Legume lipoxygenase: Strategies for application in food industry

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Abstract

Lipoxygenase (LOX) is a widely distributed enzyme in plant and animal cells. It catalyzes the oxidation of polyunsaturated fatty acid into fatty acid hydroperoxides. LOX is also associated with the production of aroma substrates, color changes, and alteration of physico-chemical characteristics. The associated reaction could be either desirable or undesirable in food production. An understanding of LOX characteristics and functional principles is essential for utilizing LOX as a natural food ingredient. Legumes are nutrient-dense food ingredient and also serve as a good source of LOX. This paper is focused on the biological function of LOX in legumes, the history of legume LOX, the application of legume LOX in the food industry, and the inhibition strategies of unwanted LOX-catalyzed reaction.

KEYWORDS
enzyme inhibition, legumes, lipoxygenase

1 | INTRODUCTION

The demand for food is always increasing with demographic growth worldwide. The agricultural world is facing the challenges of maintaining a sustainable food system while producing enough food to fulfill market demand. Moreover, there is a growing population switching from an omnivorous diet into a vegetarian diet owing to reasons such as human health and animal welfare. As a result, the global demand for nutritious plant-based food rises.

Producing novel delicious food products by using legume as ingredients or food additives is a green and remarkable solution. Legumes are inexpensive sources of dietary protein, complex carbohydrates, fibers, vitamins, and minerals (Muzquiz, 2004). Legumes, as food ingredients, can also deliver desirable functional properties for food production, such as water retention and oil-absorbing, emulsifying, foaming, pasting, and gelling properties (Du, Jiang, Yu, & Jane, 2014). Also, the cultivation and the production of legume protein require a smaller amount of energy and water usage than traditional animal protein production. As a result, legumes could serve as an excellent source of plant-based food ingredients, as well as an alternative to animal proteins.

Lipoxygenase (LOX) is an enzyme widely distributed in nature and abundant in legume and other plant cells. LOX is an oxoreductive enzyme catalyzing many oxidative reactions (Song et al., 2016). Aroma compounds produced during the LOX-catalyzed oxidation influence the flavor aspect of the food product in either a desirable way or an undesirable way. By controlling the volatile formation favorably, LOX can function as a natural flavoring agent for food production. Despite aroma production, LOX has other food-related applications, such as bleaching agent for flour and increasing the dough strength.

LOX has many potential usages in the food industry; however, it is also associated with some unwanted reactions, which include color change and nutrient deterioration. By studying and understanding the properties and reaction mechanism of LOX, food researchers could well control and utilize LOX as a natural food additive.

This review paper is mainly focused on the biological function of LOX found in the legumes, the potential application of legume LOX,
the negative implications of LOX, and strategies to inhibit the unwanted LOX-catalyzed reaction in the food industry.

2 | LIPOXYGENASE

LOXs are a group of nonheme iron, nonsulfur iron, or manganese-containing dioxygenase redox enzymes (Aanangi et al., 2016), which are widely distributed in plant and animal cells. LOX can specifically catalyze polyunsaturated fatty acids (PUFAs) and esters with cis- and cis-pentadiene structure, and hydroperoxide derivatives with conjugated double bonds are formed by intramolecular oxygen addition. The substrates of LOX in plants are mainly linoleic acid and linolenic acid. Arachidonic acid is the primary substrate for LOX in animal cells. LOXs are often classified based on their positional specificity for unsaturated fatty acid oxygenation, as shown in Table 1. Linoleic acid is oxygenated at carbon atom 9 or carbon atom 13 in the carbon backbone of the fatty acid, so the corresponding LOXs are 9-LOX and 13-LOX for oxygenation of linoleic acid (Liavonchanka & Feussner, 2006).

LOX catalyzes the regio-specific and stereo-specific deoxygenation of PUFAs, converting (1Z,4Z)-pentadiene hydroperoxy fatty acids (HPO). HPO further degrades into metabolites and produces jasmonic acid (JA), methyl jasmonate (MJ), conjugated dienoic acids, and volatile chemicals. These compounds participate in plant defense activities, including biotic and abiotic stress (Leone, Melillo, & Bleve-Zacheo, 2001).

LOX is especially abundant in legumes, and intensive studies have been done for legume LOX. Some recent studies of LOX extracted from varieties of legume sources are shown in Table 2. Soy LOX is the most widely studied among all other legume species.

2.1 | The structure of LOX

LOX is typically formed by nonheme iron and a large protein structure. Plant LOX is a one-chain monomer enzyme with a molecular weight of 90–110 kDa. It is formed by two different protein domains. N-terminal region is approximately 25–30 kDa and consists of a beta-barrel domain. The function of N-terminal region is unclear. The C-terminal is formed by alpha helix with a molecular weight of 55–65 kDa. The C-terminal of the enzyme contains a catalytic domain. It is reported that dry pea seeds (Pisum sativum var. Telephone L.) LOX has a molecular weight of 93 kDa (Szymanowska, Jakubczyk, Baraniak, & Kur, 2009), and the molecular mass of mung bean LOX is approximately 97 kDa (Aanangi et al., 2016). The molecular weight of soybean LOX is about 94–97 kDa. Soybean LOX is formed by around 853 amino acid residues. The N-terminal has 160 amino acid residues, which are formed by two beta barrels with four antiparallel plates. C-terminal has 693 amino acid residues, which are composed of 23 alpha helices, 2 antiparallel beta plates, and active site Fe3+.

2.2 | Catalytic site of LOX

It is generally believed that the catalytic center of LOX is related to iron. The active state of LOX is high-spin oxidized Fe3+, and the inactive state is high-spin reduced Fe2+ (Figure 1). The catalytic iron in the active site of liquid oxygen is coordinated by five amino acid residues and a water molecule as the sixth ligand. In legume LOX, iron complexes with the carbonyl end of C-terminal isoleucine through three conserved N-atoms of histidine, O-atoms of asparagine, and C-terminal isoleucine.

In soybean LOX-1, the iron ion center contains five endogenous ligands and one exogenous ligand. The endogenous ligand consists of three groups of histidine residues (His499, His504, and His590), one Ile639 residue, and one Asn694. The exogenous ligand is a water molecule.

### TABLE 1 Classification of lipoxygenase

| Cell type | Classification of lipoxygenase |
|-----------|-------------------------------|
| Plant     | 9-LOX, 13-LOX                 |
| Animal    | 5-LOX, 8-LOX, 12-LOX, 13-LOX  |
| Bacteria  | 10-LOX, 9-LOX, 13-LOX, Mini-LOX, 15-LOX |
| Fungi     | Mn-LOX, 15-LOX                |

### TABLE 2 Recent studies of LOX extracted from different legume sources

| Legume     | References                                      |
|------------|-------------------------------------------------|
| Alfalfa seed | Uno, Hara, and Joh (2002)                      |
| Broad bean  | Clemente, Olias, and Olias (2000)               |
| Chickpea   | Kazemi Shahandashi et al. (2013)                |
| Gunes, Pilbeam, Inal, Bagci, and Coban (2007) |
| Lentils    | Vliegenthart, Maccarrone, Zadelhoff, Veldink, and Finazzi Agrò (2000) |
| Lupins     | Stephany, Bader-Mittermaier, Schweiggert-Weizs, and Carle (2015) |
| Mung bean  | Aanangi et al. (2016)                          |
| Chow et al. | (2007)                                          |
| Pea        | Gökmen, Bahçeci, and Acar (2002)                |
| Soy        | Jiang et al. (2018)                            |
| Navicha, Hua, Masamba, Kong, and Zhang (2017) |
| Lakshmi, Madhusudhan, and Raghavaraao (2012) |
| Wang et al. | (2011)                                          |
| Whent, Lv, Luthria, Kenworthy, and Yu (2011) |
| Iassonova et al. (2009) |
| Li, Chen, Chen, and Liu (2008) |
| Wang, Zhou, and Chen (2008) |
| Chedeia, Vicas, and Socaciu (2008) |
| Kumar, Rani, Tindwani, and Jain (2003) |
2.3 | Reaction conditions of LOX

LOXs have structural specificity for their substrate. Fatty acid esters and unsaturated fatty acids with cis- and cis-pentadiene structure can serve as the substrate for LOX. As already mentioned, linoleic acid and linolenic acid serve as natural substrates for LOX in plant cells, and arachidonic acid is the natural substrate for animal cells. Different sources and substrates of LOX lead to different positions of oxygenation and thus different products. LOX-1 is active with a water-soluble substrate (e.g., linoleyl sulfate), and the products of linoleic acid oxidation are hydroperoxide at ω−6 or 13-lipohydroperoxide. Only 13-hydroperoxide is formed by linolenic acid oxidation. The substrates of LOX-2 are esterification substrates, and the products are 9- or 13-hydroperoxide. LOX-2 and LOX-3 show a higher activity with methyl esters of fatty acids and triglycerides than free fatty acids (Murphy, 2008).

The optimum pH values of LOX from different legume species are differently reported in literature. Lupinus LOX has the greatest activity at pH 6.0–8.0. The optimum pH for green pea seed LOX was found to be 5.0–7.0 (Szymanowska et al., 2009). According to Aanangi et al. (2016), mung bean LOX has the highest activity at pH 6.5. Considerable LOX activity was noted within a pH range 4.5–8.0, but mung bean LOX activity was reduced 50% in the pH below 4.5 or above 8.0.

For the same species, the optimum LOX pH could also be slightly different. This is because the protein content and enzyme activity are dependent on climate, cultivation, and storage conditions. The optimum pH values of three soybean LOXs (LOX-1, LOX-2, and LOX-3) are also different. The optimum pH for LOX-2 and LOX-3 is 6.5, whereas the optimum pH value of LOX-1 is 8.0–9.0 (Murphy, 2008). The possible reason for the decrease of enzyme activity at low pH is because the solubility of linoleic acid decreases. Linoleic acid, which is the substrate for LOX, is insoluble in acidic environment. Therefore, in acidic environment, the main factor affecting the reaction rate is the solubility of linoleic acid and its dispersion condition in water. The optimum isoelectric point (pI) for LOX-1, LOX-2, and LOX-3 is 5.68, 6.25, and 6.15, respectively (Murphy, 2008). LOX-2 and LOX-3 are inactivated immediately at 68°C, whereas LOX-1 takes up to 2 min to fully inactivate. At 60°C conditions, it takes 15–30 min for LOX-3 to inactivate and 5 min for LOX-2 to inactivate. LOX-1 is the most studied, and LOX-3 has the highest abundance (Murphy, 2008).

2.4 | LOX-catalyzed reaction mechanism

In the plant cell, the beginning of the LOX-catalyzed reaction is the incorporation of molecular oxygen into C18 PUFAs, in either the 9- or 13-position of the carbon chain. This reaction utilizes linoleic (18:2) and linolenic (18:3) acids as substrates, and it is mediated by region-specific 9- or 13-LOXs (Nemchenko, Kunze, Feussner, & Kolomiets, 2006).

In terms of catalytic reaction mechanism, the oxidation of linoleic acid catalyzed by LOX is different from the automatic oxidation of linoleic acid. First of all, linoleic acid oxygenates LOX to form a complex; then on the surface of the enzyme, it forms a double-free activator, that is, a hydrogen ion and an electric ion transfer from linoleic acid to oxygen molecule; the double-free radicals combine on the surface of the enzyme molecule to form linoleic acid hydrogen peroxyde; the hydrogen peroxyde is separated from the enzyme and falls off.

As shown in Figure 2, LOX-catalyzed reaction is believed to happen in four steps. (1) The free radical theory believes that hydrogen atoms leave the substrate, and iron ions are reduced at the same time; (2) molecular oxygen reacts with the substrate-free radicals to form peroxy free radicals, which may be accompanied by the transformation of oxygen into oxygen-free radicals; and (4) finally, peroxy free radicals are reduced by Fe2+ in LOX to form hydrogen peroxyde, whereas iron is oxidized to Fe3+, which now becomes an enzyme active form of LOX (Murphy, 2008).

In recent years, study of proton-electron transfer reaction in soybean LOX confirmed the theory of hydrogen transfer in the process of LOX reaction. It is believed that hydrogen atoms are transferred from linoleic acid to iron ions, proton, and electron transfer simultaneously between donor and acceptor, thus producing an effective hydrogen tunneling effect. Therefore, it can be seen from the above theories that the catalytic center of LOX is closely related to iron ions.

According to a previous study, LOX isolated to the plant has the highest activity towards linoleic acid. It has lower activities for arachidonic acid, linolenic acid, and methyl linolate. No activity was
detected for oleic acid. As a result, linoleic acid is always used as a substrate for LOX activity analysis (Szymanowska et al., 2009).

3 | THE HISTORY OF LOX

LOX is widely distributed in the plant and animal cells, and it has especially high activity in legume cells. Bohn and Haas (1928) first discovered the existence of an enzyme catalyzing the oxidation destruction of carotene. They noticed that the addition of small quantities of soy flour into the wheat dough generated a bleaching effect on the wheat flour. Because they believe that the color loss is due to the oxidation of carotene, they named the enzyme carotene oxidase (Hayward, Cilliers, & Swart, 2016). In 1932, Audre and Hou found that soybean contains a "lipoxidase" enzyme, which catalyzes the peroxidation of various unsaturated fatty acids. They realized that the unpleasant aroma in soybean is associated with this enzyme. Craig (1936) recognized that Lupinus albus consumed a more significant amount of oxygen than CO2 in the presence of oxygen, so he named the enzyme "fat oxidase." In 1940, Sumner (1940) and Tauber (1940) recognized that both reactions were catalyzed by the same kind of enzymes. After that, the names of carotene oxidase, unsaturated lipid oxidase, and lipid oxidase were collectively referred to as LOX. In 1946, Theorell, Holman, and Akeson (1947) first extracted LOX from soybean. In 1947, Theorell et al. successfully crystallized LOX. The second LOX (LOX-2) and the third isomers (LOX-3) along with an isozyme designated as "LOX-b" were purified in 1970–1972 (Christopher, Pistorius, & Axelrod, 1970, 1972; Yamamoto, Yasumoto, & Mitsuda, 1970). In 1972, Chen reported that each mole of soybean LOX contains 1 mol of iron. Soy contains a higher amount of LOX compare to other plants, which is 1–2% of total soy protein. As a result, soy LOX has been well studied.

4 | THE BIOLOGICAL FUNCTION OF LOX IN LEGUME

LOX gene expression happens throughout the whole lifespan of legumes. It exists in all stages of plant growth and development, including seed germination, stem formation, nodule development, fruit maturation, and plant senescence. LOX has a crucial role in the biological function as it synthesizes prostaglandins and jasmonates. LOX usually is present in the plant seed and plays a vital role in seed germination. During the seed germination, LOX may function as a storage protein and aids the lipid bodies to degrade in the seed. No adverse effect was detected on soybean line lacking LOX isoenzymes, which proves that LOX could function as a storage protein. Moreover, germination seedling of mung bean contains high levels of LOX activity. As a result, mung bean and its seedlings have been utilized as the sources of hydroperoxide lyase (HPL) enzyme.

Plants face many environmental stress factors in a natural growth environment, such as extreme temperature, ultraviolet radiation, water and oxygen shortage, insect, and pathogen infection. All these circumstances can induce the expression of one or more LOX genes. LOX protects pea roots from biotic stress. Leone et al. (2001) found that LOX is involved in defense of host–parasites. The LOX activity is elevated in the plant tissues undergoing necrosis. LOX is considered to be involved in the defense of host–parasite interactions. Cyst nematode (Heterodera goettingiana) is the primary pest that infects pea crop. Sources of resistance to the disease have been found in pea germplasm resources, but the mechanism of host resistance remains unclear (Leone et al., 2001).

High levels of LOX activity have been found in healthy, young, and rapidly growing plant tissues, and the LOX enzyme is usually located in the outer region of the organs, where it could be serve as a protection against the pathogen attack (War et al., 2012).

Foreign chemical inducers, such as chitin, salicylic acid, and potassium jasmonate, can also induce LOX gene expression. These inducers are mostly components of pathogens or components of LOX metabolic pathway (Ruan et al., 2019; Wasternack & Hause, 2013).

It has been thought that lipid peroxidation is a harmful process, which can destroy the structure of biofilm and lead to cell dysfunction.
Nita & Grzybowski, 2016). Lipid oxidation has dual effects on organisms. On the one hand, the existence of peroxyl groups disturbs the interaction between hydrophobic lipids and lipids and proteins, leading to changes in the structure of biofilms and proteins (Kurutas, 2016); at the same time, peroxyl lipids are prone to produce free radicals, which can induce modification of other biofilms and lipid proteins (Dröge, 2002; Gaschler & Stockwell, 2017). When lipid bilayer of biofilm is oxidized, it loses its barrier function, which makes the subcellular structure, and even the whole cell, unstable. On the other hand, when the oxidation of lipid is regulated in a certain way and restricted to a specific cell space, it may have a substantial impact on cells and even the whole organism. Lipid oxidation, for example, promotes the synthesis of eicosanoids, which are involved in cell differentiation, maturation, and lipid mobilization (Dennis & Norris, 2015).

Fatty acid peroxides produced by LOX reactions are transformed into different compounds through at least six pathways under the action of enzymes, collectively known as oxylipins. Plant oxylipins have negative effects on organisms. A previous study showed that oxylipins are effective biological regulators, which play an essential role in signal transduction, plant growth, development, senescence, organ formation, and homeostasis. Enzymes involved in metabolism include hydrogen oxidase, propylene oxide synthase, diethylene ether synthase, peroxidase (POD), and reductase (Porta & Rocha-Sosa, 2002).

5 | LOX EXTRACTION METHOD

The extraction methods of LOX mainly include water extraction, salt-induced precipitation, and coprecipitation. The LOX extraction methods used in recent studies are shown in Table 3. For either of the methods discussed in Table 3, generally, a higher cost and longer time are needed for higher extraction yield, albeit it might not always be necessary to achieve the highest extraction yield. The extraction method should be decided based on the end-purpose of the experiment.

5.1 | Water extraction

Crude LOX enzyme solution can be generated most easily by water (aqueous) extraction. Soybean flour was immersed in water, stirred for a certain time, and centrifuged to obtain the crude lipid oxygenase solution. The protein dissolution rate of water extraction method can reach up to 85%. However, the obtained enzyme solution contains impure proteins. This method is simple and reduces the damage of enzyme activity during operation, so the crude enzyme solution has relatively high enzyme activity (Szymanowska et al., 2009; Borhan, 1979).

5.2 | Salt-induced precipitation

Salting-out method is a traditional method, which is still widely used at present. Separation and passivation are carried out according to the difference of solubility between enzymes and heteroproteins in high concentration salt solution. The principle is that initially the solubility of the enzyme increases with the increase of salt concentration. When the salt concentration increases to a certain value, the solubility of the enzyme decreases with the increase of salt concentration, resulting in the precipitation of the enzyme. Often, a secondary salting-out method is used in order to remove impurity proteins with lower salt concentration and then to separate and purify them with higher salt concentration.

The most commonly used neutral salt is ammonium sulfate. This is because ammonium sulfate has a high solubility in water and it does not affect the activity of enzymes. It has a good separation effect and is cheap and easy to obtain. Salt-induced precipitation method is simple, safe, and reproducible. It has been widely used in recent studies. The purity of LOX extracted by salt-induced precipitation is higher than that by water extraction.

5.3 | Coprecipitation

Coprecipitation uses ionic surfactants such as sodium dodecyl sulfate (SDS) and nonionic polymers such as polyethylene glycol (PEG) to form complexes directly or indirectly with proteins under certain conditions to precipitate proteins. These are further purified by dissolving the required enzymes by appropriate methods to remove impurity proteins and precipitators. PEG is nontoxic, heat dissipation is low when dissolved, and the equilibrium time of precipitation is short. Generally, when the concentration of PEG reaches 30%, most of the protein can be precipitated. The preparation of LOX by precipitation
method includes organic solvent precipitation method and polymer precipitation method.

5.4 | Sustainability in LOX extraction

A major focus of recent studies has been towards finding sustainable LOX extraction methods. The purity of LOX obtained by the above methods is generally low and needs further purification. With the use of soybean as raw material and PEG coprecipitation to produce LOX, the steps are as follows: crushing soybean, adding water and adjusting pH value with hydrochloric acid, stirring and filtering, adding PEG into filtrate, continuous stirring, centrifugal separation, dissolving precipitation with salt buffer solution, and filtering insoluble matter to obtain enzyme solution. Further separation and purification methods include salting-out, organic solvent precipitation, polymer precipitation, bidirectional separation, dialysis, ion-exchange chromatography, gel filtration, and various electrophoresis techniques. Further, scale-up and large-scale commercialization of these methods are difficult owing to the multistep process involved. Methods such as aqueous two-phase extraction (ATPE) have been proposed that can be widely used owing to their simplicity, low cost, high yield, and ease of scale-up (Lakshmi et al., 2012). Other novel methods like supercritical (Fathordoobady, Manat, Selamat, & Pratap Singh, 2019) and ultrasound-based extraction technologies (Perez, Lopez-Nicolás, & Carmona, 2001) also offer solutions for further sustainable improvements in LOX extraction technologies.

6 | LOX ACTIVITY DETECTION METHOD

The LOX enzyme activity measurement method can be divided into three categories: spectrophotometric assays, spectroscopic assays, and oxygen consumption (Chang & Mccurdy, 1985; Hayward et al., 2016). The results of legume LOX activity are hard to compare horizontally among different studies. This is because the LOX activity results are dependent on legume sample preparation method and activity detection methods.

6.1 | Measurements via substrate co-oxidation (e.g., carotene)

In legume, LOX reacts with pigments, mainly xanthophylls, in the presence of unsaturated fatty acids. The LOX activity can be measured based on the co-oxidation of carotene in the presence of PUFAs. In this method, the bleaching rate of carotenoids is expressed by time intervals. However, the determination of bleaching efficiency is not an accurate method for activity determination, because the range of reaction activity that is proportional to the enzyme concentration is limited. Another reason is that the substrates themselves are unstable. Carotene oxidation of different LOX enzymes differs greatly by their peroxidative activities.

6.2 | Ferrous oxidation assay

This is one of the oldest methods relying on the interaction between hydrogen peroxide products and ferrous thiocyanate. In this process, Fe²⁺ is oxidized to Fe³⁺ by hydroperoxide and then reacts with thiocyanate to form a spectrophotometric colored product. However, this nonferrous product is unstable. Waslidge and Hayes (1995) subsequently replaced thiocyanate with xylene orange (a quantitative cationic indicator). The application of xylene orange in ferrous oxide xylene orange (fox) process produces a stable colored product with high molar absorption, which can be measured at 500–600 nm. Besides, the method is reproducible, fast, and insensitive to oxygen. The xylene orange method (FOX) applies to high throughput assay, and it eliminates the need for special equipment (Gay, Collins, & Gebicki, 1999).

However, owing to the requirement of acid environment for color rendering, this test can only be carried out in the end-point of the reaction. Therefore, it is not suitable for kinetic studies and can only calculate the relative amount of oxygen unless compared with known standards. Moreover, if the sample chelates iron, the results will be inaccurate.

6.3 | Oxygen consumption

Oxygen is a mandatory condition for the LOX-catalyzed oxidation reaction, so the activity of LOX can be determined by monitoring the consumption of oxygen, in either a manometric way or a polarographic way.

The polarographic assay was used by Indrawati, Ludikhuyze, Loey, and Hendrickx (2000) to measure the LOX activity in green pea juice. The substrate solution containing linoleic acid was homogenized with air-saturated phosphate buffer in a reaction cell. The green pea solution was injected into the reaction cell, and the oxygen consumption was measured and expressed as a function of reaction time. The graph of the oxygen consumption versus reaction time was generated, and the slope of the linear part of the curve was expressed as the LOX activity (Δppm O₂/s).

Monitoring the LOX activity by measuring the oxygen consumption can overcome the difficulties related to spectrophotometric analysis. The method is relatively sensitive, and the activity determination based on oxygen consumption has high accuracy (Hayward et al., 2016). The spectrophotometric analysis could not satisfy the theoretical yield on the basis of oxygen absorption when the secondary reaction occurred. For example, in the case of soybean crude extract, the results of the spectrophotometric analysis are different from the theoretical yield on the basis of oxygen absorption. This is due to the reaction of hydroxyl radicals consumed in the catalytic process, which cannot be explained by spectrophotometric analysis. Therefore, the activity measurement based on oxygen consumption has higher accuracy. This method does not require the optical clear sample for the spectrophotometer measurement, so it can measure the LOX activity in the sample with
high turbidity owing to the solubility of the substrate in an aqueous medium.

However, based on equipment requirements, difficulties exist when measuring a large quantity of samples (Hayward et al., 2016).

### 6.4 | Diene conjugated method

The most common technique used for legume LOX activity analysis is the diene conjugated method. This is a direct spectrophotometric assay. It measures the increase of absorption at 234 nm, which represents the production of diene products including cis-1,4-pentadiene and cis-1,4-unsaturated fatty acids obtained from the activity of the enzyme. Dienes directly measure the formation of diene products, so it has a high sensitivity. It can also be used for kinetic study with a recording spectrophotometer.

The diene conjugated method is separately found by Holman, Lundberg, and Burr (1945) and Theorell, Bergstrom, and Akeson (1946). They realized that the absorbance of LOX oxidized sample was increased at 234 nm, and they believe that the absorbance increase was related to the formation of a conjugated diene in linoleic acid. However, owing to the limited solubility of linoleic acid in water suspension, the application of this method was limited back then. Surrey (1963) then optimized the method by introducing a “Surrey” matrix mixture, in which linoleic acid was dissolved and clarified with 0.25% Tween® 20 and 1 M of NaOH, respectively. So far, this matrix mixture is still the most commonly used matrix.

The conjugated diene method is a standard method for the determination of LOX activity on the basis of sensitivity and reproducibility. However, owing to the low molar absorbptivity of the reaction products, the determination of low specific activity preparations is still susceptible to interference by protein absorption. This method has been widely used in recent studies of legume LOX including pea (Akyol, Alpas, & Bayndirli, 2006; Leone et al., 2001; Szymanowska et al., 2009), mung bean seeds (Aanangi et al., 2016), and soybean and soy product (Jiang et al., 2018; Manassero, Vaudagna, Sancho, Añón, & Speroni, 2016; Tedjo, Eshtiaghi, & Knorr, 2000).

### 6.5 | Spectroscopic assays

Fluorescence can function as a probe for free radical scavenging analysis, because it is degraded by peroxide free radicals and produce fluorescent products. The LOX activity can be measured based on the loss of radioactively labeled fatty acid substrate (Whent, Ping, Kenworthy, & Yu, 2010). The reagents used in this assay are inexpensive, the sensitivity of this assay is high, and only a small quantity of sample is required. This method can function as a screening tool for legume breeding (Whent et al., 2010). However, it can be interfered by the Tween® 20 and antioxidant such as reducing agents and radical scavengers in the sample. Secondary lipid oxidation products can also produce fluorescence, so care must be taken during the analysis.

Because the higher LOX concentration may affect the total analysis time, data from a samples with different LOX concentrations are not easy to compare. However, this method can be developed into high throughput analysis (Whent et al., 2010).

Chemiluminescence analysis has also been developed. Free radical process in lipid peroxidation leads to the formation of low levels of chemiluminescence. The addition of luminol and cytochrome ccan amplify this low level of chemiluminescence. The sensitivity of this method is equivalent to that of spectrophotometry when it is carried out under suitable conditions.

### 7 | THE APPLICATION OF LOX

LOX as a natural biocatalyst abundantly exists in the legume cells. It utilizes atmospheric oxygen to react with PUFA and forms potentially useful and valuable chemicals such as leukotrienes and lipoxins. Based on this, it has found some applications in the food industry.

#### 7.1 | Baking industry

LOX is the research focus of green food additives, and it has a huge market potential. In the baking industry, it functions as an effective baking conditioner and flour treatment agent. It is well documented that the LOX active soy flour has been used to fortify wheat flour (Hayward et al., 2016). It is a potential substitute for potassium bromate and benzoyl peroxide, which are commonly used as strengthening and bleaching agents, respectively (Hayward et al., 2016; Xu et al., 2014). Not only are the legume LOXs used in the bread making, but bacterial LOXs can also be used to strengthen and bleach the flour dough. However, the bacterial LOX has a lower thermal stability than plant LOX. This is probably because bacterial LOX lacks an N-terminal β-sheet domain, which is essential for high thermal stability (Xu et al., 2014). The bleaching effect is mediated by a complex interaction of soy LOX with wheat flour unsaturated fatty acid and carotenoids. However, the mechanism of the bleaching effect is not yet fully understood (Hayward et al., 2016). Studies have shown that β-carotene inhibits various free radical reactions. As such, β-carotene and other carotenoids could protect the cell membrane from lipid peroxidation by reacting with LOX (Stahl & Sies, 2004). Studies have also shown that the antioxidant activity relates to the pigment destruction in wheat flour dough (Stahl & Sies, 2004). LOX not only bleaches the wheat flour and produces white bread, but it also enhances the baking properties by increasing the mixing tolerance and dough rheology (Hayward et al., 2016). The improvement of the rheological properties of bread dough is probably related to the co-oxidation of thiol groups in wheat proteins. Moreover, enzyme active soy flour also provides extra protein content and nutritional value to the flour dough.

The thermal stability of LOX is an important element that needs to be considered when using LOX as a food additive, because baking
procedure is usually processed over 100 °C. The stability of LOX could be affected by the presence of additives. The stabilization of LOX by using additives will be further discussed in Section 8.

7.2 | Aroma production

LOX has been widely used in the food and beverage industries as a biocatalyst to produce aroma compounds at a low price and large quantity. The combined action of lipase, LOX and HPL on PUFA produces short-chain aldehyde and ketone, which are associated with a range of unique aroma (Gigot et al., 2010). C-6 or C-9 aldehyde and alcohol are associated with green leaf volatiles (Gigot et al., 2010), and they are commonly used to deliver a fresh flavor to the food product. For example, (E)-2-hexenol is used to as an alcohol enhancer because it delivers a sharp, green, fruity, unripe banana odor and is being used in apple, berry, orange, and pear flavors. (Z)-3-Nonenol has a fresh, waxy, green melon odor and is being used for fresh, tropical, melon notes (Gigot et al., 2010).

In the tea processing, especially in the process of fermenting black tea and oolong tea, people use LOX to catalyze the oxidation and decomposition of linoleic acid and linolenic acid to produce acetaldehyde, vinyl alcohol, vinyl aldehyde, and other unique aroma components of tea.

Biotechnological tools, such as stimuli-enhancing activity, production of recombinant enzymes, and stabilization of the synthesis by chemical compounds, have been used to improve aroma production using LOX. Although the easiest way to produce stable aroma compounds is by chemical synthesis, consumers in the food market have a strong preference for naturally synthesized aroma. Producing aroma compound by using legume LOX has a huge potential. However, the large-scale production of natural aroma is still challenging for the producers (Gigot et al., 2010).

7.3 | Other application of LOX

LOX can be used not only to produce many flavor substances but also to industrialize the production of dyes, coatings, detergents, and plasticizers of polyvinyl chloride. LOX can also be used as intermediates in drug synthesis. Therefore, LOX can also be used in other industrial catalytic uses to transform vegetable oils into products with high added value.

Most plasticizers are generated from petroleum. If fatty acids or fatty acid esters are selectively catalyzed by LOX to form specific derivatives of hydroperoxide fatty acids and their esters and then epoxidized to produce epoxidized oil, the amount of petroleum usage can be reduced. Hydroxyl oil can be obtained by reduction of hydroperoxide. Lipids, which are hydrolyzed by oxidase to produce unsaturated acids and aldehydes, are further reacted to obtain dibasic acids. These chemicals have important applications in food, chemical industry, biotechnology, and other fields.

LOX may have potential usage in pharmaceutical industry. LOX can catalyze the substrates to produce various kinds of arachidonic acid-like substances, which play an important role in cancer prevention and treatment. It is expected to become a new target of anticancer drugs. 5-LOX can catalyze the metabolism of arachidonic acid to form unstable white tri-dilute epoxide (LTA4), which may hydrolyze to chemicals like LTB4. LTB4 is a strong inflammatory substance that can lead to acute lung injury and asthma. The 15-LOX gene product may be a regulatory factor of anti-inflammatory factors and cell membrane remodeling, or a potential tumor suppressor. Studies have reported that 15-LOX has anticancer activity against breast, pancreatic, and colon cancers (Lee, Zuo, & Shureiqi, 2011).

8 | LOX STABILIZATION

Because LOX has many potential uses, food processors need to understand how to stabilize LOX d application. Adding sugars and polyols as food additives can improve the thermal stability of LOX (Back, Oakenfull, & Smith, 1979; Xu et al., 2014). Sucrose, glucose, mannitol, sorbitol, and glycerol could all be used as stabilizers and alleviate the thermal inactivation of enzymes (Xu et al., 2014). Sucrose of 1–2 M elevated the LOX stability by 400–600% at 70 °C. Sucrose has the highest stabilization effect among all the sugars that have been tested in this study. This is probably because the stabilization effect is affected by the number of OH groups of the sugar molecule. The experiment results showed that the order of the stabilization effect from large to small is sucrose > mannitol > sorbitol > erythritol > glycerol > glucose. The hypothesis is that the greater the number of OH groups, the stronger the stabilizer is, and glucose is an exception. This is because glucose has a strong affinity to the water molecule than LOX. Glucose–water solvent system is formed, and glucose is excluded from the LOX immediate surroundings (Busto et al., 1999).

Salts can also function as a LOX stabilizer. The order of stabilization produced by various cations was NH4+ > K+ > Na+ > Mg2+ (Busto et al., 1999). The addition of NH4Cl improved the thermal stability of pea LOX by 1.7 times (Busto et al., 1999). Polyethyleneglycols (PEGs) and nonionic surfactants (Tween® 20 and Tween® 80) had a destabilizing effect of LOX (Busto et al., 1999).

9 | THE LOX-CATALYZED UNWANTED REACTION

Products formed during LOX-catalyzed reaction are highly related to the food quality (Szymanowska et al., 2009). LOX is the primary reason for legume spoilage and quality loss. Legume usually contains a good amount of essential fatty acid, which can be degraded by the LOX-catalyzed reaction. The fatty acid deterioration involves primary autoxidation by a free radical chain reaction, photooxidation, and LOX-catalyzed enzymatic oxidation. Hydroperoxide is formed and further decomposed into carbonyl compounds, hydrocarbons, aldehyde, ketones, and other chemicals, which resulted in rancidity and volatile
compounds. These volatile compounds could be desirable and function as a natural flavoring agent, but it could also be undesirable. For example, the production of n-hexanol, n-pentanol, and n-heptanol in soy bean results in green and hay-like flavor. Mung bean is a cheap and stable food in Asia. However, in the production of green note and aroma compound, hexanal limits the application of mung bean in food production. LOX-catalyzed formation of hexanal and 2-pentyl furan produces intense green and beany aromas in winged beans. Lipid peroxidation and hydroperoxides both promote the loss of cell membrane integrity, speed up the cell ripening, and death. They also result in loss of pigments by degrading carotenoids and chlorophylls and brings a yellow color to the products. At the same time, the antioxidant status of the food product is reduced. Moreover, the production of oxidation chemicals can cause food rancidity.

LOX participates in the formation of eicosanoids in mammalian metabolism. The oxidation products of unsaturated fatty acids catalyzed by LOX form the unpleasant flavor of fruits and vegetables, such as the beany smell of soybeans and soy products. LOX also cause the fission of color, aroma, and taste in oil and oil-containing products during their processing and storage. Therefore, the inhibition of LOX becomes the focus of future research.

10 | INHIBITION OF THE LOX-CATALYZED OXIDATION

LOX-catalyzed PUFA degradation is responsible for legume senescence. The half-life, thermal inactivation constant, and activation energy of legume LOX have been extensively studied (Busto et al., 1999; Indrawati et al., 2000; Ludikhuyze, Indrawati, Van den Broeck, Weemaes, & Hendrickx, 1998). The LOX in legume can be inactivated by abiotic stress, such as thermal treatment, or thermal treatment combined with high pressure. During the food production and storage, the optimum oxidative stability can be obtained by minimizing exposure of food product to air, light, and high temperature. The turnover rate ($K_{cat}$) for LOX-1 is around 300 s$^{-1}$, which means the reaction and the aroma generation happens at an extremely fast rate.

10.1 | LOX enzyme inactivation by thermal treatment

Thermal treatment is the most widely used LOX inactivation method in the industry, and the heat inactivation of pea LOX follows first-order kinetics (Busto et al., 1999; Ludikhuyze et al., 1998). The thermal treatment can be applied by dry heating, immersion cooking, steaming, hot grinding, and extrusion cooking. Controlling the temperature of food processing is the most effective way to inactivate LOX. For example, during the soybean milk processing, grinding unsoaked shelled soybeans in 80–100°C water for 10 min can effectively remove the unpleasant flavor. Moreover, in the production of frozen legume, the legume is blanched before the freezing step for LOX inactivation. This is because freezing slows down senescence, enzymatic and chemical decay, and microbial growth, but the enzyme system remains active in a subzero environment. As a result, freezing alone does not prevent the quality loss, including undesirable volatile formation, color, and texture deterioration.

Blanching process inactivates the responsible enzyme in the food product, and the most suspicious enzyme that is responsible for the quality deterioration is LOX. Many food manufacturers blanch the food product until peroxidase (POD) is inactivated because it is one of the more stable enzymes. However, unlike LOX, POD is not responsible for the quality deterioration, and the heat treatment targeting POD is usually more severe than what is required to avoid quality loss (Bahçeçi, Serpen, Gökmen, & Acar, 2005).

According to Bahçeçi et al. (2005), blanching at 70°C for 2 min achieves >90% LOX inactivation. In order to optimize the process, food processors should blanch the legume just long enough to inactivate the targeted enzyme (Bahçeçi et al., 2005). Some studies recommended that targeting LOX enzyme is considered more significant in the blanching process (Baysal & Demirdöven, 2007). However, in some cases, targeting POD is more appropriate (Bahçeçi et al., 2005). To achieve the optimum product quality, food handlers must develop the blanching treatment on the basis of the properties of each specific food product.

It is also an effective way to deactivate the LOX by adjusting the food material to a lower pH level. For example, grinding soybean with water at pH 3.88 and then boiling can denature the LOX.

10.2 | Emerging technologies

Traditional thermal treatment is effective in LOX inactivation. However, the prolonged exposure of legume to high temperature leads to sensorial, nutritional, and functional quality loss (Rawson et al., 2011). Thermal treatment inactivates the undesirable enzyme, but it also induces nonenzymatic oxidation simultaneously. Thermal treatment can lead to unwanted changes in the food product; heat could destroy the membrane integrity and cell wall polymers. It could also degrade the pigment such as chlorophyll and results in color change. Nutrient leaching and degradation are also possible consequences. Therefore, food industries are trying to look at alternate thermal and nonthermal strategies. During thermal processing, emerging processes like reciprocating agitation thermal processing (Singh, Pratap Singh, & Ramaswamy, 2015) and controlled agitation thermal processing (Singh, Pratap Singh, & Ramaswamy, 2016) can help reduce the process time while retaining high-quality legume product. The focus of the following discussion is on non-thermal techniques.

10.2.1 | High pressure processing

High pressure processing (HPP), also known as high hydrostatic pressure (HHP) processing, has a potential to fully or partially replace the thermal processing in LOX inactivation, and it retains the food quality
better than thermal processing (Salazar et al., 2020). Previous studies have shown that HPP treatment (up to 700 MPa) can inactivate LOX either with or without the elevated temperature. Combined pressure-temperature treatment could also inactivate the LOX irreversibly, and the enzyme inactivation follows the first-order kinetics (Indrawati et al., 2000). For the green bean sample, 30 min 250 MPa HHP treatment followed by 30 min 50°C water blanching achieved 58% LOX inactivation, but 30 min 50°C water blanching only reaches 50% LOX inactivation (Akyol et al., 2006). Despite the enzyme inactivation, HPP also inactivates microorganisms. It could improve nutrient quality of the food products by inducing conformational and functional changes of macronutrients (Wang et al., 2008).

### 10.2.2 Microwave

Microwave treatment leads to heating of the samples by rotation of water molecular dipoles under oscillating electromagnetic field. This creates friction, and the samples heat up. Wang and Toledo (1987) studied the microwave inactivation of LOX in soybeans with various moisture contents. They observed that temperature and moisture content affect the enzyme inactivation, with increasing moisture content showing higher inactivation.

Thermal inactivation using microwave treatment was also studied by Kermasha, Bisakowski, Ramaswamy, and Van de Voort (1993). At microwave treatment at 750 W, 80°C for 30 s and 90°C for 5 s, the enzyme was completely inactivated with higher rate constants of 0.16 and 1.13 kJ/mol, respectively. Inactivation was reported to be linear and followed first order. Although the microwave involves heating mechanism, it is quicker than conventional thermal processes and thus could be promising.

### 10.2.3 Radio frequency

Radio frequency (RF) is also an effective LOX inactivation method. It uses electromagnetic energy at frequency ranging from 1 to 300 MHz. Alternating electric field has been implemented during the RF treatment, and heat will be generated by molar friction and space charge displacement. The heat that has been generated can inactivate the protein. RF produces product with higher functional properties and nutritional quality. This is because RF treatment has a shorter heating time and faster thermal transmission rate. Different from traditional heating technology, RF heats the sample inside and outside simultaneously (Jiang et al., 2018). According to Jiang et al. (2018), RF can inactivate rapidly and maintain the quality and functional properties of soybean. Based on their results, the inactivation rate of LOX reached over 90% for 210-s RF treatment; however, the same duration of conventional thermal treatment (110°C) reached only 3.08%. Moreover, the functional properties of RF-treated soybean protein isolate (SPI) were much higher than the conventional thermal-treated SPI samples. RF could better improve the sensory quality by reducing the content of hexanal and 1-hexanol, which are responsible for the unpleasant aroma production (Jiang et al., 2018). However, nonuniformity heating might happen especially in heterogeneous food material with irregular shape (Jiao, Tang, Wang, & Koral, 2018).

### 10.2.4 Pulsed ultraviolet light

Pulsed light, or pulsed ultraviolet light (PUV), refers to high-intensity short-duration flashes of white light ranging from the ultraviolet region to UV region to infrared region (Pollock, Pratap Singh, Ramaswamy, & Ngadi, 2017). The ultraviolet light can cause photochemical destruction of photosensitive amino acid (Mandal, Mohammadi, Wiktor, Singh, & Pratap Singh, 2020). As the absorption spectrum for proteins is 250–300 nm and the absorption spectrum for peptide bond is 190–220 nm, UV region of the pulsed light is considered the most effective in denaturing proteins. The reactive oxygen species induced by ultraviolet light can also damage the protein structure. Moreover, the PUV exposure leads to the temperature overheating (Wiktor, Mandal, Singh, & Pratap Singh, 2019) and further denatures the protein structure. According to Janve, Yang, Marshall, Reyes-De-Corcuera, and Rababah (2014), PUV illumination time and distance from light source significantly affect the LOX protein degradation. The temperature rise induced by PUV is greatly lower than that of other LOX inactivation methods. The SDS-polyacrylamide gel (SDS-PAGE) and reversed-phase high-performance liquid chromatography (RP-HPLC) results showed that LOX protein has been degraded into smaller fragments by PUV, and the LOX inactivation by PUV follows the first-order kinetics (Janve et al., 2014).

### 10.2.5 Pulsed electric field

During the pulsed electric field (PEF) treatment, short-duration and high-intensity electric field pulses affect the electrostatic interaction between peptide bonds (Wiktor, Pratap Singh, Parniak, Mykhailyk, Mandal, & Witrowa-Rajchert, 2020). The structure of the protein will be changed as a result. Based on results of Li, Chen, Chen, and Liu (2008), the PEF treatment time, treatment strength, pulse frequency, and pulse width all significantly affect the LOX denaturation. The maximum LOX inactivation achieved by PEF is around 88%, and this inactivation rate is obtained by 42 kV/cm for 1,036 μs at 25°C. However, PEF treatment can only be applied to liquid food product, which can sustain high electric field and low electrical conductivity (Rajkovic et al., 2010; Butz & Tauscher, 2002).

### 10.3 Inhibitor

LOX inhibitors can be used to avoid the unwanted LOX-catalyzed reactions. According to the mechanism of the inhibitory reaction, LOX inhibitors can be divided into the following four categories.


10.3.1 | Compete for the active sites of LOX with substrates

Inhibitors can be used as substrates to participate in the reaction, thus inhibiting LOX activity, for example, the inhibitory effect of vitamin C on LOX activity (Meščić Macan, Gazivoda Kraljević, & Račić-Malić, 2019).

10.3.2 | Chelating effect

The inhibitor chelates with Fe$^{2+}$ and keeps LOX inactivated. For example, in basil and kanai extracts, catechins interact with the active center of the enzyme, Fe$^{2+}$, which leads to the inactivation of the enzyme (Babu & Liu, 2008).

10.3.3 | Reduction action

The coenzyme of LOX contains Fe$^{3+}$, which is the active state of Fe ion. Fe$^{2+}$ is the inactive state, so the activity of LOX can be inhibited by reducing the concentration of Fe$^{3+}$. LOX activity is inhibited when Fe$^{3+}$ is reduced to Fe$^{2+}$. For example, selenide and nordihydroguaiaretic acid can inhibit LOX by transforming Fe$^{3+}$ into Fe$^{2+}$.

10.3.4 | Compete for free lipid radicals

Some inhibitors may react with free radicals in the catalytic process to terminate the chain reaction. Studies have shown that the addition of β-carotene can reduce the formation rate of conjugated dienyl groups in LOX catalytic reaction. β-Carotene reacts with linoleic acid free radicals (L) at the beginning of the chain reaction and changes to its initial state (LH), so LOX cannot complete the chain reaction (Serpen & Gökmen, 2006).

Many studies have shown that phenolic antioxidants can inhibit LOX. In the food industry, it is common to add antioxidants such as tea polyphenols, vitamin E, or rosemary to prevent the effect of oxidation and avoid food rancidity in storage. Hydrogenation of phenolic hydroxyl groups with free radicals produces stable semiquinone free radicals, thus completing the antioxidant effect by terminating the chain reaction. The free radicals can be scavenged by giving electrons directly through the reduction of antioxidants. The antioxidant functions by reducing the reaction rate of several metal-ion-catalyzed reactions.

According to Szymanowska et al. (2009), the activity of pea LOX can be successfully inhibited by phenolic compound, and the inhibition degree is related to hydroxyl groups position (ortho, meta, and para). The inhibitory effect of the phenolic compound that has been tested on pea LOX from large to small is caffeic acid > quercetin > catechin > benzoic acid > ferulic acid > kaempferol. Phenolic antioxidant effectively protects legume from lipid oxidation.

10.4 | Inhibit oxygen and light

Oxygen is a prerequisite of lipid oxidation. As a result, the most straightforward way of reducing or inhibiting food oxidative spoilage is either reducing or removing the oxygen from the food processing and storage environment. Even if eliminating oxygen is effective, it is not usually feasible during the food processing operations. Many agriculture production has happened in developing countries, and the food product is processed right after the harvest because it is easier to preserve the food product in the processed form.

Vacuum packing or shrink wrapping is a method of removing air before sealing the food product. It was firstly invented in the 1940s. It has been used on a variety of food products, including legume. It prohibits atmospheric oxygen from reacting with the food product. Moreover, oxygen is essential for all biological activities. By removing the oxygen, the growth of aerobic bacteria or fungi is all inhibited. The vacuum packaging also seals in all the volatile components, maintaining the freshness by preventing the aroma from evaporation. Today, tabletop vacuum sealer has become a popular household tabletop appliance.

A controlled atmosphere is a commonly used storage method in agriculture. The oxygen, carbon dioxide, nitrogen, temperature, and humidity are highly regulated in the storage room. By increasing the carbon dioxide content and reducing the oxygen level, the LOX-catalyzed oxidation reaction can be reduced (Cichello, 2015). Moreover, insect pests can also be eliminated. Modified atmosphere packaging (MAP), automatic misting (AM), and vent packaging (VP) are useful strategies to reduce postharvest deterioration.

Light induces both the lipid and protein oxidation, and packaging material impermeable to light is also a useful strategy to reduce the quality loss.

11 | CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

LOX is a naturally existing and inexpensive enzyme that has many potential uses in food preparation and storage. The study of LOX has a history of more than 70 years. The research on the extraction of LOX is ongoing. Thanks to excellent yields, high nutritive value, good availability, and cost-effective production, the food industry can utilize legume proteins to formulate new food products. Legume may have a future as a sustainable human food supply and a good source of LOX. It is believed that legume LOX will play a more critical role in the emerging food product development.

Meanwhile, researchers start to study the LOX in transgenic plants by gene silencing and gene deletion, and the analytical methods of LOX products have been continuously improved to promote the study of LOX pathway and the physiological role of metabolites. Hopefully, the mechanism of LOX oxidation will be fully understood in the near future; additionally, food manufacturers can inhibit the undesirable LOX activity in the food product and develop a simple,
convenient, and cost-effective method for obtaining LOX in large quantities.

The study on the kinetics and inhibition mechanism of LOX is the key research direction in the future. At the same time, molecular biological methods can be used to reveal the metabolic pathway of LOX at the molecular level, the regulatory role of LOX pathway in cancer treatment, and the mechanism of plant senescence. In the industry, the application prospect of LOX will be very broad, and it can be used in industrial production of dyes, coatings, detergents, or PVC plasticizers.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
Yuan Shi, Ronit Mandal, and Anika Singh conducted the literature review and prepared the original manuscript draft. Ronit Mandal, Anika Singh, and Anubhav Pratap Singh contributed to the critical review and editing of the manuscript. Anika Singh and Anubhav Pratap Singh conceptualized the study and were responsible for project management. Anubhav Pratap Singh secured funding for the study.

ETHICS STATEMENT
This article does not contain any studies with human or animal subjects.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article, as no new data were created or analyzed in this study.

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