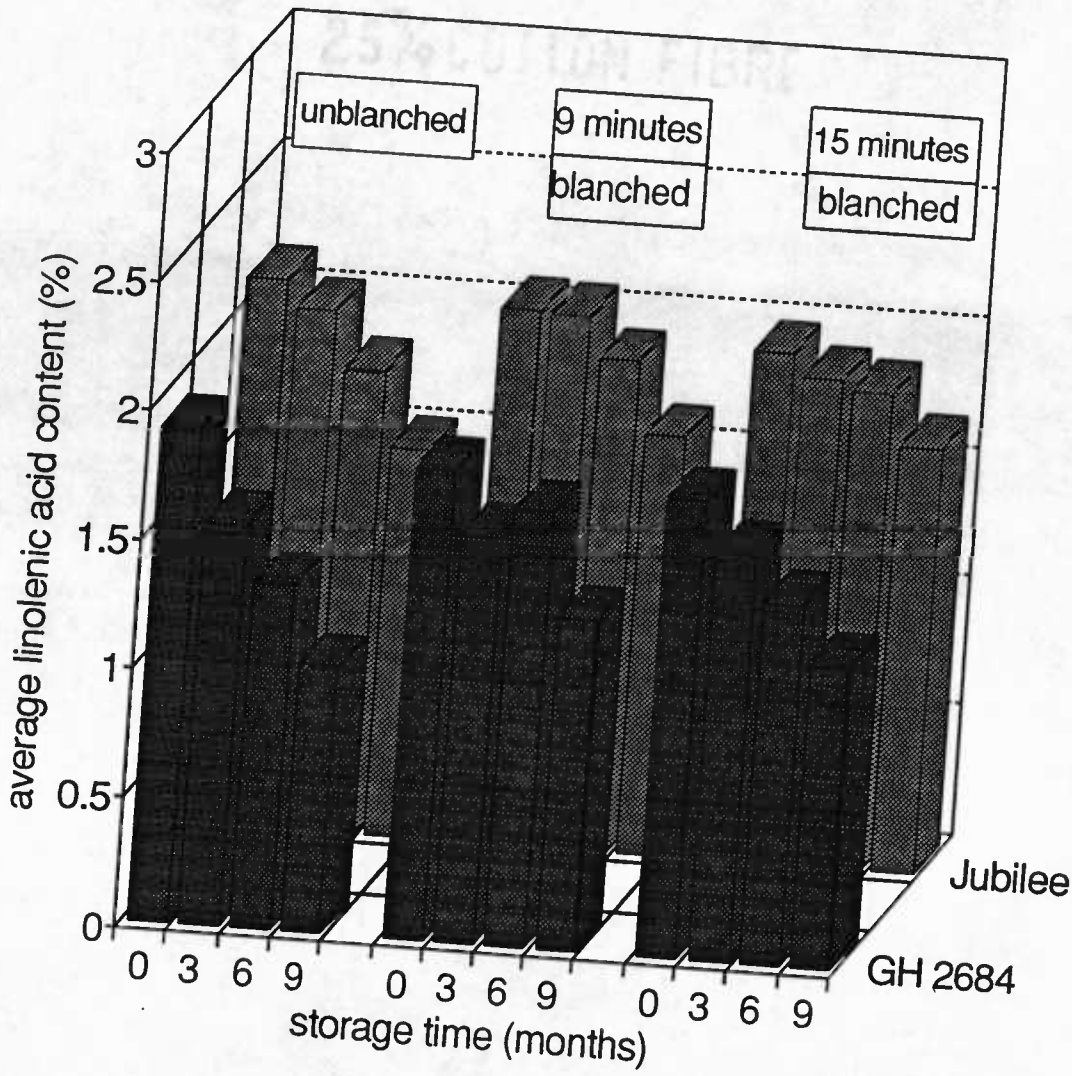

unblanched
9 minutes blanched
15 minutes blanched
average linolenic acid content (%)
3
2.5
2
1.5
1
0.5
0
0 3 6 9
0 3 6 9
0 3 6 9
storage time (months)
Jubilee
GH 2684

Figure 14: Change in peroxide value, expressed as meq O_2/kg lipid), in the whole kernels of the Jubilee and GH 2684 varieties for the unblanched and blanched (9 and 15 minutes) samples during the sixth and ninth months of storage at -23.3°C. The data presented correspond to averages over the packaging materials.

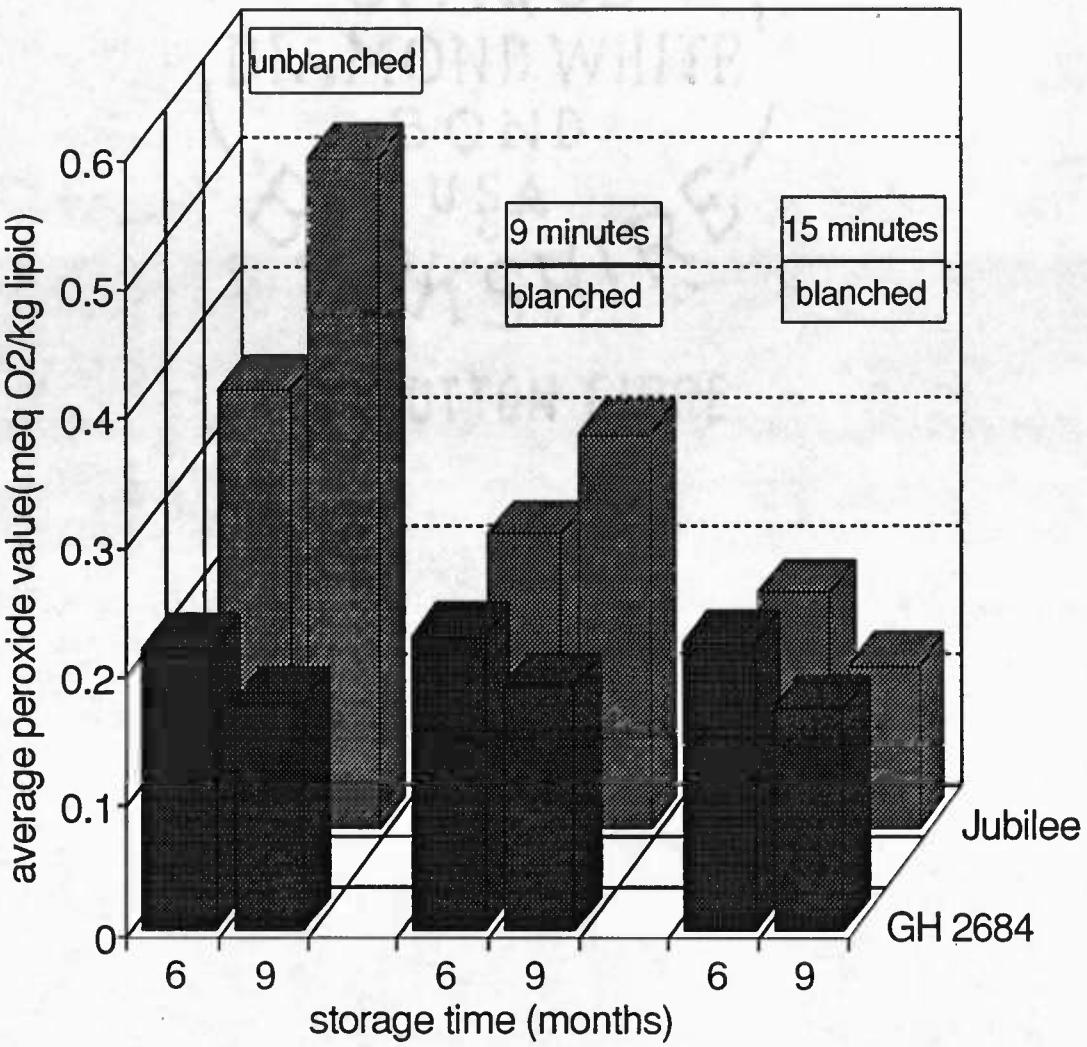
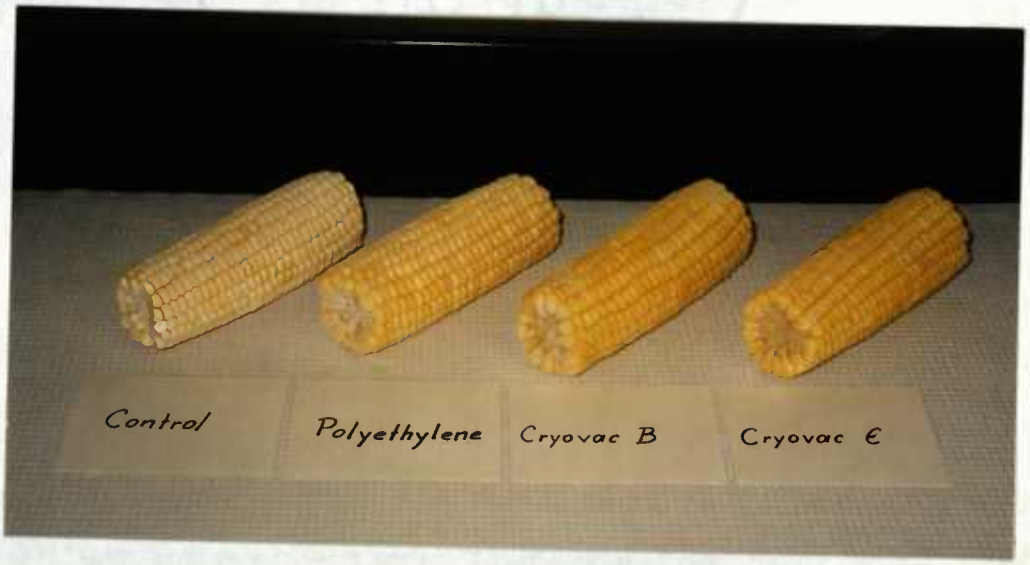

unblanched
9 minutes
blanched
15 minutes
blanched
average peroxide value(meq O2/kg lipid)
0.6
0.5
0.4
0.3
0.2
0.1
0
6
9
6
9
6
9
storage time (months)
Jubilee
GH 2684

Figure 15: Effect of the different packaging materials (A) and blanching treatments (B) in the color degradation of sweet corn after 9 months of frozen storage.

A)


Control
Polyethylene
Cryovac B
Cryovac C

B)

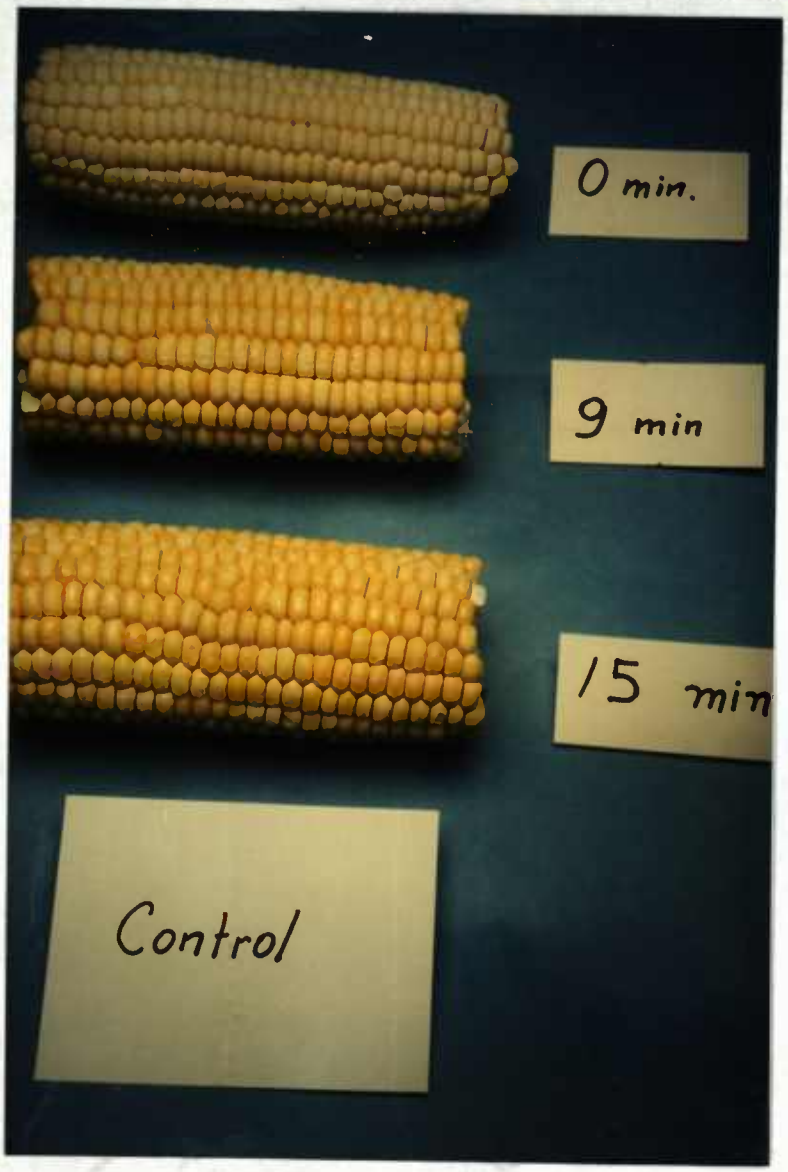

0 min.
9 min
15 min
Control

Table 5: Changes in lipoxygenase specific activity (units/mg protein) in the Jubilee and GH 2684 sweet corn kernels during 9 months of storage at 23.3°C for the different blanching treatments and packaging materials used. No significant differences (p-value > 0.05) were found among the different packaging materials.

Storage time (months)	Blanching time (minutes)	Jubilee				GH 2684			
		Packaging material				Packaging material			
		No packaging	Polyethylene	Cryovac B	Cryovac E	No packaging	Polyethylene	Cryovac B	Cryovac E
0	0	0.143 (0.016)				0.108 (0.009)			
	9	0.000				0.000			
	15	0.000				0.000			
3	0	0.099 (0.022)	0.110 (0.024)	0.106 (0.014)	0.106 (0.006)	0.098 (0.006)	0.109 (0.013)	0.097 (0.021)	0.104 (0.014)
	9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	0	0.114 (0.013)	0.099 (0.006)	0.108 (0.017)	0.109 (0.027)	0.091 (0.017)	0.091 (0.018)	0.092 (0.012)	0.103 (0.017)
	9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9	0	0.069 (0.020)	0.094 (0.008)	0.096 (0.020)	0.088 (0.019)	0.067 (0.009)	0.060 (0.012)	0.060 (0.014)	0.061 (0.004)
	9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 6: Changes in peroxidase specific activity (units/mg protein) in the Jubilee and GH 2684 sweet corn kernels during 9 months of storage at 23.3°C for the different blanching treatments and packaging materials used. No significant differences (p-value > 0.05) were found among the different packaging materials.

Storage time (months)	Blanching time (minutes)	Jubilee				GH 2684			
		Packaging material				Packaging material			
		No packaging	Polyethylene	Cryovac B	Cryovac E	No packaging	Polyethylene	Cryovac B	Cryovac E
0	0	1.082 (0.019)				1.075 (0.025)			
	9	0.048 (0.015)				0.081 (0.027)			
	15	0.000				0.000			
3	0	0.460 (0.040)	0.496 (0.025)	0.535 (0.024)	0.507 (0.007)	0.446 (0.018)	0.555 (0.008)	0.461 (0.015)	0.510 (0.017)
	9	0.035 (0.021)	0.025 (0.010)	0.022 (0.015)	0.047 (0.019)	0.040 (0.016)	0.044 (0.014)	0.014 (0.008)	0.034 (0.015)
	15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	0	0.508 (0.004)	0.600 (0.084)	0.611 (0.040)	0.581 (0.009)	0.508 (0.103)	0.527 (0.043)	0.563 (0.031)	0.565 (0.067)
	9	0.044 (0.023)	0.052 (0.007)	0.050 (0.005)	0.046 (0.033)	0.033 (0.011)	0.036 (0.020)	0.031 (0.005)	0.045 (0.025)
	15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9	0	0.424 (0.025)	0.484 (0.067)	0.477 (0.039)	0.518 (0.025)	0.460 (0.060)	0.480 (0.026)	0.455 (0.007)	0.418 (0.026)
	9	0.007 (0.004)	0.036 (0.007)	0.045 (0.016)	0.031 (0.014)	0.029 (0.004)	0.022 (0.017)	0.027 (0.011)	0.020 (0.003)
	15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 7: Initial relative percent fatty acyl content in the kernels of the two varieties of sweet corn (Jubilee and GH 2684) after the different blanching treatments (0, 9 and 15 minutes). No significant differences (p-value > 0.05) were found among linoleic and linolenic acid content for the different packaging materials.

Blanching time (min)	Fatty acyl	Jubilee	GH 2684
0	16:0	14.84 (2.44)	14.69 (0.44)
	18:0	2.44 (0.05)	2.71 (0.19)
	18:1	33.47 (1.10)	31.22 (0.81)
	18:2n-6	44.73 (2.57)	48.82 (1.44)
	18:3n-3	1.82 (0.43)	1.91 (0.25)
9	16:0	15.01 (0.53)	14.27 (0.28)
	18:0	2.83 (1.00)	2.68 (0.10)
	18:1	30.25 (0.72)	31.75 (0.43)
	18:2n-6	49.29 (2.15)	45.50 (6.03)
	18:3n-3	2.07 (0.13)	1.82 (0.09)
15	16:0	16.92 (1.04)	14.31 (0.98)
	18:0	3.44 (0.58)	2.76 (0.21)
	18:1	30.22 (0.68)	32.30) (1.50)
	18:2n-6	45.65 (0.33)	47.23 (0.73)
	18:3n-3	1.97 (0.25)	1.77 ().04)

Table 8: Relative percent fatty acid content in the kernels of the two varieties of sweet corn after 3 months of storage for the different blanching treatments and packaging materials. No significant differences (p-value > 0.05) were found among linoleic and linolenic acid content for the different packaging materials.

Blanch tim (min)	Fatty acy	Jubilee					GH 2684				
		Packaging				Average	Packaging				Average
		No packing	Polyethylene	Cryovac B	Cryovac E	(%)	No packing	Polyethylene	Cryovac B	Cryovac E	(%)
0	16:0	14.71 (1.88)	15.13 (1.71)	15.70 (1.22)	13.79 (0.25)	14.93 (1.25)	15.25 (2.04)	14.85 (0.50)	14.32 (0.82)	13.77 (0.85)	14.61 (0.93)
	18:0	3.37 (0.10)	3.41 (0.37)	3.33 (0.51)	2.40 (0.10)	3.15 (0.48)	3.02 (0.42)	2.79 (0.03)	2.59 (0.25)	2.91 (0.05)	2.8 (0.25)
	18:1	34.7 (2.14)	31.46 (0.46)	30.79 (0.16)	30.49 (0.69)	31.74 (1.80)	34.72 (4.24)	31.61 (2.11)	32.78 (1.25)	32.12 (2.35)	32.8 (2.16)
	18:2n-6	45.46 (0.55)	45.89 (1.68)	46.85 (0.33)	49.76 (0.36)	46.97 (1.70)	44.97 (0.64)	46.83 (0.10)	46.86 (1.62)	43.81 (1.94)	45.76 (1.74)
	18:3n-3	1.76 (0.20)	1.95 (0.18)	2.05 (0.06)	2.05 (0.01)	2.01 (0.19)	1.36 (0.15)	1.73 (0.36)	1.54 (0.20)	1.57 (0.19)	1.75 (0.23)
9	16:0	14.6 (1.09)	14.64 (0.92)	15.00 (0.66)	18.16 (1.48)	15.42 (1.54)	14.19 (1.03)	14.64 (0.74)	13.43 (0.86)	12.91 (0.05)	13.95 (1.02)
	18:0	2.90 (0.25)	3.17 (0.16)	2.98 (0.17)	3.72 (0.02)	3.19 (0.32)	2.84 (0.22)	2.72 (0.16)	2.43 (0.31)	2.24 (0.08)	2.57 (0.29)
	18:1	28.14 (2.93)	31.66 (2.61)	29.86 (2.08)	29.10 (0.86)	29.9 (2.22)	34.20 (2.72)	34.10 (0.09)	33.89 (3.14)	30.87 (3.63)	36.42 (2.53)
	18:2n-6	44.50 (2.36)	47.83 (1.07)	48.53 (0.35)	44.17 (2.41)	46.64 (2.36)	45.65 (0.36)	46.68 (0.46)	47.47 (2.94)	43.19 (3.99)	45.91 (2.52)
	18:3n-3	1.90 (0.05)	2.06 (0.17)	2.26 (0.19)	1.94 (0.28)	2.06 (0.21)	1.61 (0.03)	1.54 (0.06)	1.65 (0.31)	1.40 (0.02)	1.57 (0.18)
15	16:0	14.43 (1.19)	14.63 (0.47)	15.32 (1.43)	15.53 (0.98)	14.98 (0.94)	12.33 (1.55)	13.95 (1.55)	13.17 (1.44)	13.38 (0.64)	13.29 (1.12)
	18:0	2.87 (0.59)	3.14 (0.30)	3.35 (0.34)	3.38 (0.18)	3.2 (0.34)	2.38 (0.31)	2.41 (0.31)	2.40 (0.34)	2.49 (0.14)	2.42 (0.22)
	18:1	32.49 (0.87)	31.64 (0.69)	30.24 (1.88)	31.82 (1.80)	31.43 (1.39)	33.52 (1.39)	33.28 (1.39)	33.34 (0.73)	33.24 (0.77)	33.33 (0.76)
	18:2n-6	46.69 (1.400)	46.65 (0.53)	46.29 (1.46)	46.39 (0.85)	46.5 (0.93)	46.38 (1.29)	46.93 (1.29)	47.11 (1.45)	46.92 (0.35)	46.83 (1.28)
	18:3n-3	1.78 (1.16)	1.92 (0.06)	1.89 (0.05)	1.88 (0.08)	1.88 (0.08)	1.49 (0.28)	1.49 (0.02)	1.63 (0.09)	1.57 (0.07)	1.58 (0.14)

Table 9: Relative percent fatty acid content in the kernels of the two varieties of sweet corn after 6 months of storage for the different blanching treatments and packaging materials. No significant differences (p-value > 0.05) were found among linoleic and linolenic acid content for the different packaging materials.

Blanching time (min)	Fatty acyl	Jubilee					GH 2684				
		Packaging				Average (%)	Packaging				Average (%)
		No packing	Polyethylene	Cryovac B	Cryovac E		No packing	Polyethylene	Cryovac B	Cryovac E	
0	16:0	16.10 (2.72)	14.54 (1.12)	16.57 (3.3)	13.26 (0.65)	14.98 (2.07)	14.07 (1.88)	14.73 (0.74)	12.81 (0.13)	13.20) (0.23)	13.78 (1.13)
	18:0	3.26 (0.68)	3.34 (0.14)	3.36 (0.57)	2.70 (0.09)	3.15 (0.44)	2.67 (0.50)	2.77 (0.16)	2.42 (0.21)	2.48 (0.12)	2.6 (0.28)
	18:1	30.36 (2.93)	31.67 (2.92)	31.36 (1.80)	33.34 (1.45)	31.71 (2.24)	33.97 (1.10)	35.39 (1.84)	34.29 (1.95)	33.20 (1.73)	34.12 (1.43)
	18:2n-6	43.32 (5.63)	44.19 (2.94)	42.45 (5.47)	45.13 (1.52)	43.48 (3.81)	42.76 (6.40)	39.98 (2.38)	46.69 (0.82)	46.67 (1.55)	43.78 (4.32)
	18:3n-3	1.86 (0.27)	1.97 (0.31)	1.41 (0.47)	1.80 (0.18)	1.79 (0.32)	1.28 (0.04)	0.87 (0.14)	1.68 (0.23)	1.60 (0.17)	1.33 (0.39)
9	16:0	14.51 (1.26)	13.97 (1.75)	14.00 (0.39)	14.72 (0.95)	14.33 (1.06)	13.76 (1.54)	12.91 (0.11)	13.62 (0.91)	14.05 (0.66)	13.55 (0.92)
	18:0	3.05 (0.17)	2.85 (0.54)	3.17 (0.26)	2.87 (0.09)	2.99 (0.31)	2.64 (0.32)	2.55 (0.17)	2.50 (0.18)	2.89 (0.17)	2.66 (0.24)
	18:1	32.56 (1.60)	33.46 (2.72)	32.09 (0.52)	30.39 (2.11)	32.02 (1.97)	32.70 (0.17)	33.88 (0.52)	33.47 (0.91)	30.75 (1.52)	32.71 (1.48)
	18:2n-6	44.54 (1.37)	44.13 (1.35)	46.05 (1.37)	46.43 (0.77)	45.29 (1.47)	44.32 (4.25)	46.20 (0.96)	45.11 (2.92)	45.53 (2.04)	45.29 (2.50)
	18:3n-3	1.85 (0.09)	1.57 (0.23)	2.15 (0.08)	2.06 (0.15)	1.90 (0.27)	1.58 (0.29)	1.71 (0.07)	1.51 (0.16)	1.69 (0.64)	1.68 (0.25
15	16:0	14.59 (1.28)	13.72 (0.28)	13.93 (1.50)	15.40 (2.38)	14.39 (1.38)	12.87 (0.06)	12.88 (0.40)	13.53 (1.03)	14.13 (0.78)	13.44 (0.76)
	18:0	3.10 (0.14)	3.11 (0.17)	2.89 (0.48)	3.33 (0.48)	3.11 (0.34)	2.52 (0.10)	2.38 (0.16)	2.54 (0.22)	2.75 (0.20)	2.57 (0.19)
	18:1	32.26 (1.49)	31.88 (0.25)	31.79 (2.08)	30.27 (1.85)	31.52 (1.56)	33.29 (0.36)	36.02 (0.74)	32.68 (0.48)	33.78 (0.46)	33.92 (1.25)
	18:2n-6	42.90 - (3.76)	46.36 (0.47)	46.12 (0.98)	42.39 (7.50)	44.27 (4.22)	46.99 (0.08)	44.44 (0.55)	44.29 (4.75)	41.33 (3.40)	43.94 (3.27)
	18:3n-3	1.67 (0.51)	2.01 (0.01)	1.91 (0.29)	1.82 (0.69)	1.84 (0.42)	1.66 (0.44)	1.36 (0.18)	1.42 (0.28)	1.25 (0.43)	1.4 (0.29)

Table 10: Relative percent fatty acid content in the kernels of the two varieties of sweet corn after 9 months of storage for the different blanching treatments and packaging materials. No significant differences (p-value = 0.19) were found among the different packaging materials.

Blanching time (min)	Fatty acyl	Jubilee					GH 2684				
		Packaging				Average	Packaging				Average
		No packing	Polyethylene	Cryovac B	Cryovac E	(%)	No packing	Polyethylene	Cryovac B	Cryovac E	(%)
0	16:0	13.14 (0.43)	14.21 (2.02)	13.35 (1.18)	13.25 (0.76)	13.42 (0.95)	11.81 (0.96)	11.63 (0.46)	12.00 (0.48)	11.31 (0.43)	11.57 (0.86)
	18:0	2.74 (0.12)	2.84 (0.44)	2.78 (0.35)	2.71 (0.18)	2.76 (0.23)	2.31 (0.27)	2.19 (0.07)	2.36 (0.15)	2.12 (0.07)	2.23 (0.20)
	18:1	35.21 (0.35)	33.51 (3.10)	35.68 (2.52)	35.33 (3.54)	35.06 (2.21)	37.83 (1.56)	37.96 (0.71)	38.57 (0.63)	38.03 (0.61)	37.56 (1.62)
	18:2n-6	43.95 (1.39)	40.03 (4.31)	44.28 (0.37)	44.28 (2.66)	43.42 (2.55)	44.43 (0.20)	44.36 (0.44)	42.55 (0.55)	44.69 (0.85)	44.01 (1.01)
	18:3n-3	1.48 (0.06)	1.14 (0.07)	1.62 (0.30)	1.65 (0.26)	1.50 (0.26)	1.14 (0.27)	1.01 (0.08)	0.98 (0.01)	0.98 (0.06)	1.03 (0.14)
9	16:0	13.92 (0.74)	13.29 (2.78)	12.39 (1.01)	13.32 (0.83)	13.30 (1.41)	12.33 (0.21)	12.09 (0.52)	11.39 (0.45)	12.21 (0.11)	12.00 (0.46)
	18:0	2.88 (0.33)	2.60 (0.49)	2.59 (0.08)	2.94 (0.46)	2.77 (0.36)	2.36 (0.11)	2.34 (0.05)	2.22 (0.06)	2.34 (0.06)	2.31 (0.08)
	18:1	31.19 (3.17)	35.72 (4.84)	36.52 (0.52)	32.58 (1.97)	33.76 (3.38)	36.38 (1.71)	36.62 (2.69)	36.9 (1.38)	36.02 (1.46)	36.47 (1.43)
	18:2n-6	47.46 (2.49)	40.26 (5.78)	43.68 (0.91)	45.8 (1.08)	44.37 (4.11)	44.97 (1.29)	44.80 (1.56)	43.84 (1.86)	45.23 (1.54)	44.70 (1.44)
	18:3n-3	2.02 (0.36)	1.10 (0.04)	1.43 (0.02)	1.86 (0.23)	1.62 (0.44)	1.39 (0.18)	1.23 (0.23)	1.15 (0.19)	1.34 (0.11)	1.28 (0.18)
15	16:0	13.37 (0.53)	12.73 (0.52)	13.28 (0.19)	12.76 (0.44)	13.06 (0.48)	11.75 (0.61)	11.58 (0.57)	11.44 (0.26)	11.88 (0.21)	11.66 (0.42)
	18:0	2.83 (0.18)	2.70 (0.04)	2.94 (0.04)	2.72 (0.16)	2.81 (0.13)	2.30 (0.15)	2.24 (0.12)	2.23 (0.09)	2.35 (0.17)	2.28 (0.13)
	18:1	34.28 (2.65)	35.88 (0.87)	34.12 (1.34)	34.78 (0.44)	34.84 (1.47)	36.31 (0.79)	36.96 (0.49)	36.16 (1.21)	36.75 (0.88)	36.51 (0.81)
	18:2n-6	44.41 - (4.12)	44.31 (0.56)	44.79 (0.83)	45.19 (1.26)	44.67 (1.92)	45.41 (0.68)	44.71 (1.63)	45.39 (1.07)	45.13 (0.41)	45.16 (0.94)
	18:3n-3	1.61 (0.36)	1.54 (0.12)	1.79 (0.13)	1.62 (0.02)	1.64 (0.19)	1.13 (0.05)	1.11 (0.05)	1.23 (0.07)	1.20 (0.12)	1.17 (0.08)

Table 11: Peroxide value, expressed as meq O_2/Kg lipid, in the kernels of the two different sweet corn varieties (Jubilee and GH 2684) after 6 and 9 months of storage for the different blanching treatments and packaging materials.

Storage Time (months)	Blanching time (minutes)	Jubilee				GH 2684			
		Packing material				Packing material			
		No packaging	Polyethylene	Cryovac B	Cryovac E	No packaging	Polyethylene	Cryovac B	Cryovac E
6	0	0.113 (0.043)	0.075 (0.039)	0.073 (0.027)	0.070 (0.048)	0.045 (0.028)	0.044 (0.029)	0.061 (0.033)	0.067 (0.022)
	9	0.080 (0.031)	0.059 (0.032)	0.045 (0.015)	0.043 (0.011)	0.099 (0.034)	0.064 (0.016)	0.030 (0.025)	0.042 (0.001)
	15	0.044 (0.017)	0.047 (0.006)	0.051 (0.033)	0.029 (0.016)	0.043 (0.019)	0.044 (0.018)	0.086 (0.012)	0.047 (0.018)
9	0	0.132 (0.051)	0.103 (0.029)	0.160 (0.062)	0.121 (0.100)	0.040 (0.001)	0.035 (0.019)	0.041 (0.016)	0.048 (0.003)
	9	0.085 (0.044)	0.048 (0.025)	0.062 (0.003)	0.111 (0.019)	0.052 (0.014)	0.053 (0.031)	0.035 (0.019)	0.046 (0.021)
	15	0.037 (0.010)	0.022 (0.018)	0.063 (0.026)	0.058 (0.029)	0.038 (0.014)	0.043 (0.012)	0.048 (0.007)	0.041 (0.026)

SUMMARY

The evaluation of the different blanching treatments and packaging materials showed some differences in the enzymatic activity (lipoxygenase and peroxidase) and fatty acid stability for the two different varieties of sweet corn (Jubilee and GH 2684) during the nine months of storage at -23.3°C.

Both unblanched varieties of sweet corn showed similar initial peroxidase specific activities and were similar in all parameters tested during the nine months of frozen storage. The presence of lipoxygenase isozymes with different thermal stabilities in both varieties was suggested by the higher lipoxygenase specific activity found in Jubilee after freezing and nine months of frozen storage compared with the GH 2684 variety.

Complete inactivation of lipoxygenase was obtained after 9 minutes steam blanching at 100°C. Peroxidase was more heat resistant, showing some remaining specific activity after 9 minutes steam blanching but complete inactivation was obtained after 15 minutes steam blanching. Peroxidase isozymes with different thermal stabilities were found in the two sweet corn varieties studied. Jubilee showed more stable peroxidase isozymes during frozen storage while the GH 2684 variety had more heat resistant isozymes.

No regeneration was observed in any of the enzymes studied during the nine months of frozen storage, suggesting

that the heat treatment used was enough to permanently disrupt the active site of both enzymes.

Although the varieties evaluated had different genotypes, their initial relative percent fatty acid content were similar. Good stability of the polyunsaturated fatty acids was observed during the nine months storage at -23.3°C. Autoxidation was the principal oxidative mechanism responsible for the decrease in the relative percent of polyunsaturated fatty acids during storage. Some enzymatic oxidation may also have occurred in linolenic acid. During the 9 months of frozen storage, the GH 2684 variety showed better stability against lipid deterioration than the Jubilee variety.

The control of the degradation of polyunsaturated fatty acids depended mostly on the frozen storage temperature (-23.3°C) and not on the oxygen permeability of the different packaging materials.

The moisture content in the kernels of sweet corn greatly depended on the packaging material used. The best results were obtained with the Cryovac B and E bags while the control (ears without packaging material) showed severe dehydration.

Several studies have suggested the use of lipoxygenase activity as indicator of adequate blanching for leguminous vegetables (Sheu and Chen, 1991; Chem and Huang, 1988; Williams et al., 1986; Chen and Whitaker, 1986 and Lee and Wagenknecht, 1958), corn (Garrote et al., 1985; Wagenknecht, 1959) and potato (Park et at., 1988) because is closely

related to the destruction of essential fatty acids, development of off flavors and pigment degradation. The results obtained in our study suggested that blanching of the ears of sweet corn had an important effect on reducing the enzyme activity but little effect on the polyunsaturated fatty acid degradation after 9 months of storage at -23.3°C.

REFERENCES

Adams, J.B. and Onglley, M.H. 1989. The behaviour of Green Bean lipoxygenase on chromatography and isoelectric focussing. Food Chemistry. 31:57-71.

Anonymous. 1992. The almanac of the canning, freezing, preserving industries. Volume 2. Edward E. Judge and Sons. Westminster. MD. pp 190.

ASHRAE Handbook. 1990. Refrigeration, Systems and applications. SI edition.

Axelrod, B. Cheesbrough, T.M. and Laakso, S. 1981. Lipoxygenase from Soybeans. Methods in Enzymology. 71:441-450.

Aylward, F. and Haisman, D.R. 1969. Oxidation systems in fruits and vegetables: Their relation to the quality of preserved products. Advances in Food Research. pp 1-76.

Bald, W.B. 1991. Food freezing: Today and tomorrow. Springer series in applied biology. Great Britain.

Beckwar, M.R. Mansour, N.S. and Varseveld, G.W. 1977. Microwave drying: A rapid method for determining sweet corn moisture. HortScience. 12(6):562-563.

Benson, G.O. and Pearce, R.B. 1987. "Corn perspective and culture" from Corn: Chemistry and technology. Edited by S.A. Watson and P.E. Ramstad. American Association of Cereal Chemists, Inc. USA. pp 1-30.

Bligh, E.H. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Boyer, C.D. and Shannon, J.C. 1982. The use of endosperm genes in sweet corn improvement. Plant Breeding Reviews 1. Avi Publishing Co., Westport, CT.

Chen, A.O. and Hwang, W.I. 1988. Studies on enzyme selection as blanching index of frozen green beans and carrots. Food Sci. 15(2):116

Chen, A.O. and Whitaker, J.R. 1986. Purification and characterization of a lipoxygenase from immature English Peas.J. Agric. Food Chem. 34(2):203-211.

Chenchin, E.E. and Yamamoto, H.Y. 1973. Distribution and heat inactivation of peroxidase isozymes in sweet corn. J. Food Science. 38:40.

Corey, E.J. 1986. Mechanism of enzymic lipoxygenation of arachidonic acid. Key role of organoiron intermediates. Stereochemistry of organic and bioorganic transformations. Ed. W. Bartmann, K.B. Sharpless. Weinheim, F.R.G.:VCH Publishers.

Dietz, J.M. and Erdman, J.W. 1989. Effects of thermal processing upon vitamins and proteins in foods. Nutrition today.July/August. pp 6-15.

Frankel, E.W. Neff, W.E. and Selke, E. 1981. Analysis of autoxidized fats by gas chromatography-mass spectrometry. Lipids. 16(5):279-285.

Frankel, E.N. 1980. Lipid oxidation. Progress in Lipid Research. 19:1-22.

Frankel, E.N. 1991. Recent advances in lipid oxidation. J. Sci. Food Agric. 54:495-511.

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

Galliard, T. and Chan, H. W.S. 1980. "Lipoxygenases" from The biochemistry of plants. Edited by P. K. Stumpf. Academic Press. New York. pp 132-157.

Ganthavorn, C. and Powers, J.R. 1989. Partial purification and characterization of Asparagus lipoxygenase. J. Food Science. 54(2):371-373.

Gardner, H.W. 1979. Lipid hydroperoxide reactivity with proteins and amino acids. J. Agric. Food Chem. 27(2): 220-234.

Gardner, H.W. 1975. Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with nonenzymic. J.Agric. Food Chem. 23(2):129-135.

Garrote, R.L., Silva, E.R. and Bertone, R.A. 1985. Distribución e inactivación térmica de las enzimas peroxidasa y lipoxigenasa en el choclo. Rev. Agroqim. Tecnol. Aliment. 25(3): 373-383.

Gruda, Z. and Postolski, J. 1986. Tecnologia de la congelacion de los alimentos. Ed. Acribia. Spain.

Hakansson, B. and Jagerstad, M. 1990. The effect of thermal inactivation of lipoxygenase on the stability of vitamin E in Wheat. J. of Cereal Science. 12:177-185.

Halpin, B.E. and Lee, C.Y. 1987. Effect of blanching on enzyme activity and quality changes in Green Peas. J. Food Science. 52(4):1003-1005.

Hamilton, R.J. 1989. "The chemistry of rancidity in foods" from Rancidity of foods. Edited by J.C. Allen and R.J. Hamilton. Elsevier Applies Science. London and New York. pp 1-23.

Hatanaka, A. Kajiwara, T. and Sekiya, J. 1987. Biosynthetic pathway for C_6 aldehyde formation from linolenic acid in Green Leaves. Chemistry and Physics of Lipids. 44:341-361.

Henderson, S.K. Witchwoot, A. and Nawar, W.W. 1980. The autoxidation of linoleates at elevated temperatures. J. Amer. Oil Chem. Soc. 57:409-413.

Hildebrand, D.F. 1989. Lipoxygenases. Physiologia Plantarum. 76:249-253.

Hildebrand, D.F. Hamilton-Kemp, T.R. Loughrin, J.H. Ali, K. and Andersen R.A. 1990. Lipoxygenase 3 reduces hexanal production from Soybean seed homogenates. J. Agric. Food Chem. 38:1934-1936.

Jellum, M.D. 1970. Plant introductions of maize as a source of oil with unusual fatty acid composition. J. Agric. Food Chem. 18:365-370

Jul, M. 1984. The Quality of frozen foods. Academic Press. London.

Katsaboxakis, K.Z. 1984. "The influence of the degree of blanching on the quality of frozen vegetables" from Thermal processing and quality of foods. Edited by P. Zeuthen, J.C. Cheftel, C.

Erickson, M. Jul, H. Leniger, P. Linko, G. Varela, and G. Vos. Elsevier Applied Science Publishers. London and New York. pp 559-566.

Klein, B.P. 1976. Isolation of lipoxygenase from Split Pea Seeds, Snap Beans and Peas. J. Agric. Food Chem. 24(5):938-942.

Kozlowsky, A.W. 1977. Is it necessary to blanch all vegetables before freezing?. International Institute of Refrigeration. Commissions C1-C2. pp 227-237.

LaBuza, T.P. 1982. Shelf-life dating of foods. Westport, Conn., USA. Food and Nutrition Press.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. 1951. Protein measurement with the Folin phenol reagent. J.Biol. Chem. 193:265.

Lundberg, W.O. 1962. "Mechanisms and products of lipid oxidation" from Symposium on foods: Lipids and their oxidation. Edited by H.W. Schultz, E.A. Day and R.O. Sinnhuber. The AVI Publishing Company, Inc. pp 31-93.

Marshall, S.W. 1987. "Sweet Corn" from Corn: Chemistry and technology. Edited by S.A. Watson and P.E. Ramstad. American Association of Cereal Chemists, Inc. USA. pp 431-446.

Nawar, W.W. 1985. "Lipids" from Food Chemistry. Edited by O.R. Fennema. Marcel Dekker, Inc. New York. pp 139-244.

Norwig, J.F. and Thompson, D.R. 1984. Review of dehydration during Freezing. Transactions of the ASAE. pp 1619-1624.

Park, K. Kim, Y. and Lee, C. 1988. Thermal inactivation kinetics of Potato tuber lipoxygenase. J. Agric. Food Chem. 36:1012-1018.

Poca, E. Rabinovitch-Chable, H. Cook-Moreau, J. Pages, M. and Rigaud, M. 1990. Lipoxygenase from Zea Mays L.: Purification and physicochemical characteristics. Biochimica et Biophysica Acta. 1045:107-114.

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

Ramsey, F. and Schafer, D. 1992. The statistical sleuth. Department of Statistics. Oregon State University. U.S.

Ranken M.D. 1989. "Rancidity in meats" from Rancidity of foods. Edited by J.C. Allen and R.J. Hamilton. Elsevier Applied Science. London and New York. pp 225-236.

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

Remy, J. 1986. Modern freezing facilities. IIR Commission.

Rhoades, R.E. 1993. The golden grain: Corn. National Geographic. 183(6):92-117.

Richardson, T. and Hyslop, D.B. 1985. "Enzymes" from Food Chemistry. Edited by O. R. Fennema. Marcel Dekker, Inc. New York. pp 371-476.

Robards, K., Kerr, A.F., Patsalides, E. and Korth, J. 1988. Headspace gas analusis as a measure of rancidity in corn chips. J.Amer. Oil Chem. Soc. 65 (10) : 1621-1626

Schmedes, A. and Holmer, G. 1989. A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. J. Amer. Oil Chem. Soc. 66(6): 813-817.

Scott, D. 1975. "Oxidoreductases" from Enzymes in food processing. Edited by Gerald Reed. Academic Press, Inc. New York. pp 222-247.

Selivonchick D.P., Johnston P.V. and Roots B.I. 1977. Acyl and alkenyl group composition of brain subcellular fractions of goldfish (Carassius auratus L.) acclimated to different enviromental temperatures. Neurochem. Res., 2:359-366.

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. Lypoxygenase as blanching index for frozen vegetable soybeans. J.Food Science. 56:448-451.

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

Steel, R.G. and Torrie, J.H. 1980. Principles and procedures of statistics. Mc Graw Hill. Second edition.

Stine, C.M. Harland, H.A. Coulter, S.T. and Jenness, R. 1954. A modified peroxide test for detection of lipid oxidation in dairy products. J. Dairy Science. 37:202-208.

Vetter, J.L. Nelson, A.I. and Steinberg M.P. 1959. Heat inactivation of peroxidase in HTST processed whole kernel corn. Food Technol. 13(7): 410-413.

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. 1958. Enzyme action and off-flavor in frozen peas. Food Res. 23: 25-30.

Watson, S.A. 1987. "Structure and composition" from Corn: Chemistry and technology. Edited by S.A. Watson and P.E. Ramstad. American Association of Cereal Chemists, Inc. USA. pp 53-82.

Weber, E.J. 1987. "Lipids of the kernel" from Corn: Chemistry and technology. Edited by S.A. Watson and P.E. Ramstad. American Association of Cereal Chemists, Inc. USA. pp 311-350.

Wiley, R.C. Schales, F.D. and Corey K.A. 1989. "Sweet Corn" from Quality and preservation of vegetables. Edited by Michael Eskin. CRC Press. USA. pp 121-156.

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 Technology 40(6):130-140.

Wilson, C.M. 1987. "Proteins of the kernel" from Corn: Chemistry and technology. Edited by S.A. Watson and P.E. Ramstad. American Association of Cereal Chemists, Inc. USA. pp 273-310.

Wright, K.N. 1987. "Nutritional properties and nutritional values of corn and its by-products" from Corn: Chemistry and technology. Edited by S.A. Watson and P.E. Ramstad. American Association of Cereal Chemists, Inc. USA. pp 447-478.

Zhang, Q. Cavalieri, R.P. Powers, J.R. and Wu, J. 1991. Measurement of lipoxygenase activity in homogenized Green Beans tissue. J. Food Science. 56(3):719-721.

Zhuang, H. Hildebrand, D.F., Andersen, R.A. Hamilton-Kemp, T.R. 1991. Effects of polyunsaturated free fatty acids and esterified linoleoyl derivatives on oxygen consumption and C_6 aldehyde formation with soybean seed homogenates. J. Agric. Food Chem. 39(8):1357-1364.