

## Biochemical Role Of Lipoxygenase In Development Of Off Flavors, Bleaching Of Pigments And Dough Formation

Dr. Jyoti D. Vora<sup>1</sup>, Ms. Shivani Hasabnis<sup>2</sup> And Mr. Deep J. Gutka<sup>3</sup>

<sup>1</sup> Guide,

Head, Department of Biochemistry & Food Science and Quality Control  
RamnarainRuia Autonomous College, Matunga, Mumbai - 400019

<sup>2,3</sup> Student,

Department of Biochemistry & Food Science and Quality Control  
RamnarainRuia Autonomous College, Matunga, Mumbai - 400019

Corresponding Author: Dr. Jyoti D. Vora

---

**Abstract:** Soybean is one of the important ingredients in the food industry. It is one of the important protein source for all vegetarians, containing most of the essential amino acid required by the body. But its use can also have a negative consequence on the food product by means of unfavorable sensory characteristics. This is brought about by the activity of Lipoxygenase, a non-heme iron containing enzyme. It is involved in peroxidation of polyunsaturated (cis, cis-1, 4-pentadiene moiety) fatty acids in the presence of molecular oxygen. The biochemistry of the enzyme lipoxygenase has been reviewed. The mechanism of the action of Lipoxygenase has been studied but the presence of certain loopholes still exists. The aim of this review is to bridge the gap as effectively as possible. The positive and negative implications of the enzyme activity have also been discussed.

### PRACTICAL APPLICATION

The aim to present this review of literature is to study the mechanism of LOX, its positive and negative implications. These data can have practical application in food industries such as development of breads, biscuits, cereals, etc.

-----  
Date of Submission: 05-06-2018

Date of acceptance: 20-06-2018  
-----

### I. Introduction

Lipoxygenase (LOX) are a ubiquitous family of non-heme iron containing enzymes present in plant & animals. It is involved in stereo & regio-specific peroxidation of polyunsaturated fatty acid in presence of molecular oxygen. Linoleic acid is the major substrate for LOX in plant where as arachidonic acid is in animals. LOX catalyses oxidation of PUFA containing cis, cis-1, 4-pentadiene moiety. Formation of jasmonic acid (in plant) is one of the major function of LOX. LOX is abundantly found in legumes like soya bean. LOX has different & distinct isoforms which are present in various stages of germinating seedlings & mature seeds.

LOX has antimicrobial, antioxidant, anti-inflammatory properties. Studies have been carried out to use LOX for treatment of certain types of cancers. Along with these it is also involved in vegetative growth, function as storage protein & also plays a significant role in plant defense mechanism

#### SOYA BEAN

Kingdom: Plantae

Sub-kingdom: angiosperms

Order: Fabales

Family: Fabaceae

Subfamily: Faboidae

Genus: Glycine



**Species: *G. max***

Soya bean or soybean is a species of legume native to Eastern Asia. It is a terrestrial plant found in tropical regions. It is classified as oil seed rather than a pulse due to its high oil content and its more popular use as a source of vegetable oil and industrial application such as biodiesel. Soya bean contains 20% of total fat (rich in omega 6), 36% protein, 30% carbohydrates and 9% water. It is one of the leading crop commodity produced traded and utilized globally.

**LIPOXYGENASE (LOX)**

Lipoxygenase (EC 1. 13. 11) is a superfamily of enzymes containing non-heme iron which catalyzes the oxygenation reaction (addition of oxygen) of polyunsaturated fatty acids containing *cis, cis-1,4-pentadiene* moiety.

It contains central iron atom bound to four ligands molecules out of which three are histidine residues. It is abundantly and most commonly found in plants, animals and humans. In plants it is involved in growth and development of plant physiology, pest resistance, senescence and also in response to wounding, in animals in humans it is involved in metabolism of eicosanoids such as prostaglandins and leukotrienes.

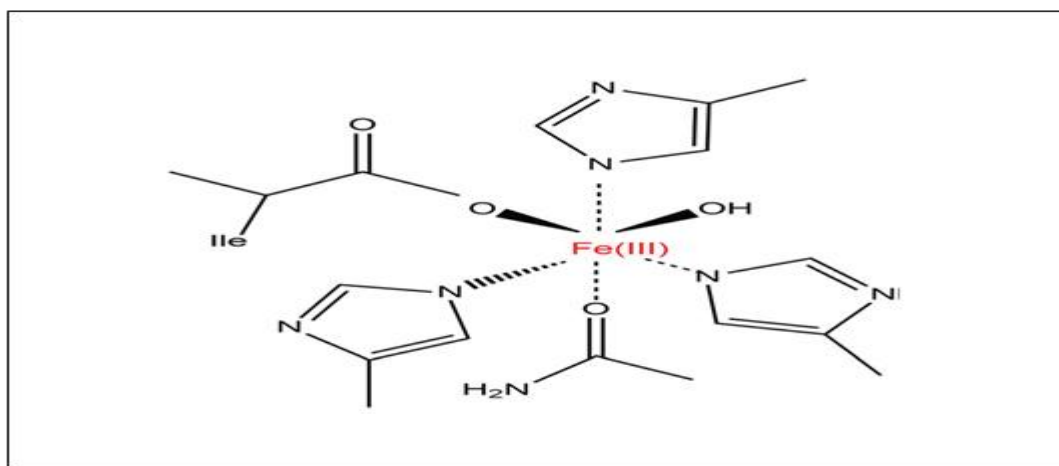
Plant exhibits a variety of LOX isoenzymes viz. cytosolic and chloroplast which functions in conjunction with hydroperoxide lyases responsible for many fragrances and other signaling molecules

**MECHANISM**

The mechanism of action of Lipoxygenase from Soybean has been studied quite well but there still are a few loop holes which are under scrutiny. Lipoxygenase from Soybean seed is the best characterized among the plant LOX. In simple words, the activity of LOX leads to addition of oxygen to fatty acids (linoleic, linolenic and arachidonic acid). Both aerobic and anaerobic pathways of LOX action are seen. These pathways result in twofold products: one which lead to production of off-flavor and the other which forms an answer to the physical hurt or wounding of the plant.

Soybeans have about 55% Linoleic and 10% Linolenic acid content. Oxidation of these fatty acids with further breakdown of these PUFAs, containing *cis,cis - 1,4-* pentadienes, leading to the generation of various medium chain aldehydes (e.g. pentanal, hexanal, heptanal and even nonanal along with their unsaturated forms) is the result of the LOX action.

The non-heme iron containing enzyme Lipoxygenase undergoes  $Fe^{2+}$  to  $Fe^{3+}$  transitions during peroxidation of fats leading to peroxidation of unsaturated fats to hydroperoxides. These inturn are responsible for the off-flavour and generating free radicals which causes bleaching of pigments. The iron atom is in the high spin (II) state in the native resting form of lipoxygenase and must be oxidized to the Fe(III) by the reaction product, fatty acid hydroperoxidase or hydrogen peroxide before activating as an oxidation catalyst. As a consequence of this requirement for oxidation of the iron I the enzyme, a lag period is observed, when the enzyme is used with pure fattyacid substrates. The active enzyme abstracts a hydrogen atom stereo-specifically from the intervening methylene group of a polyunsaturated fatty acid in a rate limiting step with the iron being reduced to Fe(II). The enzyme-alkyl radical complex is then oxidized by molecular oxygen to an enzyme-peroxy radical complex under aerobic conditions, before the transfer of electron from the ferrous atom to the peroxy group occurs. Protonation and dissociation from the enzyme allow theformation of the hydroperoxide. Under anaerobic conditions, thealkyl radical dissociates from the enzyme-alkyl radical complex and then a mixture of products including dimers, ketones andepoxides is produced by radical reactions.



**Figure 1**–Octahedral coordination of the non-heme iron in the LOX active site.

The figure was adapted from Brodhun and Feussner (2011).

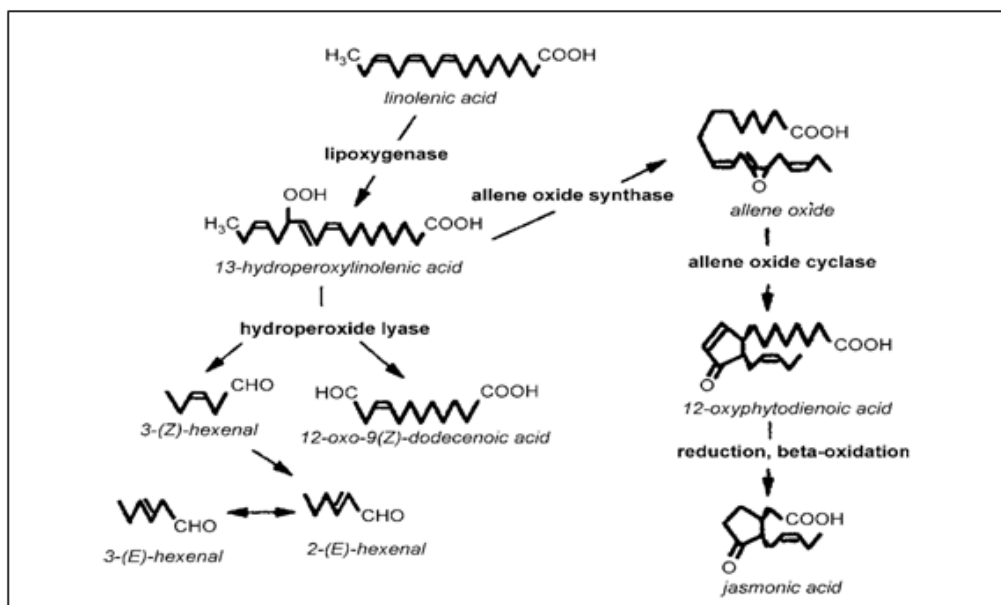


Fig. 2. Parts of the pathway of plant oxylipin metabolism leading from linolenic acid to jasmonate and volatile aldehydes.

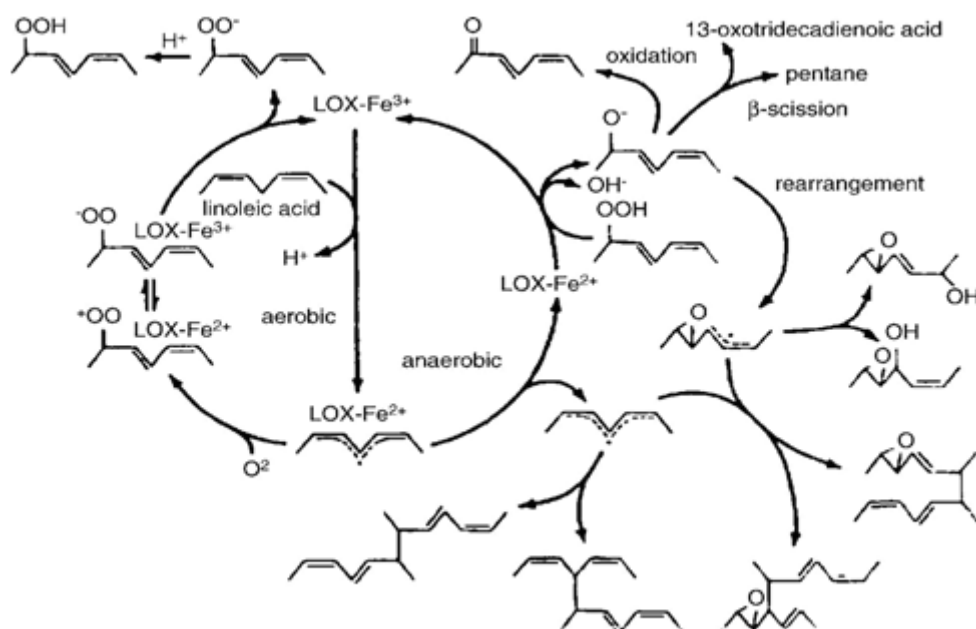
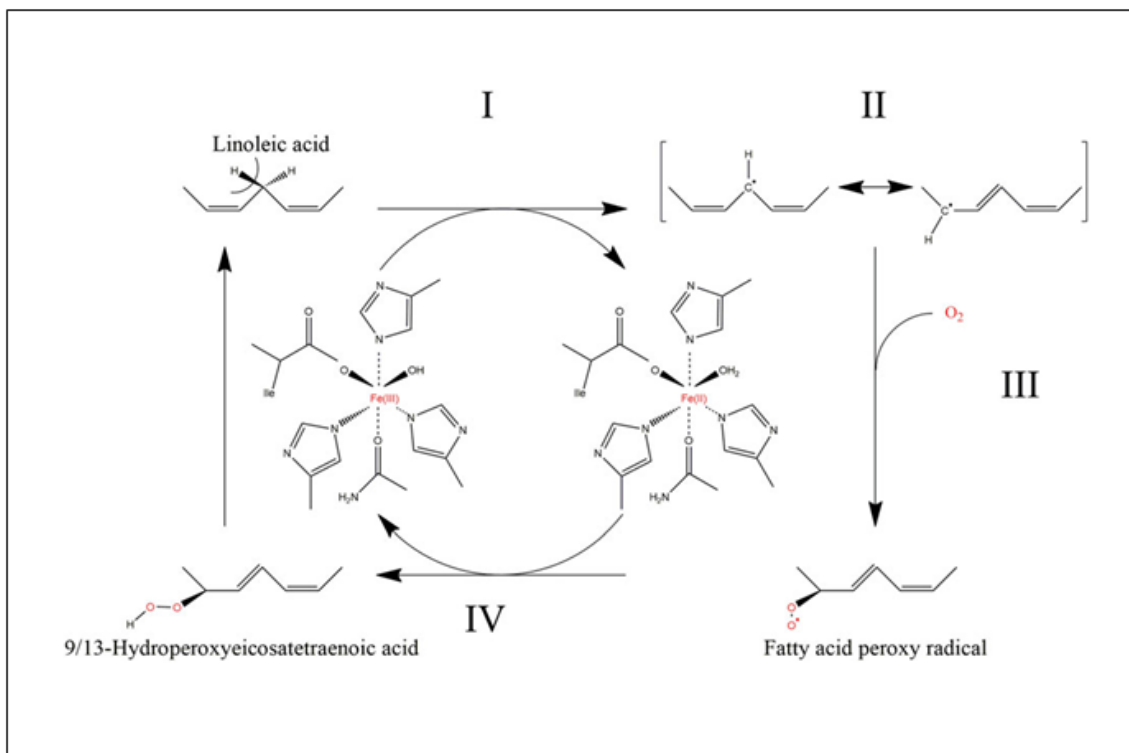


Fig.3. Pathway of Lipoxygenase catalysed oxidation

Fatty acid oxygenation is a series of biochemical reactions catalysed by specific enzymes, namely

1. Hydrogen abstraction
2. Radical rearrangement
3. Oxygen insertion
4. Per-oxyradical reduction.

These steps are depicted in the following Fig.4.



**Figure 4**—Mechanism of LOX catalysis. As depicted here, the LOX enzyme is cycled between the active Fe (III) and inactive Fe (II) states by the products and substrates of the reaction, respectively. Hydrogen abstraction results in the formation of a carbon radical which is subsequently stabilized by electron rearrangement. This is followed by oxygen insertion and reduction to form a hydroperoxide product. Figure adapted from Brodhun and Feussner (2011).

Lipoxygenase [ E.C. 1.13.11.12.] linoleate; oxygen oxidoreductase, catalyses oxidation of methylene interrupted polyunsaturated fatty acids. Lei and Boatright, 2005, revealed the importance of methanethiol to the characteristic odour of soybean which could give rise to a volatile sulphur compound; dimethyl trisulfide is responsible for the off odour. Although the means for the effect of lipoxygenase on the formation of methanethiol is not well known, but is thought to be due to release of the free radicals formed during oxidation of polyunsaturated fatty acids. Methionine residues in soy protein products are aggregated by such free radicals, resulting in the formation of methionine sulfoxide which generates methanethiol as a final product as proposed by Lei et al 2005. Therefore, the data from current studies suggest that lipoxygenase not only catalyzes the oxidation of polyunsaturated fatty acids, resulting in the formation of volatile aldehydes, ketones, and alcohols, but also promotes formation of methanethiol (Lozano et al, 2007). Flavours developed depends upon the composition of the fat with short chain fatty acids to C-10 having particularly disagreeable odours, flavours and those above C10 possessing waxy or, at alkaline pH, soapy flavours. Hydrolytic fat corrosion is not much important in terms of flavour formation when compared with flavours from oxidative deterioration of free fatty acids. Strong soybean-like flavours have been found in model systems consisting of hydroperoxides generated by the oxidative action of soy lipoxygenase on pure linoleic and linolenic acid. Volatile Compounds produced contribute to the grassy and beany flavours, while non-volatile compounds cause bitter and astringency. Privett et al. (1955) provided evidence that the principle initial products of lipoxygenase catalysis are optically active, *cis-trans* conjugated, monomeric hydroperoxides.

The isozymes of Lipoxygenase as studied before, have slight variation in the mechanism of action. They are:

- (1) Soy isozyme has an optimum pH of 9.0. It only acts on free polyunsaturated fatty acids and it forms 9- and 13-hydroperoxides in the ratio of 1:9 at room temperature. Some types of lipoxygenases can also catalyze the co-oxidation of carotenoids in the presence of PUFAs. Soybean lipoxygenase type-1 (LOX-1) has been used for the bleaching of wheat flour and also been shown to act as a bread improver and a valuable processing aid during dough development.
- (2) Soy isozyme has an optimum pH of 6.8, it acts on triglycerides as well as free polyunsaturated fatty acids and it forms 9- and 13-hydroperoxide in the ratio of about 1:1 at room temperature. A bleached color can also indicate deterioration in either fresh vegetables, such as yellow French beans or fruits and processed food

products, where carotenoids are important natural colorants and antioxidants. It has been reported that type-2 lipoxygenases (LOX-2 and -3) of soybean, pea and wheat are pigment bleachers in the presence of linoleic acid, but most of the reported studies for the co-oxidation of carotenoids have been for soybean LOX-1. It has been claimed that, under anaerobic conditions, this enzyme shows strong co-oxidizing activities in the presence of PUFA or a corresponding acyl hydroperoxide, whereas, under aerobic conditions, it is not an efficient catalyst for the bleaching reaction.

(3) Soy isozyme is similar to isozyme 2, but its activity is inhibited by calcium ions, whereas lipoxygenase-2 is stimulated by the metal.

(4) Lipoxygenase is very similar to isozyme 3, but can be separated by gel chromatography or electrophoresis.

### **NEGATIVE IMPLICATIONS**

Soybeans are an excellent protein source especially for the vegetarian people around the world. The consumption of soy protein has been encouraged and accelerated as it is an economic high protein source. Various food products are made or manufactured using soy and soy proteins. But certain undesirable effects due to enzymatic activity are observed. They are as follows:

1. Destruction of chlorophyll and carotenes.
2. Development of oxidative off-flavors and off-aromas.
3. Oxidative damage to vitamins and some proteins.
4. Oxidation of essential fatty acids; linoleic, linolenic and arachidonic acids.

Lipoxygenase activity produces the leaf aldehyde 'hexanal' which is important in tea aroma. But as opposed to this, lipoxygenase activity in Soybean produces off-flavours. Linoleate and linolenate esters in triacylglycerols are the most important LOX substrates in soybeans. The predominant hydroperoxides formed at the C-9 and C-13 positions on the fatty acid chain resulting in, from linoleic acid, 9(Z),11(E)-13-hydroperoxy-9,11-octadecadienoic acid (13-HPOD) or 10(S)-12(Z)-9-hydro-peroxy-10,12-octadecadienoic acid (9-HPOD) and from linolenic acid 10(E),12(Z),15(Z)-9-hydroperoxy-10,12-15-octadecatrienoic acid (9-HPOT) and 9(Z),11(E)15(Z)-13-hydroperoxy-9,11,15-octadecatrienoic acid (13-HPOT). These hydroperoxides are involved in further enzymatic reactions resulting in spontaneous or enzymatically caused cleavage of the fatty acid chain. When this occurs, aldehydes (hexanal, nonenal) and ketones are released causing the off flavor that is characteristic of soybeans.

Amongst all the types of lipoxygenases, LOX-2 is the most responsible for undesirable aroma compounds.

Lipoxygenase oxidation of polyunsaturated fatty acids (linoleic, linolenic and arachidonic acids) certainly decrease as the amount of these essential fatty acids in foods. This is directly related to production of off-flavors as discussed above.

The free radicals generated by LOX catalyzed oxidation of PUFAs decrease carotenoids (vitamin A precursors), tocopherols (Vitamin E), Ascorbate (Vitamin C) and Folate content of foods. The free radicals are also damaging to cysteine, tyrosine, tryptophan and histidine residues of proteins. These affect the amino acid content of Soy foods.

### **STRATEGIES ADOPTED TO REDUCE THE BEANY FLAVOR**

The unpleasant flavor of soy has cast a shadow on its nutritional properties. Therefore, many methods have been considered in order to get rid of this lipid derived beany off flavor in soybean which is due to lipoxygenase activity. Soybean proteins can be readily be modified by chemical, physical and enzymatic treatments changes the functional properties of the protein such as leading to flavor change. Methods such as heat treatment, pH adjustment, hydrolysis, enzyme treatment, ingredients addition, thermal modification, and breeding of soybean with low beany flavors have been used to eliminate/ reduce unpleasant beany flavor of soybean (Heywood et al, 2002; Suratman et al, 2004). According to Heywood et al, 2002, value enhanced soybeans (genetic modified/breed) have transformed fatty acid/ protein composition which means volatile compounds are removed that cause unpleasant beany flavor. According to Iassonova et al, 2009; removing lipoxygenase (LOX) isozymes can reduces the amounts of volatile off-flavor compounds in soybeans and soy products drastically, but are not completely eliminated.

Farkas and Goldblith (1962) showed that the inactivation of lipoxygenase by heat follows a first order reaction rate for 90% of the reaction.

They also found that heat inactivation of lipoxygenase is very sensitive to pH. The rate of inactivation increases below pH 4 or above pH 8.

Beckel et al. (1948) observed improvement in color and decreased bitterness of soybean products by ethanol extraction. Mustakas et al. (1961) reported that 95% ethanol and 91% isopropanol are effective in removing bitterness and color from defatted soy flakes.

Eldridge et al. (1977) observed that soybeans soaked in 40 to 60% alcohol give the best overall flavor score and minimum grassy, beany response as well as minimum lipoxygenase activity.

Borhan and Snyder (1979) investigated lipoxygenase destruction in whole soybeans by combinations of heating and soaking in ethanol. They observed that lipoxygenase assay at pH 9 is suitable for the detection of the most heat resistant lipoxygenase. They found that the concentration of ethanol required for complete inactivation of lipoxygenase in 24 hours.

soaking decreases as temperature increases. Higher nitrogen solubility was obtained with lower temperatures of soaking. Increasing pH using sodium carbonate and sodium bicarbonate is effective in rapid inactivation of lipoxygenase. With emphasis on protein solubility, they recommended 15 to 45% ethanol, 40 to 60°C and 2 to 6 hr for soaking soybeans.

Biotechnology has also been very useful in solving this issue by knocking off the LOX-2 genes; which has resulted in reduced perception of off-flavors. Thus, production of LOX-null varieties of Soybean has increased and are now fully fledged used for production of Soy milk or Tofu.

## **APPLICATIONS**

The applications of LOX are actually the positive or as can be called the desirable effects produced by LOX in foods. They are:

### **1. Bleaching of pigments.**

The ability of lipoxygenase to bleach or decolorize several pigments is one of its oldest abilities but ironically it is the least understood. Theories have been put forth that the bleaching effect is byproduct of its oxidation process. Also there is some relation between Peroxidation and lipid oxidation reaction which causes generation of free radicals which bleach the pigments.

### **2. Formation of -s-s- bonds in gluten during dough formation.**

During the bread making process, different types of bonds are formed to allow for protein structure development. These bonds include: hydrogen, hydrophobic, ionic, disulfide and possibly dityrosine cross-links. These bonds formed in the proteins have indirect effect on dough formation, its texture and leavening property and also affects bread making quality (Tilley et al., 2001). Since gluten is composed of glutenin and gliadin, upon the addition of water, dough formation and manipulation, the glutenin will form cross-links around gliadin to form the gluten complex (McWilliams, 2001). Lipoxygenase gives stability to the disulfide bonds and prevents its breakdown. This in turn increases the mixing tolerance of the dough.

## **II. Conclusion**

Lipoxygenase is a ubiquitous non heme iron containing enzyme. It has many different types of isoenzymes. In soybean its main function is of development of off flavors and odours by peroxidation reaction of triacylglycerides like linoleic, linolenic and arachidonic acids. It catalyzes the redox reaction. The product formed is medium chain aldehydes. There are various other side reactions and byproducts also generated. The side reactions involve the co-oxidation reaction which generates free radicals that bleach the pigment. This property is used in bleaching various flour. LOX is used in development of good quality bread dough. The byproducts like dimers, ketones and epoxides are formed. Different isoenzymes have different activators and inhibitors. Amongst all the types of lipoxygenases, LOX-2 is the most responsible for undesirable aroma compounds. The negative implications of LOX can be reduced by various methods like temperature, pH change, heat, chemical treatments and genetic engineering.

## **References**

- [1]. Addition of Soybean Lipoxygenase to All-Purpose Flour and its Effects on Dough Gluten Strength and Bread Quality, Erin M. Danielson.
- [2]. Biological Roles and Biochemistry of the Lipoxygenase Pathway, Harold W. Gardner.
- [3]. Colorimetric Method for the Determination of Lipoxygenase Activity, Gordon E. Anthon and Diane M. Barrett.
- [4]. Oxygenation of Unsaturated Fatty Acids by Soybean Lipoxygenase, William L. Smith and William I.C. M. Lands.
- [5]. Lipoxygenases: From Isolation to Application, Stefan Hayward, Tertius Cilliers, and Pieter Swart.
- [6]. Lipoxygenase in fruits and vegetables: A review, Taner Baysal, Ashlan Demirdoven.
- [7]. Lipoxygenase: Its biochemistry and role in breadmaking, J. M. Faubion and R. C. Hoseney.

Dr. Jyoti D. Vora. "Biochemical Role Of Lipoxygenase In Development Of Off Flavors, Bleaching Of Pigments And Dough Formation." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 4.3 (2018): 52-57.