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Can Soybean Lipoxygenases be Real Models for Mammalian Lipoxygenases? A Bioinformatics Approach

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Abstract: Soybean lipoxygenase-1 is one of well-studied enzymes because it is considered as a model enzyme for mammalian lipoxygenases. In general, the soybean lipoxygenase-1 is used in the test of inhibitory activities of various compounds. The present study provides a bioinformatics approach for comparison of various lipoxygenases in the databases. Their various physical and chemical parameters such as molecular weight, theoretical pI, amino acid composition, aliphatic index, and grand average of hydropathicity and the multiple sequence alignments of the lipoxygenases were computed by using several bioinformatics tools. In order to see phylogenetic relationships among lipoxygenases, a phylogenetic tree was constructed. The first three most abundant amino acids in soybean lipoxygenase-1 and 15-lipoxygenase (human) are L (10.3 %), S (7.4 %), A (6.7 %) and L (13.3 %), G (7.4 %), V (7.1 %), respectively. According to the phylogenetic tree, the soybean lipoxygenases are within separate clade compared to the mammalian lipoxygenases. In conclusion, soybean lipoxygenase-1 may not fully characterize the human lipoxygenase-15 since there are remarkable sequence-based differences, which are obtained by using bioinformatics tools between soybean lipoxygenase-1 and lipoxygenases from other sources especially from human. In this context, the researchers, who are aware of the problems described above and who have similar concerns, have begun to use recombinant DNA technology to produce recombinant human lipoxygenase-15 enzyme.

Keywords: Bioinformatics, lipoxygenases, soybean lipoxygenase.

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INTRODUCTION

Lipoxygenases (EC 1.13.11.12, linoleate:oxygen, oxidoreductases, LOXs) which are a large protein family are widely distributed in animals, plants and fungi. These enzymes are also found in cyanobacteria, although they are rarely found in other prokaryotes (1,2). Its general structure contained non-heme, non-sulfur iron atom which is responsible for enzymic activity. LOXs belong to dioxygenase family and they catalyze the oxidation of the polyunsaturated fatty acids (PUFA) such as linoleate, linolenate and arachidonate to form hydroperoxides utilizing Fe^{+2}/Fe^{+3} redox potential and molecular oxygen (3,4). Mammalian LOXs are named as 5-LOX, 8-LOX, 11-LOX 12-LOX and 15-LOX, whereas it is 9-LOX and 13-LOX in plants based on the peroxidation position of PUFAs (5-7). Boyington et al (8) identified the first crystalline structure of LOXs from soybean that is previously named as soybean LOX-1. Although this enzyme was firstly characterized in 1947, the comprehensive analyses of soybean LOX-1 biochemistry were conducted following the structural identification in 1993 (9). Among LOXs, soybean LOX-1 is one of the well-studied LOX enzymes due to its ease of purification and activation stability (10,11) and also it has been considered as a model LOX for

mammalian LOXs, especially for human due to the insufficient purified human LOXs (5,8,12-19). According to the results of X-ray crystalline analysis, soybean LOX-1 has two domains: a smaller β-barrel domain and a much larger α-helix domain included the catalytic iron and substrate binding cavity (8,20,21). LOX enzymes generally have a highly conserved Fe-coordination sphere within the α-helix structure. Three His and one Ile ligands in the active site are also conserved in all lipoxygenases except from rat leukocyte 5-LOX (22,23). Minor et al (24) reported that the active site had two additional ligands as a water and Asn694 which can be responsible for the coordination flexibility of the active site. Although the highly conserved region in catalytic site motivate researchers to use soybean LOX-1 as a model for 15-LOX (human), it should be kept in mind that minor changes in conserved structure may lead significant functional differences between related enzymes (2). For instance, the reaction mechanism of 15-LOX (human) is considered to be similar to soybean LOX-1, but the k_{cat} value of 15-LOX (human) is approximately 45-fold lower than that of soybean LOX-1. The decrease in the k_{cat} value might have been caused by the change in redox potential of iron as a result of ligation difference (23).

LOXs have stimulated interests of many researchers so far because of the roles of these enzymes in many diseases such as bronchial asthma, allergic rhinitis, inflammatory bowel, rheumatoid arthritis, osteoporosis, stroke, cardiovascular diseases, Alzheimer's disease, and cancer. Moreover, LOX products, leukotrienes, and lipoxins, have a part in significant metabolic functions such as organelle degradation (25), transcription regulation (26), and possibly tumor cell metastasis (27). Therefore, the inhibition of LOXs is considered an important target for the treatment of LOX-related diseases (5-7,16,28-35). There are some reports which show the results of LOX inhibition studies in the literature. The inhibitory effects of three Malian plant extracts with different polarities on soybean 15-LOX were determined by Maiga et al (14). The inhibitory potential of substituted benzoic acids which is widely found in plants on soybean 15-LOX was studied by Russell et al (17). In another report conducted by Bouriche et al (36), the effects of *Cleome arabica* leaf extract, rutin, and quercetin on soybean LOX were examined. Mahesha et al (16) investigated the inhibitory effect of genistein and daidzein compounds against soybean LOX. Plant polyphenols known as sinapic acid, caffeic acid, chlorogenic acid, ferulic acid, naringenin, and chrysin were evaluated as an inhibitor of lipoxygenase and chlorogenic acid was determined as the strongest inhibitor for soybean LOX with an IC_{50} values of 61.27 μmol/L (37). The inhibitory effect of sesamol, the phenolic degradation product of sesamolin, on lipoxygenase was determined in another investigation. It is reported that sesamol inhibited the soybean LOX-1 in a dose dependent manner with IC_{50} value of 51.84 μM and the enzyme kinetic results indicated that the inhibition occurred in a competitive manner with the Ki value of 4.9 μM (6). In above mentioned studies, soybean LOX has been used as a model enzyme for mammalian LOXs. On the other hand, the developments in bioinformatics and computational biology have provided a big contribution to understanding of proteins more comprehensively. Bioinformatics tools such as Clustal Omega (38,39), some tools in expasy.ch (40,41) such as PROTPARAM (41) and also phylogenetic tree construction (38,39) might help for a detailed comparison of LOXs.

This investigation aims at answering the following research questions:

- Is soybean LOX family phylogenetically close to mammalian LOX family?
- Are there any differences among various physical and chemical parameters of LOXs; such as molecular weight, theoretical pI, amino acid composition, aliphatic index and grand average of hydropathicity (GRAVY)?
- Is it reliable enough to use soybean LOXs in inhibition studies as a model for mammalian LOXs?

IN SILICO EXPERIMENTAL SECTION

The sequences and related biochemical reactions of the LOXs studied were retrieved from proteomics server of the Swiss Institute of Bioinformatics – ExPASy (Expert Protein Analysis System) (40). LOXs analyzed and their corresponding codes were presented in Tables 1 and 2. Their various physical and chemical parameters such as molecular weight, theoretical pI, amino acid composition, aliphatic index, and GRAVY were computed by using PROTPARAM tool of Expasy.ch (40,41). These parameters were also shown in Tables 3 and 4. The multiple sequence alignments of the LOXs were also computed by using Clustal Omega (38,39). In order to understand the evolutionary relationship between the LOXs studied, a phylogenetic analysis was carried out. Phylogenetic tree was constructed via phylogenetic tool at Clustal Omega web tool (38,39). In order to achieve the secondary structure prediction of soybean LOX-1 and 15-LOX (human), the PSIPRED Protein Structure Prediction Server was used (42). Table 7 represents the conserved regions of the aligned sequences.

Table 1. Coding used for analyzed lipoxygenases and related database information.

Code	Protein Name	Abbreviation	Gene Name	Primary Accession Number in Swiss-Prot	Species
1	Polyunsaturated fatty acid 5-lipoxygenase	5-LO	ALOX5	P09917	*Homo sapiens* (Human)
2	Arachidonate 12-lipoxygenase, 12R-type	12R-LOX	ALOX12B	O75342	*Homo sapiens* (Human)
3	Polyunsaturated fatty acid 5-lipoxygenase	5-LO	Alox5	P12527	*Rattus norvegicus* (Rat)
4	Arachidonate 12-lipoxygenase, 12R-type	12R-LOX	Alox12b	O70582	*Mus musculus* (Mouse)
5	Polyunsaturated fatty acid lipoxygenase ALOX12	12S-LOX	ALOX12	P18054	*Homo sapiens* (Human)
6	Seed linoleate 13S-lipoxygenase-1	L-1	LOX1.1	P08170	*Glycine max* (Soybean)
7	Seed linoleate 9S-lipoxygenase-3	L-3	LOX1.3	P09186	*Glycine max* (Soybean)
8	Polyunsaturated fatty acid lipoxygenase ALOX15	15-LOX	ALOX15	P16050	*Homo sapiens* (Human)
9	Polyunsaturated fatty acid 5-lipoxygenase	5-LO	Alox5	P48999	*Mus musculus* (Mouse)
10	Arachidonate 12-lipoxygenase, 12R-type	12R-LOX	Alox12b	Q2KMM4	*Rattus norvegicus* (Rat)
11	Polyunsaturated fatty acid 5-lipoxygenase	5-LO	ALOX5	P51399	*Mesocricetus auratus* (Golden hamster)
12	Polyunsaturated fatty acid lipoxygenase ALOX15	15-LOX	ALOX15	P12530	*Oryctolagus cuniculus* (Rabbit)
13	Polyunsaturated fatty acid lipoxygenase ALOX15B	15-LOX-B	ALOX15B	O15296	*Homo sapiens* (Human)
14	Polyunsaturated fatty acid lipoxygenase ALOX8	8-LOX	Alox8	O35936	*Mus musculus* (Mouse)
15	Polyunsaturated fatty acid (12S)/(13S)-lipoxygenase, epidermal-type	12-LOX-e	Alox12e	P55249	*Mus musculus* (Mouse)
16	Polyunsaturated fatty acid lipoxygenase ALOX12	12S-LOX	Alox12	P39655	*Mus musculus* (Mouse)
17	Hydroperoxide isomerase ALOXE3	e-LOX-3	ALOXE3	Q9BYJ1	*Homo sapiens* (Human)
18	Seed linoleate 9S-lipoxygenase	-	LOX1.4	P24095	*Glycine max* (Soybean)
19	Seed linoleate 9S-lipoxygenase-2	L-2	LOX1.2	P09439	*Glycine max* (Soybean)
20	Polyunsaturated fatty acid lipoxygenase ALOX15	15-LOX	Alox15	P39654	*Mus musculus* (Mouse)
21	Polyunsaturated fatty acid lipoxygenase ALOX15	15-LOX	Alox15	Q02759	*Rattus norvegicus* (Rat)
22	Polyunsaturated fatty acid lipoxygenase ALOX15	15-LOX	ALOX15	P16469	*Sus scrofa* (Pig)
23	Linoleate 9S-lipoxygenase-4	L-4	LOX1.5	P38417	*Glycine max* (Soybean)
24	Polyunsaturated fatty acid lipoxygenase ALOX15B	15-LOX-B	Alox15b	Q8K4F2	*Rattus norvegicus* (Rat)
25	Polyunsaturated fatty acid lipoxygenase ALOX15	15-LOX	ALOX15	P27479	*Bos taurus* (Bovine)
26	Hydroperoxide isomerase ALOXE3	e-LOX-3	Aloxe3	Q9WV07	*Mus musculus* (Mouse)

RESULTS AND DISCUSSION

The functions and the catalytic activities of LOXs studied were summarized in Table 2. When the Table 2 is evaluated, it is very clear that LOXs from plants and vertebrates do not reveal completely same biochemical catalytic reactions.

Some chemical and physicochemical properties of LOXs studied such as number of amino acids, molecular weight (Da), theoretical pI, number of negatively and positively charged amino acids, aliphatic index and GRAVY were shown in Table 3. The molecular weight of soybean LOX-1 is higher than that of 15-LOX (human). The length of sequences for soybean LOXs are generally longer than those for mammalian ones. Although the soybean LOX have more amino acid residues in its general structure than mammalian LOX, approximately 200 amino acids, there is a little difference between the pI values of these species. The solubility characteristics of the proteins can be expressed via GRAVY index as the positive and negative values of it shows hydrophobic and hydrophilic characters, respectively (43). As it is seen in the Table 3, the value of GRAVY for soybean LOXs is more negative than those of mammalians. So it can be said that soybean LOXs are more soluble in polar solvents. The minimum and maximum theoretical pI values were observed as 5.51 and 8.31 for polyunsaturated fatty acid 5-lipoxygenase and polyunsaturated fatty acid (12S)/(13S)-lipoxygenase, epidermal-type, respectively. It is well known that the amino acids in active site are responsible for the enzymic activity. Although these differences mentioned above exhibit the difference of evolutionary origins of these enzymes, the similarities in active site composition reveal convergent evolution. As can be seen from Table 3, the amino acid residues in active sites of all LOXs studied are very similar. Mogul et al (44) suggested that the active site of soybean LOX-1 and 15-LOX (human) is slightly different according to the results of inhibition studies conducted with alkenyl-sulfate substrates. It was reported that alkenyl-sulfates are not inhibitors for soybean LOX-1. They cannot inhibit 15-LOX (human) at low concentrations, either. But 15-LOX (human) is irreversibly inhibited by alkenyl-sulfates at high concentrations. Moreover, different enzymic activities were reported for LOXs from different origins in the scientific literature. For example, the activity of soybean LOX-1 was reported as 280 μmol/min.mg (45). On the other hand, the enzymic activity of 15-LOX from *Pseudomonas aeruginosa* (46) was reported to be 747130.2 IU/mg protein with linoleic acid. Therefore, there might be another parameter such as water or different ligand-substrate interactions except from the amino acid residues in active sites which can help researchers to a better understanding about the activity variations among LOXs. Iron atom in the active site of LOXs is essential for the enzymatic activity and it is believed that the micro environment of this atom is crucial for the reaction (23,31). Iron in the active site has six coordinates which were identified as His499, His504, His690, Asn694, the terminal carboxylate of Ile839 and an additional water ligand (8,24). The enzymatic activity (or the rate of the reaction) is affected by the various changes in this environment. For example, the Asn694 residue is converted His in mammalian LOX (15-LOX (rabbit)). As the His is a stronger ligand than Asn, the rate of the reaction is decreased as a result of the changes in the reduction potential of iron ion and the pKa value (23,31).

The amino acid compositions of LOXs are shown in Table 4. The first three most abundant amino acids in soybean LOX-1 and LOX-15 (human) are L (10.3 %), S (7.4 %), A (6.7 %) and L (13.3 %), G (7.4 %), V (7.1 %), respectively. It is known that the number of amino acids between plant and mammalian LOXs are significantly different. Mammalian LOXs have nearly 662-711 amino acids (in the range of 75-81 kDa for molecular weight) while plant LOXs have 838-923 amino acids (in the range of 94-103 kDa for molecular weight). It is very surprising that despite the difference between the amino acid number of plant and mammalian LOXs, the topologies and the catalytic site are highly conserved in LOX family (21,47).

Table 2. Comparison of functions and catalytic activities of lipoxygenases analyzed (40,41).

Protein Name	Function	Catalytic activity
Polyunsaturated fatty acid 5-lipoxygenase (Human)	It catalyzes the oxygenation of arachidonic acid to 5-hydroperoxyicosatetraenoic acid (5-HPETE) followed by the dehydration of the hydroperoxide into an epoxide, 5,6-oxidoicosatetraenoic acid (LTA4), thereby participates in the first step in leukotriene biosynthesis and in the inflammatory processes.	(5Z,8Z,11Z,14Z)-eicosatetraenoate + O_2 = H_2O + leukotriene A_4
Arachidonate 12-lipoxygenase, 12R type (Human)	It catalyzes the regio and stereo-specific incorporation of a single molecule of dioxygen into free and esterified polyunsaturated fatty acids generating lipid hydroperoxides that can be further reduced to the corresponding hydroxy species.	(5Z,8Z,11Z,14Z)-eicosatetraenoate + O_2 = (12R)-hydroperoxy-(5Z,8Z,10E,14Z)-eicosatetraenoate
Polyunsaturated fatty acid 5-lipoxygenase (Rat)	It catalyzes the oxygenation of arachidonic acid to 5-hydroperoxyicosatetraenoic acid (5-HPETE) followed by the dehydration of the hydroperoxide into an epoxide, 5,6-oxidoicosatetraenoic acid (LTA4), thereby participates in the first step in leukotriene biosynthesis and in the inflammatory processes.	(5Z,8Z,11Z,14Z)-eicosatetraenoate + O_2 = H_2O + leukotriene A_4
Arachidonate 12-lipoxygenase, 12R-type (Mouse)	It catalyzes the regio and stereo-specific incorporation of a single molecule of dioxygen into free and esterified polyunsaturated fatty acids generating lipid hydroperoxides that can be further reduced to the corresponding hydroxy species. Does not convert arachidonic acid to (12R)-hydroperoxyeicosatetraenoic acid/(12R)-HPETE.	1-*O*-methyl-(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = 1-*O*-methyl (5*Z*,8*Z*,10*E*,12*R*,14*Z*)-hydroperoxyiecosatetraenoate
Polyunsaturated fatty acid lipoxygenase ALOX12 (Human)	It catalyzes the regio and stereo-specific incorporation of a single molecule of dioxygen into free and esterified polyunsaturated fatty acids generating lipid hydroperoxides that can be further reduced to the corresponding hydroxy species. Mainly converts arachidonic acid to (12S)-hydroperoxyeicosatetraenoic acid/(12S)-HPETE but can also metabolize linoleic acid.	(5Z,8Z,11Z,14Z)-eicosatetraenoate + O_2 = (12S)-hydroperoxy-(5Z,8Z,10E,14Z)-eicosatetraenoate
Seed linoleate 13S-lipoxygenase-1 (Soybean)	Plant lipoxygenase may be involved in a number of diverse aspects of plant physiology including growth and development, pest resistance, and senescence or responses to wounding. With linoleate as substrate, L-1 shows a preference for carbon 13 as the site for hydroperoxidation (in contrast to L-2 and L-3, which utilize either carbon 9 or 13).	(9*Z*,12*Z*)-octadecadienoate + O_2 = (13*S*)-hydroperoxy-(9*Z*,11*E*)-octadecadienoate
Seed linoleate 9S-lipoxygenase-3 (Soybean)	Plant lipoxygenase may be involved in a number of diverse aspects of plant physiology including growth and development, pest resistance, and senescence or responses to wounding. It catalyzes the hydroperoxidation of lipids containing a cis,cis-1,4-pentadiene structure.	(9Z,12Z)-octadecadienoate + O_2 = (9S)-hydroperoxy-(10E,12Z)-octadecadienoate
Polyunsaturated fatty acid lipoxygenase ALOX15 (Human)	It catalyzes the stereo-specific peroxidation of free and esterified polyunsaturated fatty acids generating a spectrum of bioactive lipid mediators. It converts arachidonic acid into 12-hydroperoxyeicosatetraenoic acid/12-HPETE and 15-hydroperoxyeicosatetraenoic acid/15-HPETE. Also converts linoleic acid to 13-hydroperoxyoctadecadienoic acid. May also act on (12S)-hydroperoxyeicosatetraenoic acid/(12S)-HPETE to produce hepoxilin A3.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (12*S*)-hydroperoxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoate

Polyunsaturated fatty acid 5-lipoxygenase (Mouse)	It catalyzes the oxygenation of arachidonic acid to 5-hydroperoxyicosatetraenoic acid (5-HPETE) followed by the dehydration of the hydroperoxide into an epoxide, 5,6-oxidoicosatetraenoic acid (LTA4), thereby participates in the first step in leukotriene biosynthesis and in the inflammatory processes.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (5S)-hydroperoxy-(6E,8*Z*,11*Z*,14*Z*)-eicosatetraenoate
Arachidonate 12-lipoxygenase, 12R-type (Rat)	It catalyzes the regio and stereo-specific incorporation of a single molecule of dioxygen into free and esterified polyunsaturated fatty acids generating lipid hydroperoxides that can be further reduced to the corresponding hydroxy species.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (12*R*)-hydroperoxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoate
Polyunsaturated fatty acid 5-lipoxygenase (Golden hamster)	It catalyzes the oxygenation of arachidonic acid to 5-hydroperoxyicosatetraenoic acid (5-HPETE) followed by the dehydration of the hydroperoxide into an epoxide, 5,6-oxidoicosatetraenoic acid (LTA4), thereby participates in the first step in leukotriene biosynthesis and in the inflammatory processes.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = H_2O + leukotriene A_4
Polyunsaturated fatty acid lipoxygenase ALOX15 (Rabbit)	It catalyzes the stereo-specific peroxidation of free and esterified polyunsaturated fatty acids generating a spectrum of bioactive lipid mediators. It converts arachidonic acid into 12-hydroperoxyeicosatetraenoic acid/12-HPETE and 15-hydroperoxyeicosatetraenoic acid/15-HPETE. Also converts linoleic acid to 13-hydroperoxyoctadecadienoic acid. May also act on (12S)-hydroperoxyeicosatetraenoic acid/(12S)-HPETE to produce hepoxilin A3.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (12*S*)-hydroperoxy-(5*Z*,8*Z*,10E,14*Z*)-eicosatetraenoate
Polyunsaturated fatty acid lipoxygenase ALOX15B (Human)	It catalyzes the stereo-specific peroxidation of free and esterified polyunsaturated fatty acids (PUFAs) generating a spectrum of bioactive lipid mediators. It converts arachidonic acid to 15S-hydroperoxyeicosatetraenoic acid/(15S)-HPETE. Also acts on linoleic acid to produce 13-hydroxyoctadecadienoic acid/13-HPODE.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (15*S*)-hydroperoxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoate
Polyunsaturated fatty acid lipoxygenase ALOX8 (Mouse)	It catalyzes the stereo-specific peroxidation of free and esterified polyunsaturated fatty acids (PUFAs) generating a spectrum of bioactive lipid mediators. It catalyzes the peroxidation of arachidonate and linoleate into (8S)-HPETE and (9S)-HPODE respectively.	(9Z,12Z)-octadecadienoate + O_2 = (9S)-hydroperoxy-(10E,12Z)-octadecadienoate
Polyunsaturated fatty acid (12S)/(13S)-lipoxygenase, epidermal-type (Mouse)	It catalyzes the regio and stereo-specific incorporation of a single molecule of dioxygen into free and esterified polyunsaturated fatty acids generating lipid hydroperoxides that can be further reduced to the corresponding hydroxy species.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (12*S*)-hydroperoxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoate
Polyunsaturated fatty acid lipoxygenase ALOX12 (Mouse)	It catalyzes the regio and stereo-specific incorporation of a single molecule of dioxygen into free and esterified polyunsaturated fatty acids generating lipid hydroperoxides that can be further reduced to the corresponding hydroxy species. Mainly converts arachidonic acid to (12S)-hydroperoxyeicosatetraenoic acid/(12S)-HPETE but can also metabolize linoleic acid. In contrast does not react towards methyl esters of linoleic and arachidonic acids.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (12*S*)-hydroperoxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoate
Hydroperoxide isomerase ALOXE3 (Human)	Non-heme iron-containing lipoxygenase which is atypical in that it displays a prominent hydroperoxide isomerase activity and a reduced lipoxygenases activity. The hydroperoxide isomerase activity catalyzes the isomerization of hydroperoxides, derived	a hydroperoxyeicosatetraenoate = a hydroxy-epoxy-eicosatetraenoate

	from arachidonic and linoleic acid by ALOX12B, into hepoxilin-type epoxyalcohols and ketones. In presence of oxygen, oxygenates polyunsaturated fatty acids, including arachidonic acid, to produce fatty acid hydroperoxides.	
Seed linoleate 9S-lipoxygenase (Soybean)	Plant lipoxygenase may be involved in a number of diverse aspects of plant physiology including growth and development, pest resistance, and senescence or responses to wounding. It catalyzes the hydroperoxidation of lipids containing a cis,cis-1,4-pentadiene structure.	(9*Z*,12*Z*)-octadecadienoate + O_2 = (9*S*)-hydroperoxy-(10*E*,12*Z*)-octadecadienoate
Seed linoleate 9S-lipoxygenase-2 (Soybean)	Plant lipoxygenase may be involved in a number of diverse aspects of plant physiology including growth and development, pest resistance, and senescence or responses to wounding. It catalyzes the hydroperoxidation of lipids containing a cis,cis-1,4-pentadiene structure.	(9*Z*,12*Z*)-octadecadienoate + O_2 = (9*S*)-hydroperoxy-(10*E*,12*Z*)-octadecadienoate
Polyunsaturated fatty acid lipoxygenase ALOX15 (Mouse)	It catalyzes the stereo-specific peroxidation of free and esterified polyunsaturated fatty acids generating a spectrum of bioactive lipid mediators. It converts arachidonic acid into 12-hydroperoxyeicosatetraenoic acid/12-HPETE and 15-hydroperoxyeicosatetraenoic acid/15-HPETE. Also converts linoleic acid to 13-hydroperoxyoctadecadienoic acid. May also act on (12S)-hydroperoxyeicosatetraenoic acid/(12S)-HPETE to produce hepoxilin A3. Probably plays an important role in the immune and inflammatory responses.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (12*S*)-hydroperoxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoate
Polyunsaturated fatty acid lipoxygenase ALOX15 (Rat)	It catalyzes the stereo-specific peroxidation of free and esterified polyunsaturated fatty acids generating a spectrum of bioactive lipid mediators. It converts arachidonic acid into 12-hydroperoxyeicosatetraenoic acid/12-HPETE and 15-hydroperoxyeicosatetraenoic acid/15-HPETE. Also converts linoleic acid to 13-hydroperoxyoctadecadienoic acid. May also act on (12S)-hydroperoxyeicosatetraenoic acid/(12S)-HPETE to produce hepoxilin A3. Probably plays an important role in the immune and inflammatory responses.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (12*S*)-hydroperoxy-(5*Z*,8*Z*,10E,14*Z*)-eicosatetraenoate
Polyunsaturated fatty acid lipoxygenase ALOX15 (Pig)	It catalyzes the stereo-specific peroxidation of free and esterified polyunsaturated fatty acids generating a spectrum of bioactive lipid mediators. It converts arachidonic acid into 12-hydroperoxyeicosatetraenoic acid/12-HPETE and 15-hydroperoxyeicosatetraenoic acid/15-HPETE. Also converts linoleic acid to 13-hydroperoxyoctadecadienoic acid. May also act on (12S)-hydroperoxyeicosatetraenoic acid/(12S)-HPETE to produce hepoxilin A3. Probably plays an important role in the immune and inflammatory responses.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (12*S*)-hydroperoxy-(5*Z*,8*Z*,10E,14*Z*)-eicosatetraenoate
Linoleate 9S-lipoxygenase-4 (Soybean)	Plant lipoxygenase may be involved in a number of diverse aspects of plant physiology including growth and development, pest resistance, and senescence or responses to wounding. It catalyzes the hydroperoxidation of lipids containing a cis,cis-1,4-pentadiene structure.	(9*Z*,12*Z*)-octadecadienoate + O_2 = (9*S*)-hydroperoxy-(10*E*,12*Z*)-octadecadienoate
Polyunsaturated fatty acid lipoxygenase ALOX15B (Rat)	It catalyzes the stereo-specific peroxidation of free and esterified polyunsaturated fatty acids generating a spectrum of bioactive lipid mediators. It converts arachidonic acid to 15S-hydroperoxyeicosatetraenoic acid/(15S)-HPETE. Also acts on linoleic acid to produce 13-hydroxyoctadecadienoic acid/13-	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (15*S*)-hydroperoxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoate

	HPODE.	
Polyunsaturated fatty acid lipoxygenase ALOX15 (Bovine)	It catalyzes the stereo-specific peroxidation of free and esterified polyunsaturated fatty acids generating a spectrum of bioactive lipid mediators. It converts arachidonic acid into 12-hydroperoxyeicosatetraenoic acid/12-HPETE and 15-hydroperoxyeicosatetraenoic acid/15-HPETE. Also converts linoleic acid to 13-hydroperoxyoctadecadienoic acid. May also act on (12S)-hydroperoxyeicosatetraenoic acid/(12S)-HPETE to produce hepoxilin A3. Probably plays an important role in the immune and inflammatory responses.	(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate + O_2 = (12*S*)-hydroperoxy-(5*Z*,8*Z*,10E,14*Z*)-eicosatetraenoate
Hydroperoxide isomerase ALOXE3 (Mouse)	Non-heme iron-containing lipoxygenase which is atypical in that it displays a prominent hydroperoxide isomerase activity and a reduced lipoxygenases activity. The hydroperoxide isomerase activity catalyzes the isomerization of hydroperoxides, derived from arachidonic and linoleic acid by ALOX12B, into hepoxilin-type epoxyalcohols and ketones. In presence of oxygen, oxygenates polyunsaturated fatty acids, including arachidonic acid, to produce fatty acid hydroperoxides.	a hydroperoxyeicosatetraenoate = a hydroxy-epoxy-eicosatetraenoate

Table 3. Protein characteristics of lipoxygenases analyzed.

Proteins	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Number of amino acids	674	701	673	701	663	839	857	662	674	701	673	663	676	677	662	663
Molecular Weight (Da) x 10^3	78.0	80.4	78.1	80.6	75.7	94.4	96.8	74.8	78.0	80.7	77.9	75.3	75.9	76.2	75.5	75.4
Theoretical pI	5.51	7.57	5.85	6.25	5.82	5.96	6.26	6.14	5.78	6.43	5.81	6.15	5.73	6.25	8.31	5.79
Number of negatively charged residues (Asp + Glu)	92	75	91	77	82	101	106	80	89	76	89	75	73	75	78	79
Number of positively charged residues (Arg + Lys)	74	76	77	69	69	86	96	72	74	70	75	68	58	66	82	65
Aliphatic index	87.06	89.17	85.75	86.66	88.60	89.73	89.42	94.70	86.93	85.82	86.92	93.95	90.47	89.62	95.53	90.75
Grand average of hydropathicity (GRAVY):	-0.278	-0.197	-0.337	-0.223	-0.268	-0.309	-0.337	-0.166	-0.281	-0.238	-0.285	-0.126	-0.173	-0.192	-0.210	-0.229
Active site residues	H_{368} H_{373} H_{551} N_{555} I_{674}	H_{398} H_{403} H_{578} N_{582} I_{701}	H_{367} H_{372} H_{550} N_{554} I_{673}	H_{398} H_{403} H_{578} N_{582} I_{701}	H_{360} H_{365} H_{540} N_{544} I_{663}	H_{499} H_{504} H_{690} N_{694} I_{839}	H_{518} H_{523} H_{709} N_{713} I_{857}	H_{360} H_{365} H_{540} I_{662}	H_{368} H_{373} H_{551} N_{555} I_{674}	H_{398} H_{403} H_{578} N_{582} I_{701}	H_{367} H_{372} H_{550} N_{554} I_{673}	H_{361} H_{366} H_{541} H_{545} I_{663}	H_{373} H_{378} H_{553} I_{676}	H_{374} H_{379} H_{554} I_{677}	H_{360} H_{365} H_{540} I_{662}	H_{360} H_{365} H_{540} N_{544} I_{663}

(TABLE CONTINUED)

Proteins	17	18	19	20	21	22	23	24	25	26
Number of amino acids	711	864	865	663	663	663	853	677	663	711
Molecular Weight (Da) x 10^3	80.5	96.8	97.1	75.4	75.4	75.0	96.5	76.1	75.1	80.5
Theoretical pI	6.53	5.78	6.27	5.76	6.15	5.86	5.71	5.86	6.09	6.35
Number of negatively charged residues (Asp + Glu)	69	107	102	79	77	80	115	78	81	72
Number of positively charged residues (Arg + Lys)	65	92	93	67	70	67	99	64	72	66
Aliphatic index	84.92	89.59	90.05	87.93	87.06	93.68	86.89	89.48	93.24	87.26
Grand average of hydropathicity (GRAVY):	-0.188	-0.280	-0.293	-0.211	-0.188	-0.153	-0.361	-0.170	-0.190	-0.148
Active site residues	H_{408} H_{413} H_{588} N_{592} I_{711}	H_{525} H_{530} H_{716} N_{720} I_{864}	H_{527} H_{532} H_{718} N_{722} I_{865}	H_{361} H_{366} H_{541} I_{663}	H_{361} H_{366} H_{541} I_{663}	H_{361} H_{366} H_{541} I_{663}	H_{513} H_{518} H_{704} N_{708} I_{853}	H_{374} H_{379} H_{554} I_{677}	H_{361} H_{366} H_{541} I_{663}	H_{408} H_{413} H_{588} N_{592} I_{711}

Table 4. The amino acid composition of lipoxygenases analyzed.

Protein	1		2		3		4		5		6		7		8		9	
Amino acids (aa)	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%
Ala (A)	43	6.4	44	6.3	40	5.9	40	5.7	52	7.8	56	6.7	49	5.7	46	6.9	43	6.4
Arg (R)	36	5.3	46	6.6	34	5.1	44	6.3	42	6.3	37	4.4	46	5.4	40	6.0	32	4.7
Asn (N)	26	3.9	23	3.3	26	3.9	29	4.1	18	2.7	40	4.8	41	4.8	16	2.4	25	3.7
Asp (D)	44	6.5	33	4.7	43	6.4	37	5.3	38	5.7	46	5.5	50	5.8	36	5.4	42	6.2
Cys (C)	13	1.9	16	2.3	13	1.9	20	2.9	17	2.6	4	0.5	7	0.8	13	2.0	12	1.8
Gln (Q)	31	4.6	22	3.1	30	4.5	27	3.9	40	6	27	3.2	25	2.9	33	5.0	30	4.5
Glu (E)	48	7.1	42	6	48	7.1	40	5.7	44	6.6	55	6.6	56	6.5	44	6.6	47	7.0
Gly (G)	33	4.9	43	6.1	33	4.9	41	5.8	39	5.9	53	6.3	61	7.1	49	7.4	33	4.9
His (H)	17	2.5	22	3.1	20	3	21	3	18	2.7	25	3	26	3	17	2.6	20	3.0
Ile (I)	44	6.5	43	6.1	46	6.8	43	6.1	29	4.4	54	6.4	50	5.8	26	3.9	46	6.8
Leu (L)	59	8.8	77	11	59	8.8	75	10.7	83	12.5	86	10.3	96	11.2	88	13.3	59	8.8
Lys (K)	38	5.6	30	4.3	43	6.4	25	3.6	27	4.1	49	5.8	50	5.8	32	4.8	42	6.2
Met (M)	16	2.4	14	2	14	2.1	17	2.4	18	2.7	15	1.8	12	1.4	16	2.4	15	2.2
Phe (F)	35	5.2	33	4.7	34	5.1	33	4.7	26	3.9	30	3.6	37	4.3	26	3.9	35	5.2
Pro (P)	34	5.0	50	7.1	32	4.8	49	7	43	6.5	51	6.1	51	6	41	6.2	33	4.9
Ser (S)	33	4.9	34	4.9	34	5.1	34	4.9	29	4.4	62	7.4	56	6.5	32	4.8	36	5.3
Thr (T)	30	4.5	41	5.8	33	4.9	40	5.7	30	4.5	46	5.5	47	5.5	26	3.9	31	4.6
Trp (W)	15	2.2	13	1.9	14	2.1	13	1.9	19	2.9	14	1.7	15	1.8	17	2.6	15	2.2
Tyr (Y)	30	4.5	36	5.1	33	4.9	36	5.1	17	2.6	37	4.4	31	3.6	17	2.6	32	4.7
Val (V)	49	7.3	39	5.6	44	6.5	37	5.3	34	5.1	52	6.2	51	6	47	7.1	46	6.8
Pyl (O)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sec (U)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(TABLE CONTINUED)

Protein	10		11		12		13		14		15		16		17		18	
Amino acids (aa)	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%	Number of aa	%
Ala (A)	39	5.6	46	6.8	41	6.2	49	7.2	49	7.2	34	5.1	46	6.9	46	6.5	50	5.8
Arg (R)	43	6.1	34	5.1	36	5.4	35	5.2	37	5.5	46	6.9	39	5.9	40	5.6	41	4.7
Asn (N)	32	4.6	26	3.9	19	2.9	22	3.3	18	2.7	15	2.3	19	2.9	26	3.7	35	4.1
Asp (D)	35	5.0	42	6.2	31	4.7	28	4.1	33	4.9	37	5.6	37	5.6	34	4.8	53	6.1
Cys (C)	20	2.9	13	1.9	15	2.3	11	1.6	10	1.5	12	1.8	14	2.1	24	3.4	2	0.2
Gln (Q)	26	3.7	31	4.6	37	5.6	36	5.3	27	4.0	34	5.1	41	6.2	34	4.8	24	2.8
Glu (E)	41	5.8	47	7.0	44	6.6	45	6.7	42	6.2	41	6.2	42	6.3	35	4.9	54	6.2
Gly (G)	39	5.6	32	4.8	45	6.8	46	6.8	43	6.4	41	6.2	41	6.2	40	5.6	64	7.4
His (H)	22	3.1	19	2.8	15	2.3	19	2.8	24	3.5	14	2.1	19	2.9	18	2.5	19	2.2
Ile (I)	41	5.8	46	6.8	29	4.4	32	4.7	34	5.0	26	3.9	25	3.8	32	4.5	64	7.4
Leu (L)	75	10.7	58	8.6	86	13.0	84	12.4	80	11.8	91	13.7	87	13.1	85	12.0	83	9.6
Lys (K)	27	3.9	41	6.1	32	4.8	23	3.4	29	4.3	36	5.4	26	3.9	25	3.5	51	5.9
Met (M)	18	2.6	15	2.2	17	2.6	11	1.6	14	2.1	17	2.6	18	2.7	16	2.3	10	1.2
Phe (F)	34	4.9	34	5.1	30	4.5	32	4.7	30	4.4	27	4.1	27	4.1	30	4.2	39	4.5
Pro (P)	50	7.1	31	4.6	38	5.7	53	7.8	48	7.1	42	6.3	44	6.6	48	6.8	56	6.5
Ser (S)	33	4.7	35	5.2	39	5.9	41	6.1	51	7.5	39	5.9	29	4.4	47	6.6	59	6.8
Thr (T)	40	5.7	30	4.5	27	4.1	37	5.5	33	4.9	31	4.7	35	5.3	51	7.2	59	6.8
Trp (W)	13	1.9	15	2.2	18	2.7	16	2.4	17	2.5	13	2.0	17	2.6	15	2.1	14	1.6
Tyr (Y)	35	5.0	32	4.8	18	2.7	18	2.7	19	2.8	17	2.6	16	2.4	30	4.2	35	4.1
Val (V)	38	5.4	46	6.8	46	6.9	38	5.6	39	5.8	49	7.4	41	6.2	35	4.9	52	6.0
Pyl (O)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sec (U)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(TABLE CONTINUED)

Protein	19		20		21		22		23		24		25		26	
Amino acids (aa)	**Number of aa**	**%**	**Number of aa**	**%**	**Number of aa**	**%**	**Number of aa**	**%**	**Number of aa**	**%**	**Number of aa**	**%**	**Number of aa**	**%**	**Number of aa**	**%**
Ala (A)	55	6.4	42	6.3	44	6.6	44	6.6	56	6.6	52	7.7	42	6.3	48	6.8
Arg (R)	41	4.7	34	5.1	37	5.6	40	6.0	33	3.9	34	5.0	43	6.5	38	5.3
Asn (N)	41	4.7	24	3.6	23	3.5	18	2.7	37	4.3	17	2.5	17	2.6	25	3.5
Asp (D)	48	5.5	37	5.6	36	5.4	35	5.3	54	6.3	36	5.3	38	5.7	36	5.1
Cys (C)	5	0.6	16	2.4	18	2.7	14	2.1	3	0.4	10	1.5	14	2.1	27	3.8
Gln (Q)	28	3.2	34	5.1	31	4.7	31	4.7	24	2.8	29	4.3	30	4.5	32	4.5
Glu (E)	54	6.2	42	6.3	41	6.2	45	6.8	61	7.2	42	6.2	43	6.5	36	5.1
Gly (G)	61	7.1	40	6.0	40	6.0	46	6.9	61	7.2	41	6.1	48	7.2	38	5.3
His (H)	24	2.8	15	2.3	15	2.3	19	2.9	18	2.1	21	3.1	18	2.7	19	2.7
Ile (I)	53	6.1	25	3.8	26	3.9	23	3.5	62	7.3	33	4.9	28	4.2	34	4.8
Leu (L)	88	10.2	81	12.2	78	11.8	93	14.0	78	9.1	80	11.8	90	13.6	86	12.1
Lys (K)	52	6.0	33	5.0	33	5.0	27	4.1	66	7.7	30	4.4	29	4.4	28	3.9
Met (M)	13	1.5	17	2.6	21	3.2	15	2.3	12	1.4	14	2.1	14	2.1	13	1.8
Phe (F)	34	3.9	32	4.8	31	4.7	30	4.5	38	4.5	33	4.9	29	4.4	32	4.5
Pro (P)	51	5.9	39	5.9	39	5.9	38	5.7	49	5.7	51	7.5	38	5.7	49	6.9
Ser (S)	59	6.8	38	5.7	40	6.0	43	6.5	49	5.7	49	7.2	40	6.0	46	6.5
Thr (T)	47	5.4	33	5.0	30	4.5	26	3.9	46	5.4	31	4.6	29	4.4	45	6.3
Trp (W)	14	1.6	16	2.4	16	2.4	17	2.6	16	1.9	17	2.5	18	2.7	14	2.0
Tyr (Y)	37	4.3	21	3.2	20	3.0	16	2.4	42	4.9	18	2.7	15	2.3	29	4.1
Val (V)	60	6.9	44	6.6	44	6.6	43	6.5	48	5.6	39	5.8	40	6.0	36	5.1
Pyl (O)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sec (U)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

In order to obtain genetic relationships among LOXs studied, a phylogenetic tree is constructed in Figure 1. According to phylogenetic tree, soybean LOXs are within separate clade compared to mammalian LOXs. The human LOXs are in the same clade with the mouse, rat and golden hamster. The sequence identity between the plant and mammalian LOXs are reported as 21%-27% only while it is 43%-86% for plant LOXs and 39%-93% for mammalian ones (47). The phylogenetic tree shows that soybean LOXs are different compared to mammalian LOXs.

The similarities among sequences of LOXs studied are analyzed by Clustal Omega for multiple alignments. According to these results, the best similarities are observed with the score of 98 between 5-LO (rat) – 5-LO (mouse). Besides, 5-LO (rat), 5-LO (mouse) and 5-LO (golden hamster) can be a better model for 5-LO (human) as the scores of Clustal Omega are observed 93. 12R-LOX (human) can be best represented by 12R-LOX (mouse) and 12R-LOX (rat) with the score of 86. 12S-LOX (human) can be best represented by 12S-LOX (mouse) with the score of 86. 15-LOX (human) can be best represented by 15-LOX (pig or bovine) with the score of 87 (Table 5).

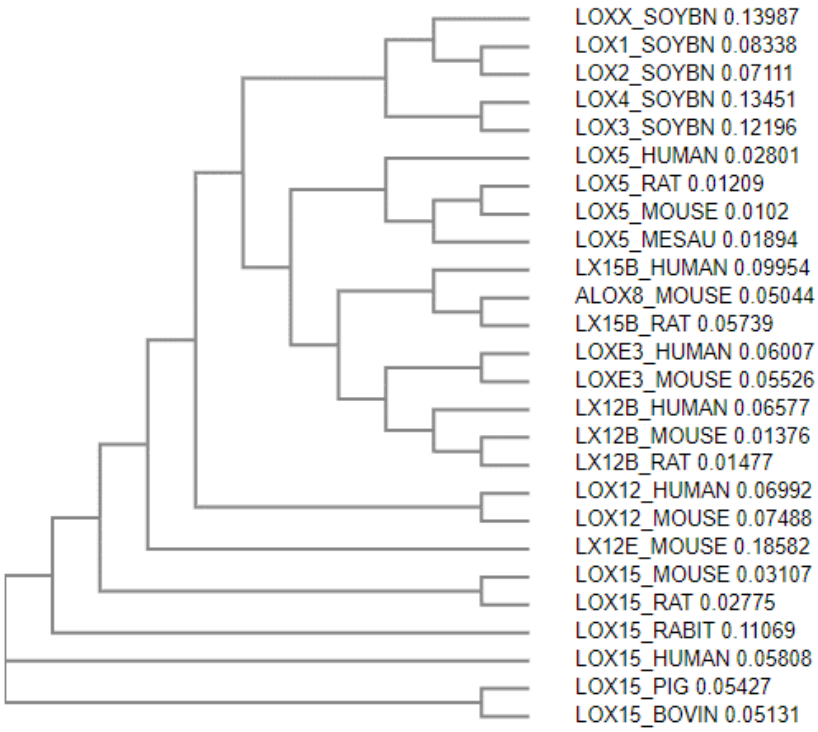

Figure 1. Phylogenetic tree of lipoxygenases examined.

Table 5. The similarity scores of lipoxygenases obtained from Clustal Omega analysis.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	LOXX_SOYBN	100	67.84	71.09	70.3	71.93	26.4	26.4	26.79	25.51	25.04	24.42	25.86	26.44
2	LOX4_SOYBN	67.84	100	74.35	66.51	67.45	24.73	24.73	26.68	23.83	24.14	23.52	24.49	25.39
3	LOX3_SOYBN	71.09	74.35	100	72.25	75.03	26.36	27.75	27.99	25.93	25.78	25.78	26.28	27.17
4	LOX1_SOYBN	70.3	66.51	72.25	100	84.55	24.53	24.69	27.12	25.2	24.57	24.26	25.24	25.98
5	LOX2_SOYBN	71.93	67.45	75.03	84.55	100	26.13	25.82	27.77	26.17	25.23	24.77	25.43	26.64
6	LOX12_HUMAN	26.4	24.73	26.36	24.53	26.13	100	85.52	59.76	57.4	58.61	61.78	65.51	65.71
7	LOX12_MOUSE	26.4	24.73	27.75	24.69	25.82	85.52	100	60.06	58.16	58.16	60.57	63.69	64.35
8	LX12E_MOUSE	26.79	26.68	27.99	27.12	27.77	59.76	60.06	100	59.52	59.67	62.39	66.41	65.86
9	LOX15_MOUSE	25.51	23.83	25.93	25.2	26.17	57.4	58.16	59.52	100	94.12	71.49	73.72	70.74
10	LOX15_RAT	25.04	24.14	25.78	24.57	25.23	58.61	58.16	59.67	94.12	100	71.79	74.77	71.79
11	LOX15_RABIT	24.42	23.52	25.78	24.26	24.77	61.78	60.57	62.39	71.49	71.79	100	81.27	79.34
12	LOX15_HUMAN	25.86	24.49	26.28	25.24	25.43	65.51	63.69	66.41	73.72	74.77	81.27	100	86.56
13	LOX15_PIG	26.44	25.39	27.17	25.98	26.64	65.71	64.35	65.86	70.74	71.79	79.34	86.56	100
14	LOX15_BOVIN	26.28	24.92	27.33	25.51	26.17	65.11	64.35	66.31	72.25	73	78.73	86.86	89.44
15	LOX5_HUMAN	28.91	28.96	29.08	25.97	27.99	42.86	42.69	40.43	41.69	41.69	40.03	41.2	42.19
16	LOX5_RAT	27.37	28.42	27.22	24.54	26.53	40.85	41.01	38.93	40.55	40.24	38.26	40.09	40.85
17	LOX5_MOUSE	27.79	28.53	27.63	24.96	26.95	40.94	41.1	39.02	40.79	40.49	38.36	40.18	40.79
18	LOX5_MESAU	27.68	28.57	27.52	24.69	26.84	41.31	41.16	38.47	40.24	40.09	38.11	39.48	40.7
19	LX15B_HUMAN	27.37	26.19	27.33	25.42	26.07	38.05	38.66	36.28	38.36	38.2	37.75	38.36	38.66
20	ALOX8_MOUSE	27.63	26.91	27.59	25.38	26.19	38.05	38.81	36.89	38.36	38.66	36.99	38.05	38.2
21	LX15B_RAT	26.56	25.54	26.37	24.77	25.88	37.6	37.9	35.82	37.44	37.29	36.07	36.99	37.14
22	LOXE3_HUMAN	27.22	26.71	27.33	25.34	26.41	37.78	37.48	35.71	37.63	37.63	37.78	37.48	37.78
23	LOXE3_MOUSE	27.96	27	27.47	26.23	27.45	38.09	37.48	37.08	38.54	38.09	37.48	37.78	37.63
24	LX12B_HUMAN	25.98	26.05	25.34	23.6	25	38.09	37.18	34.65	35.96	36.12	35.05	35.51	34.6
25	LX12B_MOUSE	25.08	25	24.89	23.3	24.7	37.33	36.57	34.5	35.36	35.36	35.36	35.05	34.6
26	LX12B_RAT	25.08	25.15	24.74	23.3	24.55	37.03	36.42	34.5	35.2	35.36	35.05	34.75	33.99

(TABLE CONTINUE)

		14	15	16	17	18	19	20	21	22	23	24	25	26
1	LOXX_SOYBN	26.28	28.91	27.37	27.79	27.68	27.37	27.63	26.56	27.22	27.96	25.98	25.08	25.08
2	LOX4_SOYBN	24.92	28.96	28.42	28.53	28.57	26.19	26.91	25.54	26.71	27	26.05	25	25.15
3	LOX3_SOYBN	27.33	29.08	27.22	27.63	27.52	27.33	27.59	26.37	27.33	27.47	25.34	24.89	24.74
4	LOX1_SOYBN	25.51	25.97	24.54	24.96	24.69	25.42	25.38	24.77	25.34	26.23	23.6	23.3	23.3
5	LOX2_SOYBN	26.17	27.99	26.53	26.95	26.84	26.07	26.19	25.88	26.41	27.45	25	24.7	24.55
6	LOX12_HUMAN	65.11	42.86	40.85	40.94	41.31	38.05	38.05	37.6	37.78	38.09	38.09	37.33	37.03
7	LOX12_MOUSE	64.35	42.69	41.01	41.1	41.16	38.66	38.81	37.9	37.48	37.48	37.18	36.57	36.42
8	LX12E_MOUSE	66.31	40.43	38.93	39.02	38.47	36.28	36.89	35.82	35.71	37.08	34.65	34.5	34.5
9	LOX15_MOUSE	72.25	41.69	40.55	40.79	40.24	38.36	38.36	37.44	37.63	38.54	35.96	35.36	35.2
10	LOX15_RAT	73	41.69	40.24	40.49	40.09	38.2	38.66	37.29	37.63	38.09	36.12	35.36	35.36
11	LOX15_RABIT	78.73	40.03	38.26	38.36	38.11	37.75	36.99	36.07	37.78	37.48	35.05	35.36	35.05
12	LOX15_HUMAN	86.86	41.2	40.09	40.18	39.48	38.36	38.05	36.99	37.48	37.78	35.51	35.05	34.75
13	LOX15_PIG	89.44	42.19	40.85	40.79	40.7	38.66	38.2	37.14	37.78	37.63	34.6	34.6	33.99
14	LOX15_BOVIN	100	41.69	40.85	40.94	40.24	39.57	38.05	37.6	37.63	37.94	35.51	35.05	34.6
15	LOX5_HUMAN	41.69	100	92.99	93.16	93.31	43.64	44.46	43.8	41.22	42.36	40.07	39.74	39.57
16	LOX5_RAT	40.85	92.99	100	97.77	96.14	42.53	43.59	42.23	40.63	41.83	40.03	40.18	40.03
17	LOX5_MOUSE	40.94	93.16	97.77	100	96.58	42.92	43.52	42.47	41.02	42.22	40.27	40.42	40.27
18	LOX5_MESAU	40.24	93.31	96.14	96.58	100	42.68	43.74	42.53	40.93	42.13	39.73	39.73	39.73
19	LX15B_HUMAN	39.57	43.64	42.53	42.92	42.68	100	78.11	80.77	55.07	54.63	50.9	50.75	51.04
20	ALOX8_MOUSE	38.05	44.46	43.59	43.52	43.74	78.11	100	89.22	53.73	53.58	50.3	49.1	49.4
21	LX15B_RAT	37.6	43.8	42.23	42.47	42.53	80.77	89.22	100	52.09	52.39	50.6	49.7	50.15
22	LOXE3_HUMAN	37.63	41.22	40.63	41.02	40.93	55.07	53.73	52.09	100	88.47	54.92	53.78	53.21
23	LOXE3_MOUSE	37.94	42.36	41.83	42.22	42.13	54.63	53.58	52.39	88.47	100	55.49	54.21	53.64
24	LX12B_HUMAN	35.51	40.07	40.03	40.27	39.73	50.9	50.3	50.6	54.92	55.49	100	86.02	86.59
25	LX12B_MOUSE	35.05	39.74	40.18	40.42	39.73	50.75	49.1	49.7	53.78	54.21	86.02	100	97.15
26	LX12B_RAT	34.6	39.57	40.03	40.27	39.73	51.04	49.4	50.15	53.21	53.64	86.59	97.15	100

The sequence alignments may present the deletion and conserved amino acids residues during the evolution process. According to the results from the sequence alignments, a deletion site which consists of 177 amino acids is observed in 15-LOX (human) compared to soybean LOX-1 (Table 6a). On the other hand, the high similarities are seen between 15-LOX (human) and 15-LOX (rabbit). There is only one amino acid deletion which is localized at 90th position of the 15-LOX (human) compared to 15-LOX (rabbit). There are 535 conserved residues, 70 residues with very similar physical characteristics, 21 residues with similar physical characteristics in LOXs analyzed which is presented in Table 6b. The results presented in Table 7 that there is an important structural similarity between soybean LOX-1 and 15-LOX (human) enzymes and the alpha-helical structure is more common model than β-strand. It is important to note that the enzymes compared have remarkable similarity, although there is quite a difference between the amino acid residues of these sequences. As can be seen from that figure the same structured regions contained 135 conserved amino acid residues. Moreover, all of the amino acid residues replaced in active site have the same secondary structure prediction (Table 7). Amagata et al (48) investigated the inhibitory activities and the selectivity of some compounds isolated from marine sponge on soybean LOX-1 and 15-LOX (human). The different IC_{50} values obtained for these compounds and the variations in selectivity presented that the structures of studied enzymes were different. Prigge et al (49) use the identified molecular structure of soybean LOX-1 as a model in order to develop the possible structures of human 5-, 12-, and 15- LOXs. Although the structures of human enzymes were not identified fully, the molecular models of active sites that are highly conserved of these enzymes were studied by Prigge et al (49). Besides, the differences among sequences were analyzed in details. There has not been any report which identified the 3D structure of 15-LOX (human). The known 3D structure of LOX from mammalians is only reticulocyte-type 15-LOX (rabbit). According to alignments between the sequences of soybean LOX-1, 15-LOX (human) and 15-LOX (rabbit), the 3D structure of 15-LOX (rabbit) can be a better model for 15-LOX (human) than that of soybean LOX-1. The Clustal Omega score was 81 between 15-LOX (human) and 15-LOX (rabbit). These results are well in line with the results of Gillmor et al (50) and Choi et al (51) who reported that the crystal structure of 15-LOX (rabbit) had an important role for understanding the properties of mammalian LOXs.

Table 6a. Sequence alignments between Soybean LOX-1 – 15-LOX (human) ((*) conserved, (:) very similar, (.) somewhat similar physicochemical character).

```
SP|P08170|LOX1_SOYBN  MFSAGHKIKGTVVLMPKNELEVNPDGSAVDNLNAFLGRSVSLQLISATKADAHGKGKVGK 60
SP|P16050|LOX15_HUMAN --------------MGLYRIRVSTGA------SLYAGSNNQVQLW---LVGQHGEAALGK 37
                                    *   .:.*. ..      . : * . .:**     .. **:. :**
SP|P08170|LOX1_SOYBN  DTFLEGINTSLPTLGAGESAFNIH-FEWDGSMGIPGAFYIKNYMQVEFFLKSLTLEAISN 119
SP|P16050|LOX15_HUMAN RLWPA--------RGK-ETELKVEVPEYLGPLLFVKLRKRHLLKDDAWFCNWISVQGPGA 88
                         :          *  *: :::.  *: * : :      :   :  :* : ::::. .
SP|P08170|LOX1_SOYBN  QGTIRFVCNSWVYNTKLYKSVRIFFANHTYVPSETPAPLV-SYREEELKSLRGNGTGERK 178
SP|P16050|LOX15_HUMAN GDEVRFPCYRWVEGNGVLSLPE----GTGRTVGEDPQGLFQKHREEE-----------LE 133
                        . :** *  ** .. : .  .    .   . .* *  *. .:****           :
SP|P08170|LOX1_SOYBN  EYDRIYDYDVYNDLGNPDKSEKLARPVLGGSSTFPYPRRGRTGRGPTVTDPNTEKQGEVF 238
SP|P16050|LOX15_HUMAN ERRKLYRWGNWKDGLI---------LNMAGA-------------------------KLY 158
                       *  ::* :. ::*             :.*:                       :::
SP|P08170|LOX1_SOYBN  YVPRDENLGHLKSKDALEIGTKSLSQIVQPAFESAFDLKSTPIEFHSFQDVHDLYEGGIK 298
SP|P16050|LOX15_HUMAN DLPVDERFLED-------------------------KRVDFEVSLAKG---LADLAIK 188
                        :* **.: .                          * . :*.   :.   * : .**
SP|P08170|LOX1_SOYBN  LPRDVISTIIPLPVIKELYRTDGQHILKFPQPHVVQVSQSAWMTDEEFAREMIAGVNPCV 358
SP|P16050|LOX15_HUMAN DSLNVLTC------WKDL--DDFNRIFWCGQSKLAERVRDSWKEDALFGYQFLNGANPVV 240
                          :*::        *:*   * ::*:   * ::.:  :.:*  *  *. ::: *.** *
SP|P08170|LOX1_SOYBN  IRGLEEFPPKSNLDPAIYGDQSSKITADSLDLDGYTMDEALGSRRLFMLDYHDIFMPYVR 418
SP|P16050|LOX15_HUMAN LRRSAHLPARLVFPPGMEELQAQ-------------LEKELEGGTLFEADFSLLD-GIKA 286
                       :*   .:* :  : *.:   *:.            ::: * .  **  *:  :
SP|P08170|LOX1_SOYBN  QINQLNSAKTYATRTILFLREDGTLKPVAIELSLPHSAGDLSAAVSQVVLPAKEGVESTI 478
SP|P16050|LOX15_HUMAN NVILCSQQHLAAPLVMLKLQPDGKLLPMVIQLQLPRTGSPPPP----LFLPT---DPPMA 339
                       ::   .. :  *  .:* *: **.* *:.*:*.**::..        :.**:
SP|P08170|LOX1_SOYBN  WLLAKAYVIVNDSCYHQLMSHWLNTHAAMEPFVIATHRHLSVLHPIYKLLTPHYRNNMNI 538
SP|P16050|LOX15_HUMAN WLLAKCWVRSSDFQLHELQSHLLRGHLMAEVIVVATMRCLPSIHPIFKLIIPHLRYTLEI 399
                       *****.:*  .*   *:* ** *. *   * :*:** * *  :***:**: ** * .::*
SP|P08170|LOX1_SOYBN  NALARQSLINANGIIETTFLPSKYS-VEMSSAVYKNWVFTDQALPADLIKRGVAIKDPST 597
SP|P16050|LOX15_HUMAN NVRARTGLVSDMGIFDQIMSTGGGGHVQLLKQAGAFLTYSSFCPPDDLADRGL------- 452
                       *. ** .*:.  **::  :  .  . *:: . .    .::. . * ** .**:
SP|P08170|LOX1_SOYBN  PHGVRLLIEDYPYAADGLEIWAAIKTWVQEYVPLYYARDDDVKNDSELQHWWKEAVEKGH 657
SP|P16050|LOX15_HUMAN -----LGVKSSFYAQDALRLWEIIYRYVEGIVSLHYKTDVAVKDDPELQTWCREITEIGL 507
                            * ::.  ** *.*.:*  *  :*:  * *:*  *  **:* *** * :* .* *
SP|P08170|LOX1_SOYBN  GDLKDKPWWPKLQTLEDLVEVCLIIIWIASALHAAVNFGQYPYGGLIMNRPTASRRLLPE 717
SP|P16050|LOX15_HUMAN QGAQDRGFPVSLQARDQVCHFVTMCIFTCTGQHASVHLGQLDWYSWVPNAPCTMRLPPPT 567
                        . :*: :  .**: ::: ..  : *: .:. **:*::**  : . : * * : *   *
SP|P08170|LOX1_SOYBN  KGTPEYEEMINNHEKAYLRTITSKLPTLISLS----VIEILSTHASDEVYLGQRDNPHWT 773
SP|P16050|LOX15_HUMAN TKDATLE-----------TVMATLPNFHQASLQMSITWQLGRRQPVMVAVGQHEEE-YF 614
                       .    *           *: :.**.: . *    :   *. :    * :**:::  :
SP|P08170|LOX1_SOYBN  SDSKALQAFQKFGNKLKEIEEKLVRRNNDPSLQGNRLGPVQLPYTLLYPSSEEGLTFRGI 833
SP|P16050|LOX15_HUMAN SGPEPKAVLKKFREELAALDKEIEIRN----------AKLDMPYEYLRPSVVENSV---- 660
                       *. :   .::** ::*  :::::  **          . :::**  * **  *. .
SP|P08170|LOX1_SOYBN  PNSISI 839
SP|P16050|LOX15_HUMAN ----AI 662
                          :*
```

Table 6b. Sequence alignments between 15-LOX (rabbit) – 15-LOX (human) ((*) conserved, (:) very similar, (.) somewhat similar physicochemical character).

```
SP|P16050|LOX15_HUMAN MGLYRIRVSTGASLYAGSNNQVQLWLVGQHGEAALGKRLWPARGKETELKVEVPEYLGPL 60
SP|P12530|LOX15_RABIT MGVYRVCVSTGASIYAGSKNKVELWLVGQHGEVELGSCLRPTRNKEEEFKVNVSKYLGSL 60
                      **:**: ******:****:*:*:*********. **. * *:*.** *:**:* :*** *
SP|P16050|LOX15_HUMAN LFVKLRKRHLLKDDAWFCNWISVQGPGAG-DEVRFPCYRWVEGNGVLSLPEGTGRTVGED 119
SP|P12530|LOX15_RABIT LFVRLRKKHFLKEDAWFCNWISVQALGAAEDKYWFPCYRWVVGDGVQSLPVGTGCTTVGD 120
                      ***:***:*:**:**********. **. *:  ******* *:** *** *** *.  *
SP|P16050|LOX15_HUMAN PQGLFQKHREEELEERRKLYRWGNWKDGLILNMAGAKLYDLPVDERFLEDKRVDFEVSLA 179
SP|P12530|LOX15_RABIT PQGLFQKHREQELEERRKLYQWGSWKEGLILNVAGSKLTDLPVDERFLEDKKIDFEASLA 180
                      **********:*********:**.**:*****:**:** ************::***.***
SP|P16050|LOX15_HUMAN KGLADLAIKDSLNVLTCWKDLDDFNRIFWCGQSKLAERVRDSWKEDALFGYQFLNGANPV 239
SP|P12530|LOX15_RABIT WGLAELALKNSLNILAPWKTLDDFNRIFWCGRSKLARRVRDSWQEDSLFGYQFLNGANPM 240
                       ***:**:*:***:*: ** ***********:****.******:**:************:
SP|P16050|LOX15_HUMAN VLRRSAHLPARLVFPPGMEELQAQLEKELEGGTLFEADFSLLDGIKANVILCSQQHLAAP 299
SP|P12530|LOX15_RABIT LLRRSVQLPARLVFPPGMEELQAQLEKELKAGTLFEADFALLDNIKANVILYCQQYLAAP 300
                      :****.:************************:.*********:***.******* .**:****
SP|P16050|LOX15_HUMAN LVMLKLQPDGKLLPMVIQLQLPRTGSPPPPLFLPTDPPMAWLLAKCWVRSSDFQLHELQS 359
SP|P12530|LOX15_RABIT LVMLKLQPDGKLMPMVIQLHLPKIGSSPPPLFLPTDPPMVWLLAKCWVRSSDFQVHELNS 360
                      ************:******:**: ** ************.**************:***:*
SP|P16050|LOX15_HUMAN HLLRGHLMAEVIVVATMRCLPSIHPIFKLIIPHLRYTLEINVRARTGLVSDMGIFDQIMS 419
SP|P12530|LOX15_RABIT HLLRGHLMAEVFTVATMRCLPSIHPVFKLIVPHLRYTLEINVRARNGLVSDFGIFDQIMS 420
                      ************:.************:****:**************.*****:********
SP|P16050|LOX15_HUMAN TGGGGHVQLLKQAGAFLTYSSFCPPDDLADRGLLGVKSSFYAQDALRLWEIIYRYVEGIV 479
SP|P12530|LOX15_RABIT TGGGGHVQLLQQAGAFLTYRSFCPPDDLADRGLLGVESSFYAQDALRLWEIISRYVQGIM 480
                      **********:******** ***************:*************** ***:**:
SP|P16050|LOX15_HUMAN SLHYKTDVAVKDDPELQTWCREITEIGLQGAQDRGFPVSLQARDQVCHFVTMCIFTCTGQ 539
SP|P12530|LOX15_RABIT GLYYKTDEAVRDDLELQSWCREITEIGLQGAQKQGFPTSLQSVAQACHFVTMCIFTCTGQ 540
                      .*:**** **:** ***:**************.:***.***:  *.**************
SP|P16050|LOX15_HUMAN HASVHLGQLDWYSWVPNAPCTMRLPPPTTKDATLETVMATLPNFHQASLQMSITWQLGRR 599
SP|P12530|LOX15_RABIT HSSIHLGQLDWFTWVPNAPCTMRLPPPTTKDATLETVMATLPNLHQSSLQMSIVWQLGRD 600
                      *:*:*******::*********************************:**:******.*****
SP|P16050|LOX15_HUMAN QPVMVAVGQHEEEYFSGPEPKAVLKKFREELAALDKEIEIRNAKLDMPYEYLRPSVVENS 659
SP|P12530|LOX15_RABIT QPIMVPLGQHQEEYFSGPEPRAVLEKFREELAIMDKEIEVRNEKLDIPYEYLRPSIVENS 660
                      **:** :***:*********:***:******* :*****:** ***:********:****
SP|P16050|LOX15_HUMAN VAI 662
SP|P12530|LOX15_RABIT VAI 663
                      ***
```

Table 7. Sequence alignments and secondary structure predictions for Soybean LOX-1 and 15-LOX (human). Residues estimated to be in coil conformation shown in yellow; residues estimated to be in β-sheet conformation shown in blue and residues estimated to be in alpha-helical conformation shown in green.

```
SP|P16050|LOX15_HUMAN ----------MGLYRIRVSTGA------SLYAGSNNQVQLW---LVGQHGEAALGK 37
SP|P08170|LOX1_SOYBN  MFSAGHKIKGTVVLMPKNELEVNPDGSAVDNLNAFLGRSVSLQLISATKADAHGKGKVGK 60
                                *   .:.*. ..      . : * . .:**      .. **:. :**
SP|P16050|LOX15_HUMAN RLWPA--------RGK-ETELKVEVPEYLGPLLFVKLRKRHLLKDDAWFCNWISVQGPGA 88
SP|P08170|LOX1_SOYBN  DTFLEGINTSLPTLGAGESAFNIH-FEWDGSMGIPGAFYIKNYMQVEFFLKSLTLEAISN 119
                        :          *  *: :::.  *: * : :      :   :  :* : :::::. .
SP|P16050|LOX15_HUMAN GDEVRFPCYRWVEGNGVLSLPE----GTGRTVGEDPQGLFQKHREEE----------LE 133
SP|P08170|LOX1_SOYBN  QGTIRFVCNSWVYNTKLYKSVRIFFANHTYVPSETPAPLV-SYREEELKSLRGNGTGERK 178
                       . :** *  ** .. : .  .    .  . .* *  *. .:****           :
SP|P16050|LOX15_HUMAN ERRKLYRWGNWKDGLI--------LNMAGA------------------------KLY 158
SP|P08170|LOX1_SOYBN  EYDRIYDYDVYNDLGNPDKSEKLARPVLGGSSTFPYPRRGRTGRGPTVTDPNTEKQGEVF 238
                      *  ::* :. ::*             :.*:                       :::
SP|P16050|LOX15_HUMAN DLPVDERFLED-------------------------KRVDFEVSLAKG---LADLAIK 188
SP|P08170|LOX1_SOYBN  YVPRDENLGHLKSKDALEIGTKSLSQIVQPAFESAFDLKSTPIEFHSFQDVHDLYEGGIK 298
                       :* **.: .                          * . :*.   :.   * : .**
SP|P16050|LOX15_HUMAN DSLNVLTC------WKDL--DDFNRIFWCGQSKLAERVRDSWKEDALFGYQFLNGANPVV 240
SP|P08170|LOX1_SOYBN  LPRDVISTIIPLPVIKELYRTDGQHILKFPQPHVVQVSQSAWMTDEEFAREMIAGVNPCV 358
                         :*::        *:*   * ::*:   * ::.:  :.:*  *  *. ::: *.** *
SP|P16050|LOX15_HUMAN LRRSAHLPARLVFPPGMEELQAQ------------LEKELEGGTLFEADFSLLD-GIKA 286
SP|P08170|LOX1_SOYBN  IRGLEEFPPKSNLDPAIYGDQSSKITADSLDLDGYTMDEALGSRRLFMLDYHDIFMPYVR 418
                      :*   .:* :  : *.:   *:.              ::: * .   **  *:  :
SP|P16050|LOX15_HUMAN NVILCSQQHLAAPLVMLKLQPDGKLLPMVIQLQLPRTGSPPPP----LFLPT---DPPMA 339
SP|P08170|LOX1_SOYBN  QINQLNSAKTYATRTILFLREDGTLKPVAIELSLPHSAGDLSAAVSQVVLPAKEGVESTI 478
                      ::   .. :  *  .:* *: **.* *:.*:*.**::..        :.**:
SP|P16050|LOX15_HUMAN WLLAKCWVRSSDFQLHELQSHLLRGHLMAEVIVVATMRCLPSIHPIFKLIIPHLRYTLEI 399
SP|P08170|LOX1_SOYBN  WLLAKAYVIVNDSCYHQLMSHWLNTHAAMEPFVIATHRHLSVLHPIYKLLTPHYRNNMNI 538
                      *****.:*  .*   *:* ** *. *   * :*:** * *  :***:**: ** *  .::*
SP|P16050|LOX15_HUMAN NVRARTGLVSDMGIFDQIMSTGGGGHVQLLKQAGAFLTYSSFCPPDDLADRGL------- 452
SP|P08170|LOX1_SOYBN  NALARQSLINANGIIETTFLPSKYS-VEMSSAVYKNWVFTDQALPADLIKRGVAIKDPST 597
                      *. ** .*:.  **::  :  .  . *:: . .    .::. . * ** .**:
SP|P16050|LOX15_HUMAN -----LGVKSSFYAQDALRLWEIIYRYVEGIVSLHYKTDVAVKDDPELQTWCREITEIGL 507
SP|P08170|LOX1_SOYBN  PHGVRLLIEDYPYAADGLEIWAAIKTWVQEYVPLYYARDDDVKNDSELQHWWKEAVEKGH 657
                         * ::.  ** *.*.:*  *  :*:  * *:*  *  **:* *** * :*  .* *
SP|P16050|LOX15_HUMAN QGAQDRGFPVSLQARDQVCHFVTMCIFTCTGQHASVHLGQLDWYSWVPNAPCTMRLPPPT 567
SP|P08170|LOX1_SOYBN  GDLKDKPWWPKLQTLEDLVEVCLIIIWIASALHAAVNFGQYPYGGLIMNRPTASRRLLPE 717
                       . :*: :  .**: ::: ..  : *: .:. **:*::**  : . : * * : *   *
SP|P16050|LOX15_HUMAN TKDATLE-----------TVMATLPNFHQASLQMSITWQLGRRQPVMVAVGQHEEE-YF 614
SP|P08170|LOX1_SOYBN  KGTPEYEEMINNHEKAYLRTITSKLPTLISLS----VIEILSTHASDEVYLGQRDNPHWT 773
                      .     *           *: :.**.: . *    :   *. :    * :**:::  :
SP|P16050|LOX15_HUMAN SGPEPKAVLKKFREELAALDKEIEIRN----------AKLDMPYEYLRPSVVENSV---- 660
SP|P08170|LOX1_SOYBN  SDSKALQAFQKFGNKLKEIEEKLVRRNNDPSLQGNRLGPVQLPYTLLYPSSEEGLTFRGI 833
                      *. :   .::** ::*  :::::  **          . :::**  * **  *. .
SP|P16050|LOX15_HUMAN ----AI 662
SP|P08170|LOX1_SOYBN  PNSISI 839
                         :*
```

CONCLUSION

Soybean LOX-1 might not be accepted as a model enzyme for 15-LOX from other sources as mentioned by several researchers that soybean LOX-1 used as a model enzyme for 15 LOX (human) (14,16-18,52) since there are remarkable sequence-based differences which are obtained by using bioinformatics tools between soybean LOX-1 and LOXs from other sources especially from human. The future works are strongly warranted by using newly developed bioinformatics tools to find the appropriate enzyme model for human LOX. At this point, various genetic methods can be introduced and developed. The relevant enzymes can be produced in large scale by micro-organisms with the help of recombinant DNA technology for instance. Recently, the use of recombinant human lipoxygenase enzymes has become increasingly common due to such sensitivities in lipoxygenase inhibition studies (35,48,53,54).

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