# Some properties of lipoxygenase in mature (edible) corn (*Zea mays*) and its inactivation by microwave

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## Abstract

The enzyme peroxidase has been used as an indicator enzyme for adequacy of blanching in frozen vegetables. However more recent research indicates that enzymes other than peroxidase may be responsible for quality characteristics of frozen blanched vegetables. Lipoxygenase has been identified as the key enzyme responsible for quality characteristics in peas and beans and cysteine lyase in broccoli and cauliflower. Lipoxygenase is thought to be important for flavour quality in processed corn on the cob. The predominant lipoxygenase found in mature corn was class II; sharp acidic pH maximum, and the reaction activated by calcium. A $K_m$ value of 0.051 mM linoleic acid was calculated. The kernel and germ tissues contained 75% of the lipoxygenase with the remainder found in the outer part of the cob. The microwave was efficient in deactivating peroxidase and 80% of the lipoxygenase in 5 minutes.

***Additional key words:*** *peroxidase, blanching.*

## Introduction

Although peroxidase (POD) has been used as a marker enzyme for adequacy of blanching for thirty years it has been known that peroxidase, completely inactivated on the day of blanching, can recover up to 40% of it's original activity after storage at low temperature. Asparagus POD reached a peak of regenerated activity of 40% after 50 days (Gavanthorn and Powers, 1989). Peroxidase is generally regarded as the most heat-stable enzyme and therefore loss of POD activity would mean the loss of activity of other enzymes such as catalase, lipase, lipoxygenase (LOX), and cysteine lyase. Recent research has shown that POD is not always the most heat-stable enzyme; in asparagus tips LOX is more heat-stable than POD (Gavanthorn *et al.*, 1991). These authors advise independently evaluating different fruits and vegetables for LOX and POD heat-stability.

Lipoxygenase (linoleate:oxidoreductase - E.C.1.13.-11.12) in the presence of dissolved oxygen, catalyses the formation of fatty acid hydroperoxides from poly-unsaturated fatty acids. The breakdown products from this reaction have been implicated in aroma and taste changes in many processed foods.

Iso-enzymes of lipoxygenase have been identified in a large variety of fruits and vegetables including corn and have been divided into 3 classes according to their properties (Siedow, 1991). The most common LOX substrate, linoleic acid, represents 40-65% of the oil content of corn of which 85% is found in the germ. Suggested roles of LOX are the biosynthesis of jasmonic acid required for plant growth (Vick and Zimmermann, 1983), synthesis of traumatic acid, released on plant wounding (Zimmermann and Coudron, 1979), and possible involvement in the pathway of carotenoids to abscisic acid (Vick and Zimmermann, 1987).

The effects of different enzymes on the quality of frozen vegetables have been demonstrated by the addition of purified enzymes in combination to green pea purée and independently to green bean purée (Williams *et al.*, 1986). The changes in taste and aroma were examined by a trained taste panel. Lipoxygenase was shown to be responsible for the overall aroma intensity more than lipase, catalase and peroxidase and responsible for the taste characteristics of unripe banana, grass, straw, ammonia and partly responsible for the sour component.

Lipoxygenase may also affect the nutritional quality of the corn. Corn has high levels of carotene and cryptoxanthin (hydroxy-β-carotene), the precursors to vitamin A, when compared to other fruits and vegetables. Different varieties of corn have levels of provitamin A content (total carotene + 50% cryptoxanthin content) ranging from 0.134 to 0.302mg/100g fresh weight

(Cabulea, 1971); in comparison mango has 2.3 (one of the highest levels found in fruits), banana 0.13 and kiwifruit 0.08mg/100g (Wills *et al.*, 1986). Class II and III lipoxygenases co-oxidise carotenoids in the presence of molecular oxygen and fatty acids (Arens *et al.*, 1973; Ben-Aziz *et al.*, 1971 and Zamora *et al.*, 1988), whereas class I LOX is not involved in co-oxidation reactions.

This work was carried out to obtain an indepth understanding of lipoxygenase activity in Jubilee sweet corn so that inactivation by microwave heating could be studied to improve the flavour, colour and texture of the blanched frozen product.

## Materials and Methods

### Sample preparation

Corn on the cob was divided into four tissues in order to locate the LOX. These are defined as kernel (pericarp, endosperm and the tip cap but not including the germ), germ, outer cob (brown tissue, the outer 1.5 cm of the cob), and the inner cob (white tissue, the centre of the cob about 1 cm in diameter) for the experiments in locating LOX. In all following experiments, kernel refers to kernel with germ. The inner cob did not make a significant contribution to LOX and POD and only the outer cob was analysed in the microwave experiments. Tissue was either ground by mortar and pestle or macerated by ultra-turrax in a ten-fold dilution (w/v) of ice-cold Tris buffer (50 mM, pH 7). A sample was taken from each homogenate and spun in a micro-centrifuge for 5 minutes at 10,000*g. The supernatant contained the LOX. Addition of varying concentrations of non-ionic detergent (Triton X-100) to the precipitate did not release any further LOX. The LOX is therefore thought to be mainly a soluble rather than a membrane-bound enzyme. The fatty layer on the surface of the supernatant was carefully removed and the remaining supernatant used in the LOX assay. Samples survived well when kept at 0 - 4°C for 3-4 days with only small losses of activity; generally less than 10%.

### pH profile

Four buffers were used in the pH range 4.0 to 8.2; citrate (4.2-6.5), piperazine (6.0-6.8), phosphate (6.3-7.5) and Tris (7.0-8.25). At overlapping areas of pH the buffers were mixed.

### Microwave experiment

A 1 Kw, 2.45 Ghz magnetron mounted on a launching section of waveguide fed microwave power into a horizontal cylindrical multimode cavity fitted with a mode stirrer. Corn was positioned on a polyethylene supporting plate mounted horizontally in the cavity a third of the diameter from the bottom. The power was fed from the waveguide launching system to the four stab tuner via a circulator to prevent reflected power returning to the magnetron. Forward and reverse power were measured and various representative corn temperatures were recorded.

The ears of corn were individually wrapped in plastic film and microwave blanched for 3.0, 3.5, 4.0 and 5.0 minutes, 15 ears for each microwave program. Immediately following the blanching the surface, inner cob and cross section temperatures were recorded with a digital thermometer with a metal probe. The corn was then immersed in ice-cold water for 15 mins. Five ears of corn from each program were analysed for their LOX, POD and protein content. The remainder were stored in aluminium lined plastic bags, vacuum packed, and stored at -20°C to await taste trials by the HortResearch sensory evaluation group, Auckland.

### Lipoxygenase assay

A modification of the Surrey assay (1964) was used so that a continual change in the absorbance at 234nm could be monitored on a Varian Cary 1 spectrophotometer, similar to the method described by Al-Obaidy and Siddiqi (1981). The stock linoleic acid substrate was dispersed in an alkaline solution containing 0.28% (v/v) Triton X-100 and the assay carried out at 25°C. New substrate was prepared every 3-4 days. The sample cuvette contained Tris (pH 7) or piperazine buffer (pH 6.25), 100µl of the stock substrate (assay concentration 0.30 mM), and 10 - 50µl of the sample in a total volume of 3mL. The reference cuvette contained a heat-treated corn LOX as a reaction blank. The initial velocity of reaction was read over the linear change in absorbance which generally varied from 20 to 60 seconds. One unit of activity was defined as the change in absorbance of 0.001/minute.

### Peroxidase assay

Peroxidase was measured in potassium phosphate buffer (10 mM, pH 6.0) at 25°C with hydrogen peroxide (1 mM) as the substrate and o-dianisidine hydrochloride (0.5 mM) as the hydrogen donor. The linear change in absorbance was read at 460nm. One unit of activity was defined as the change in absorbance of 0.001/minute.

### Protein assay

Protein concentration was determined by the Coomassie Blue assay (Bradford, 1976) with BSA as the protein standard.

## Results and Discussion

### The lipoxygenase reaction

The scan of the lipoxygenase reaction (Fig. 1) shows a concomitant depletion of the peak at 280nm and an increase at 234nm, the absorbance of the diene bond of the linoleic acid hydroperoxide. The initial velocity of reaction of LOX in all tissues showed an initial linear increase in absorbance which then gradually plateaued, characteristic of a LOX catalysed reaction and distinct from the non-specific catalysis by heme proteins (Fig. 2).

### Distribution of lipoxygenase

Kernel (without the germ), germ, outer cob and inner cob were examined for the concentration and specific activity of LOX (Table 1). The LOX concentration/g tissue was similar in the germ and kernel, and the outer cob concentration about 25% of this value.

LOX was not distributed evenly in the kernel tissue (including the germ). In a 20cm cob about 3-4cm of the base kernels are larger and irregularly shaped when compared to the uniformly spaced and shaped kernels in the mid section of the cob. The kernels 4-5 cms from the tip of the cob are smaller in size, more so as they approach the tip and are round in shape. These kernels at the extremes of the cob contained a significantly higher concentration of LOX, both activity/g tissue and specific activity, particularly the kernels at the tip which contained 150% compared to the mid-section kernels, (Table 2). Lipoxygenase has been associated with growth and development in a number of fruits and vegetables and the large increases of LOX 14 days following pollination indicate that it plays a similar role in corn (Belefant and Fong, 1991).

Peroxidase had a different distribution to LOX, 80% of activity/g tissue was found in the cob and 20% in the kernel. Peroxidase followed the pattern of LOX distribution in the kernel with higher concentrations in

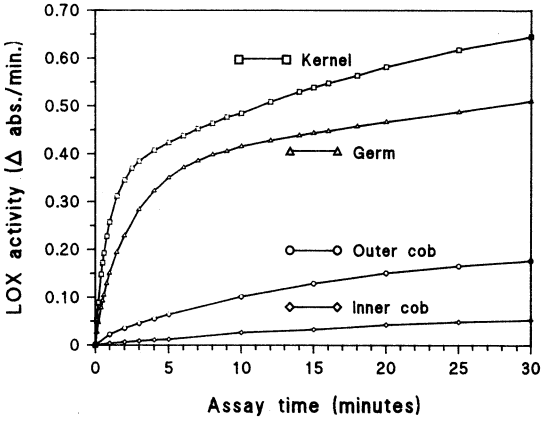


**Figure 2. Relative activity of LOX in different tissues. Tissues samples were prepared in identical manner for comparison of tissue activities. The activity was measured in Tris buffer at pH 7.**

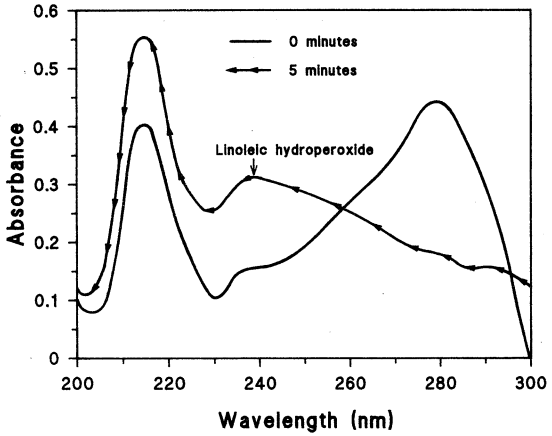


**Figure 1. Corn LOX reaction with linoleic acid. The absorbance spectrum (200-300nm) at 0 and 5 mins reaction time. The assay measured the breakdown of linoleic acid to linoleic hydroperoxide which had a peak absorbance at 234nm. The reaction scanned at 0 mins was carried out in ice-cold Tris buffer to almost inactivate the reaction. At 5 minutes the reaction was > 90% complete.**

**Table 1. Concentration and specific activities of LOX in corn tissues.**

| | ΔA/min/g tissue | Protein mg/g tissue | ΔA/min/mg protein |
|---|---|---|---|
| Kernel | 78.0 | 6.78 | 11.50 |
| Germ | 68.0 | 14.52 | 4.68 |
| Outer cob | 22.8 | 3.86 | 5.90 |
| Inner cob | 3.0 | 0.40 | 7.50 |

the base and tip kernel when compared to the mid-section.

**Table 2. Lipoxygenase concentration and specific activity in corn kernels.**

| | ΔA/min/g tissue | Protein mg/g tissue | ΔA/min/mg protein |
|---|---|---|---|
| **pH 7.0 (standard assay)** | | | |
| Base | 62.2 (122%) | 6.22 (102%) | 10.26 (126%) |
| Mid-section | 51.3 (100%) | 6.09 (100%) | 8.14 (100%) |
| Tip | 77.0 (150%) | 6.56 (108%) | 11.91 (146%) |
| **pH 6.25 (maximal activity)** | | | |
| Base | 281.2 (115%) | 7.80 (109%) | 36.05 (105%) |
| Mid-section | 244.8 (100%) | 7.16 (100%) | 34.18 (100%) |
| Tip | 342.8 (140%) | 8.11 (113%) | 42.32 (124%) |

Base refers to the 4-5 cm. of the cob, large irregularly shaped kernels; tip, 4-5 cm. at the tip of the cob, small round shaped kernels. Percentages are the activity/g tissue and specific activity of LOX found in the base and tip kernels in comparison to the mid-section kernels. The values of the activity/g and specific activity are significant to $p < 0.05$.

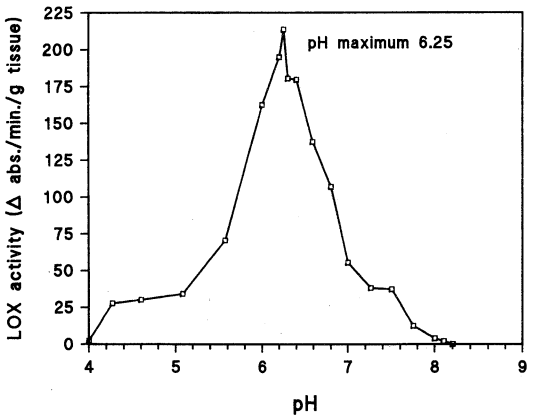


**Figure 3. Corn kernel lipoxygenase pH profile. The reactions were carried out in citrate buffer (pH 4.2 - 6.5), piperazine (pH 6.0 - 6.8), phosphate (pH 6.3 - 7.5) and Tris (7.0 - 8.25). At overlapping pH points the buffers were mixed to the appropriate pH.**

**pH profile**

The pH profile (Fig. 3) was measured in piperazine, citrate, phosphate and Tris buffers (50 mM). LOX shows a sharp peak activity at pH 6.25, typical of class II type LOX and an activity range of 4.0 to 8.2. Poca *et al.* (1990) isolated 2 LOX iso-enzymes,denominated L1 and L2, from 5 day old germinating corn seeds, which had broad peak activities at pH 6.0-8.2 and pH 7.0-9.0 respectively. Three iso-enzymes from corn embryos 25 days after pollination had pH maxima of 5.5, 6.8, and 7.5 with the enzyme with the pH 6.8 maximum as the predominant LOX (Belefant and Fong, 1991). Ida *et al.* (1983) identified 3 LOX isoenzymes in rice embryos with pH maxima at 4.5, 5.5 and 7.0, with the isoenzyme with the pH maxima 7.0 as the predominant enzyme.

**Temperature stability**

Samples from kernel preparation were held at each temperature (from 4°C to 65°C) for two minutes and were measured for LOX activity immediately, before cooling took place. Lipoxygenase reached maximal activity at 35-40°C, consistent with the maximal activities found in other fruits and vegetables that contain class II type LOX; 40°C in tomato (Bonnet and Crouzet, 1977) and 36-46°C for kiwifruit (Boyes *et al.*, 1992). All corn LOX activity was lost at 62°C with 50% loss of activity at 50-55°C. LOX exhibits a wide range of heat stabilities according to the iso-enzyme and within the iso-enzyme classes but the corn LOX is comparable to apple, 100% loss of activity at 50°C for 2 minutes (In-Sook and Grosch, 1977) and cucumber, 100% loss of activity at 70°C for 2 minutes (Wardale and Lambert, 1980). Class II and III type lipoxygenases isolated from 3 day old rice seedlings differed in heat stability; LOX III lost 90% activity after heating to 60°C for 25 mins, whereas LOX II lost only 10% activity (Ohta *et al.*, 1986). The heat-lability of an enzyme which has been partially purified does not always relate to the survival in vivo. Generally enzymes in vivo survive at higher temperatures for longer periods of time than partially purified enzymes.

**Effect of calcium**

Calcium acetate from a 12 mM stock solution was added to the Tris buffer (pH 7) and linoleic acid substrate, vortexed and the reaction was immediately initiated by the addition of a sample prepared from kernel. The reaction reached peak activity, 320% of the control activity, at 0.3 mM calcium concentration; at equimolar concentration to the linoleic acid substrate concentration (Fig. 4). It has been suggested that the calcium reacts with the substrate rather than the LOX

(Reynolds and Klein, 1982). This reaction activation is characteristic of class II type lipoxygenases. Calcium has no effect on the class I LOX reaction, and inhibits the class III LOX reaction. Belefant and Fong (1991) showed an activation of the LOX reaction to the major LOX enzyme present, 180% of the control activity, at a calcium concentration of 10 mM. This activation was thought not to be of physiological significance at this high calcium level, but the 10 mM calcium peak activity may be due to the linoleic acid concentration of 8.9 mM used in the analysis.

**Enzyme kinetics**

The activity of the germ LOX was measured over a substrate range of 0.06 to 0.30 mM linoleic acid. A $K_m$ of 0.051 mM linoleic acid and a $V_{max}$ of 0.139 A/min were determined (Fig. 5). At linoleic acid concentrations greater than 0.3 mM the initial velocity of reaction did not follow linear kinetics. This $K_m$ is lower than the values obtained for corn isoenzymes extracted from 5 day old pollinated seeds, denominated L1 and L2, which had values of 0.179 and 0.240 mM respectively (Poca *et al.*, 1990). Class II and III lipoxygenases isolated from 3 day old rice seedlings had $K_m$ values of 0.170 and 0.059 mM respectively (Ohta *et al.*, 1986).

**Microwave experiments**

Corn on the cob, when cooked in a microwave without covering had a crinkly appearance due to water loss, and some colour discolouration. The surface temperature did not rise above 65°C when microwaved for 3.5 minutes, although the inner cob which has a "fleshy" texture reached 73-86°C. However the outer cob which has a dry texture was always 5-15°C below the temperature of centre cob. When the corn was heated wrapped in a plastic film there was no water loss or colour change to the kernels. There was an increase in the surface temperature of the kernels (5-10°C), although only a small increase in the temperature of the inner and outer cob was observed. However the difference between the temperature of the inner and outer cob was still of the same magnitude (5-15°C).

The soluble protein of both the cob and kernel fell sharply after 3 mins microwave time, to 45% and 33% of their original levels respectively and continued to fall in approximately a linear manner (Fig. 6a). However POD was heat-resistant up to 3 minutes, particularly in the cob because of the long time for the heat to disseminate from both the inner cob and the kernel to the outer cob (Fig. 6b). At 5 minutes microwave time there was no measurable POD activity in the kernel and only

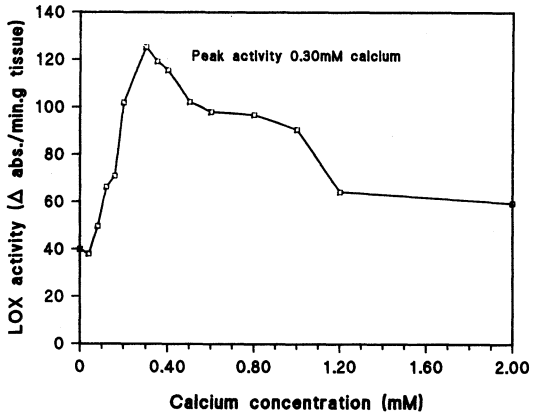


**Figure 4. Effect of calcium on kernel lipoxygenase. An aliquot of the 12 mM calcium acetate stock solution was added to the Tris buffer and substrate (pH 7), mixed and the reaction immediately initiated by the addition of LOX.**

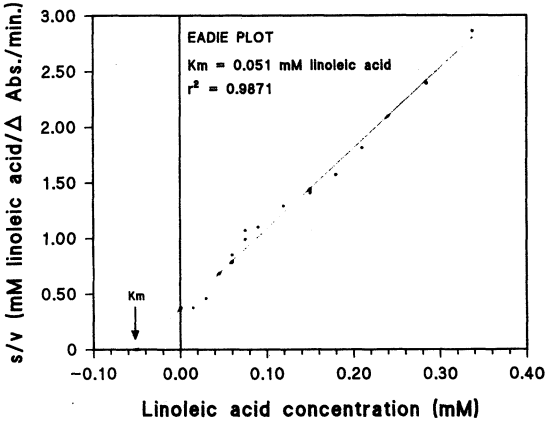


**Figure 5. Kinetics of corn germ lipoxygenase. The linoleic acid substrate range was 0.06 - 0.30 mM. At concentrations greater than 0.30 mM the reaction rate slowed, possibly due to substrate inhibition.**

1.5% of the original activity remaining in the cob. Adequate steam blanching of corn on the cob required 15 mins at 99°C. When blanched for a lesser time, the corn developed an off-flavour and colour deterioration, which correlated with the surviving peroxidase content in the outer cob and kernel (Lee and Hammes, 1979). However this does not indicate that peroxidase is solely responsible for the off-flavour and colour deterioration.

Lipoxygenase appeared to be more heat-resistant than POD (Fig. 6c). Most of the activity/g tissue was found in the kernel and after 5 mins 21% of the original activity survived. As a result the specific activity of the kernel LOX (A/min./mg protein) steadily increased over the 5 min. period of microwaving (Fig. 6d). This shows that at 5 mins LOX was more heat-stable than the other corn proteins.

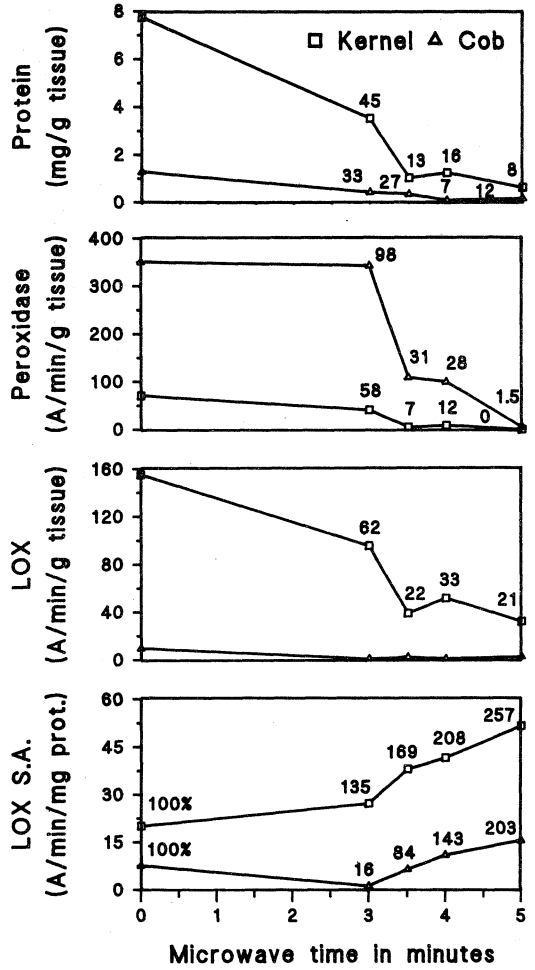


**Figure 6. Analysis of microwaved corn. a) Soluble protein; b) Peroxidase activity (measured at 460nm with o-dianisidine/$H_2O_2$ as the substrate); c) Lipoxygenase activity (measured in piperazine buffer at pH 6.25); d) Lipoxygenase specific activity. Percentages are relative to y-axis values at time = 0.**

## Conclusion

Mature Jubilee (edible) corn on the cob contained at least one iso-enzyme of LOX which showed the characteristics of a class II type (sharp acidic pH peak, and the reaction activated by calcium). It may also contain other iso-enzymes in lesser quantities of activity which would only be shown by purification. Initial experiments on the deactivation of LOX and POD by microwave blanching for times up to 5 mins are awaiting taste evaluation. All POD activity is virtually eliminated after 5 mins microwave time whereas some LOX activity remains. This initial study was undertaken to show that microwave blanching was a viable alternative to high energy steam and water blanching. Based on these results a larger study of the effects of the microwave blanching is planned in the following season.

## Acknowledgment

We thank Watties Frozen Foods for supplying the corn samples.

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