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Review Article

Hexanal Treatment for Improving the Shelf-life and Quality of Fruits: A Review

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ABSTRACT

Fruits are rich sources of bioactive compounds such as lycopene, tannins, β-carotene, resveratrol, and lignan. These bioactive compounds' antioxidative, antimicrobial, and antidiabetic properties are important in the human diet. Since fruits are one of the major sources of health-promoting nutrients for human consumption, they have high economic value. Ripening is a developmental process which involves changes in the colour, texture, taste, and metabolite composition of fruits, thus affecting their quality. In the market, the good quality of fruits depends on the ripening stage. Rapid ripening could shorten the shelf-life and quality of fruits. Shortened shelf-life causes fruit spoilage during post-harvest, transport, storage, and distribution. In turn, it will cause economic losses in the fruit market. Low-temperature storage is one of the techniques to prolong the shelf-life of fruits. However, this technique requires an expensive facility to achieve and maintain the low temperature. On the other hand, it has been reported that hexanal treatment could prolong the shelf-life and quality of fruits. It is also cheaper and easier to apply. However, the ripening inhibition mechanism of hexanal is not yet fully understood. The effectiveness of hexanal treatment on different fruits is also unclear. Numerous publications on hexanal

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treatment reported different formulations, techniques, and effectiveness rates on different fruits. Optimised formulation and technique are important to develop an efficient hexanal treatment strategy. Therefore, the mechanism, effectiveness, formulation, technique, and development of hexanal-based products to prolong the shelf-life of fruits are discussed in this review.

Keywords: Antimicrobial, biosafety, hexanal, phospholipase D, post-harvest, ripening, shelf-life

INTRODUCTION

Fruits are major nutrient sources for human consumption. Thousands of bioactive compounds, such as lycopene, tannins, β-carotene, resveratrol, and lignan, are found in fruits (Dhalaria et al., 2020). Studies have shown that the bioactive compounds have antioxidant, antimicrobial, and antidiabetic properties (Karasawa & Mohan, 2018). The consumption of bioactive compounds has a beneficial impact on human health. In addition, fruits have high economic value as fruit production contributes to the economic development of a country. The production and demand for fruits have increased worldwide. According to the Food and Agriculture Organization of the United Nations (FAO) (2023), about 933 million tonnes of fruits were produced in 2022. The major fruits, such as watermelons, bananas, grapes, oranges, and apples, contributed to 52% of total production worldwide in 2022. Bananas, a tropical fruit, are one of the major traded fruits globally, with an estimated value of up to USD11 billion in 2017 (Voora et al., 2020). Therefore, studying fruit ripening and extending its shelf-life is crucial to improving fruit production to meet the growing demand for fruit.

Ripening is a fruit developmental process. As the fruit ripens, coordinated and complex metabolic pathways are involved. The texture, taste, colour, and metabolite content of fruits change due to ripening. Ethylene plays an important role in fruit ripening. The ripening molecular mechanisms mediated by ethylene were reported in apples (*Malus domestica*) (Lv et al., 2023) and bananas (*Musa acuminata*) (Chang & Brecht, 2023). In durian (*Durio zibethinus*), a gene related to ethylene perception, *DzETR2*, was up-regulated during ethylene-induced ripening (Thongkum et al., 2018). Fruit softening is an important event during ripening that affects the quality of fruits, which is associated with membrane deterioration (El Kayal et al., 2017). Phospholipase D (PLD) is a key enzyme in the membrane degradation pathway (Jincy et al., 2017). The inhibition of PLD has resulted in the enhanced shelf-life and quality of bananas (L. Li et al., 2022). Therefore, studying the ripening mechanism is important for developing post-harvest techniques to improve the shelf-life and quality of fruits.

Rapid ripening shortens fruits' shelf-life. Fruit spoilage can occur at any point during harvest, transport, storage, and distribution, which leads to profit losses in the fruit market (dos Santos et al., 2020;

Gustavsson & Stage, 2011). For example, durian has a limited shelf-life of three to four days at room temperature (Tan et al., 2019). During the durian season, typically from May to August, durians are spoiled in abundance before reaching consumers due to their limited shelf-life. Therefore, an efficient method to improve or prolong the shelf-life of fruit is important. Low or chilling temperature storage is one of the most used methods to prolong the shelf-life of fruits. However, it requires expensive facilities to achieve and maintain a low or chilling temperature for storage. Furthermore, certain fruits are prone to chilling injury due to chilling temperature (Razali et al., 2022). In addition, low or chilling temperatures could also alter the taste, aroma, and quality of fruits.

Hexanal is a naturally produced compound from plants. It is generally recognised as safe (GRAS) material. Hexanal treatment could prolong the shelf-life and quality of several fruits. Delayed ripening of fruits treated with hexanal was observed in many fruits, such as cherry (*Prunus avium*) (Sharma et al., 2010), blueberry (*Vaccinium cyanococcus*) (Songe et al., 2010), and mango (*Mangifera indica*) (Silué et al., 2022). Hexanal treatment inhibits the expression and activity of the enzyme PLD (El Kayal et al., 2017). However, the complete mechanism of delayed ripening by hexanal treatment remains unclear. The formulation and effectiveness of hexanal treatment on different types of fruits also vary. Therefore, these are discussed in the present review.

HEXANAL

Hexanal is an organic compound with a molecular formula of $C_6H_{12}O$. Hexanal exists as a colourless, highly volatile liquid. Hexanal is naturally produced by plants with high concentrations found in maise (*Zea mays*) (K. Zhang et al., 2022), tea (*Camellia sinensis*) (Ho et al., 2015), and black walnut (*Juglans nigra*) (Lee et al., 2011), while low concentrations found in thornless blackberry (*Rubus ulmifolius*) (Du et al., 2010) and grapes (*Vitis vinifera*) (Kaya et al., 2022). The United States Food and Drug Administration (USFDA) has approved hexanal as a food additive with a lethal dose (LD_{50}) of 3,700 mg/kg (Khan & Ali, 2018). Figure 1 shows the molecular structure of hexanal.

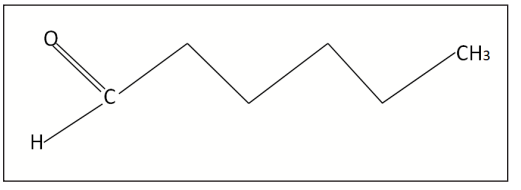

Figure 1. 2D molecular structure of hexanal (National Library of Medicine [NIH], n.d.)

Hexanal Biosynthesis

Hexanal is naturally biosynthesised in trace amounts by certain species of plants. Linoleic and linolenic acids are the biological precursors of hexanal biosynthesis (Pérez et al., 1999). Lipoxygenase (LOX) and hydroperoxide lyase (HPL) metabolic pathways are involved in hexanal biosynthesis (Pérez et al., 1999). Enzymes responsible for hexanal biosynthesis include LOX, HPL, lipolytic acyl hydrolase, and (E,Z)-2,3-enal isomerase (Pérez et al.,

1999). The metabolic pathway of hexanal biosynthesis is shown in Figure 2.

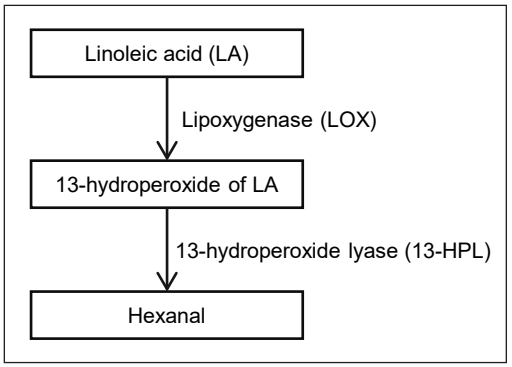

Figure 2. Metabolic pathway of hexanal biosynthesis (Pérez et al., 1999)

Mechanism of Shelf-life Enhancement by Hexanal Treatment

Fruit softening is an important event that occurs during ripening. The decrease in texture and firmness of fruits due to the loss of membrane integrity leads to senescence, which causes post-harvest losses in the fruit market (Padmanabhan et al., 2020). The mechanism of fruit softening involves coordinated and complex enzymatic reactions and pathways (Marangoni et al., 1996). Cell wall degradation is a major cause of fruit softening (Payasi et al., 2009). Cell wall degradation is a complex process that involves diverse protein families such as expansins and cellulase (Payasi et al., 2009; Whitney et al., 2000). In plant cell walls, cellulose microfibrils and xyloglucans are bound by a hydrogen bond (Payasi et al., 2009). Expansins disrupt the hydrogen bond between cellulose microfibrils and xyloglucans (Whitney et al., 2000).

Cellulase hydrolyses β-1,4 glucan linkages in cellulose and xyloglucan (Payasi et al., 2009). These enzyme activities cause cell wall degradation and play an important role in fruit softening. The structure of cellulose and xyloglucan are shown in Figure 3. Understanding ripening mechanisms, including ethylene biosynthesis cell wall, chlorophyll, and phospholipid membrane degradation, is important to develop pre- or post-

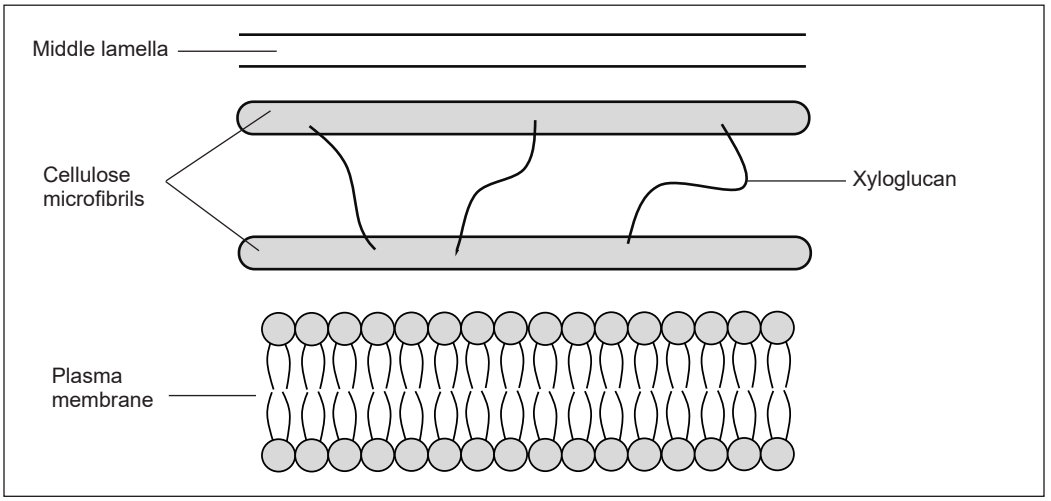

Figure 3. The structure of cellulose, xyloglucan and plasma membrane (Payasi et al., 2009)

harvest techniques to enhance the shelf-life and quality of fruits. For example, 1-methylcyclopropene (1-MCP), which is commercially available as Ethylbloc™ or SmartFresh™, is used to inhibit the ethylene binding to the ethylene receptors in fruits (Thongkum et al., 2018), thus delaying the ripening process, such as in durian (*Durio zibethinus*) and apple (*Malus domestica*) (Lv et al., 2023; Thongkum et al., 2018).

Hexanal treatment is another effective method to enhance the shelf-life and quality of fruits by inhibiting PLD, which is the key enzyme in phospholipid membrane degradation associated with fruit softening during ripening and decay (Jincy et al., 2017; Marangoni et al., 1996). The pre- or post-harvest techniques used to improve the shelf-life of fruits affect the ripening mechanism. Numerous studies have revealed the mechanism of fruit ripening at the genomic, transcriptomic, proteomic, and metabolomic levels in response to hexanal treatment as a promising method to enhance the shelf-life and quality of fruits.

PHOSPHOLIPASE D (PLD)

PLD is a key enzyme in phospholipid membrane degradation during fruit ripening, leading to the loss of membrane integrity, fruit softening, and decay (El Kayal et al., 2017). PLD belongs to the phospholipase enzyme superfamily along with phospholipase A (PLA), phospholipase B (PLB), and phospholipase C (PLC). PLD activity was first described in carrots (*Daucus carota*) (Hanahan & Chaikoff, 1947). The choline content in phospholipids extracted from carrots exposed to steam was higher than in untreated carrots. Therefore, it was suggested that the presence of an enzyme later known as phospholipase D (PLD) in the carrot could split choline from phospholipid in untreated carrot. In addition, the enzyme was inactive due to the steam treatment (Hanahan & Chaikoff, 1947).

The amount of PLD enzyme which can be extracted from plants is limited. The cloning and expression of PLD genes are important to provide an adequate amount of PLD protein to study the biochemical properties of PLD. PLD was cloned from castor bean (*Ricinus communis*) heterologously expressed in *E. coli* (Wang et al., 1994). Recombinant PLD with the size of 2,834 bp cDNA encoding 808 amino acids exhibited PLD activity, catalysing transphosphatidylation reaction (Wang et al., 1994). PLD catalyses the hydrolysis of phosphodiester bonds of glycerophospholipids such as phosphatidylcholine (PC) as a substrate to generate phosphatidic acid (PA) and choline. PC is a major substrate of PLD. However, PLD also exhibits activity on other substrates such as phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS), which are the constituents of phospholipid membrane in many cellular processes, including membrane deterioration in plant cells associated with ripening (J. Li et al., 2020). The advancement of genomic study has paved the way for discovering PLD in other

organisms. PLD genes can be found in plants (Qin & Wang, 2002), animals (Frohman et al., 1999), and bacteria (Zambonelli & Roberts, 2005). In plants, PLD is encoded by a multigene family. There are 12 copies of PLDs reported in *Arabidopsis thaliana* (Qin & Wang, 2002).

In eukaryotes, PLD has two domains, an N-terminal PX/PH or C2 domain and a C-terminal catalytic domain comprising two histidine (H), lysine (K), and aspartic acid (D) motifs in their gene structure (Hammond et al., 1997; J. Li et al., 2020). Therefore, PLDs are classified as PX/PH-PLDs and C2-PLDs based on their N-terminal domains. *Arabidopsis* has two PX/PH-PLDs and ten C2-PLDs. Moreover, plant PLD comprises α, β, γ, δ, ε, and ζ subtypes. The specific function of each isoenzyme remains unclear. Numerous studies have suggested that each isoform of PLD in the plant genome is differentially regulated in response to different types of stresses on plants. Different PLD genes have different expression patterns in tissues, organs, and developmental stages. It has been reported that PLDα1 in *Arabidopsis* was highly expressed in root cells and suggested to be involved in membrane assembly and vesicle trafficking (Novák et al., 2018).

A previous study showed that PLDα in *Arabidopsis* (AtPLDα) is involved in abscisic acid (ABA) and ethylene-induced senescence (Fan et al., 1997). Meanwhile, PLDδ was expressed near the plasma membrane binding to the cortical microtubule in hypocotyl cells (Novák et al., 2018; Q. Zhang et al., 2017). PLD is activated by calcium ions (Ca^{2+}) to perform its catalytic function. The Ca^{2+} binding site is present in the structure of PLD (Hammond et al., 1997). The cDNA sequence encoded for forming a Ca^{2+} binding site is highly conserved among C2-PLDs (J. Li et al., 2020). The Ca^{2+}-mediated activation is essential for the activity of C2-PLDs but not for PX/PH-PLDs (J. Li et al., 2020). It indicates that the PLD isoforms have different specific functions and are regulated by different plant molecules or hormones. Therefore, specific isoforms of PLD play an important role in the catabolism of phospholipids during ripening and senescence. The elevated levels of Ca^{2+} and pH in the cytoplasm stimulate the activity of PLD due to ripening.

Membrane Lipid Catabolism

The cell membrane, composed of fatty acid-based lipids and proteins, is an important structure of all cells. Phospholipid bilayer and proteins are the basic structures of the plasma membrane. Phospholipids and their catalytic products are important for various cellular mechanisms as signalling molecules, including fruit ripening. There are five types of phospholipids in the membrane, including phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphoinositides (PIPs). The structure of the plasma membrane can be seen in Figure 3. The structure and composition of plasma membrane undergo modification catalysed by several major enzymes, namely PLA,

PLB, PLC, and PLD, as the fruit ripens, thus leading to membrane deterioration and softening of the fruit. These enzymes cleave their substrate at different positions of phospholipid molecules.

Ethylene binds to the ethylene receptor at the onset of ripening to produce a signal for the expression of ripening-related genes, including PLD. In addition, an increase in Ca^{2+} and a decrease in pH in the cytoplasm trigger the PLD autocatalytic activity. PLD cleaves the substrate at the phosphodiester bond to release the head group. PLD catalyses the conversion of PC as a main substrate to PA and choline. PA is then converted to diacylglycerols by phosphatidate phosphatase. Then, diacylglycerols are further catabolised to free fatty acids such as linoleic and linolenic acids by lipolytic acyl hydrolase (LAH). Lipoxygenase (LOX) catalyses the conversion of linoleic and linolenic acids to hydroperoxides. Hydroperoxide lyase (HPL) will convert hydroperoxide products from linoleic and linolenic acids to aldehydes such as hexanal, *trans*-2-hexenal, or *cis*-3-hexenal. These aldehydes are converted to 1-hexanol, *trans*-2-hexen-1-ol, and *cis*-3-hexen-1-ol by alcohol dehydrogenase (ADH) (Genovese et al., 2021). The intermediate compounds or products generated along the pathway, such as alkanes, fatty alcohols, and fatty acids, cause membrane destabilisation by forming lipid microvesicles, gel-phase lipids, and non-bilayer lipid structures. Therefore, the biophysical property of the membrane is altered, thus contributing to the softening of the fruit.

Inhibition of Ripening by Hexanal Treatment

The activity of PLD leads to membrane deterioration during ripening. Rapid ripening shortens fruits' shelf-life. The binding of ethylene to the ethylene binding receptor results in the expression of PLD and other ripening-related genes (Tiwari & Paliyath, 2011). PLD activity is also triggered by the cytoplasmic Ca^{2+} and hydrogen ion (H^{+}) elevation through stresses, membrane disruption, and ion leakage (Paliyath et al., 2008). Therefore, inhibiting PLD activity is important to prolong fruits' shelf-life. Many studies have been done to develop pre- or post-harvest technologies by inhibiting PLD activity. Alcohols such as *n*-butanol and 2-butanol can also inhibit PLD activity (L. Li et al., 2022). In addition, PLD activity can be inhibited by hexanal (El Kayal et al., 2017). It has been reported that PLD inhibition by hexanal treatment resulted in significant shelf-life enhancement in bananas (*Musa acuminata*) (L. Li et al., 2022).

The application of hexanal treatment on raspberry increased the expression of transcription activator for calcium-binding protein called calmodulin (El Kayal et al., 2017). Calcium signalling is crucial in a wide array of cellular functions, which involve calcium ions as a messenger in response to stresses. Calmodulin is a calcium sensor which binds to calcium to produce a signal that triggers the expression of genes related to cell wall metabolism. Cell wall degradation is one of the important events that causes fruit softening and decay.

Ca^{2+} inhibits leaf abscission and tissue senescence by crosslinking pectic acid residues to rigidify the cell wall (Hepler, 2005). Hexanal treatment affects the cell wall and lipid membrane metabolism, thus leading to shelf-life improvement. Nevertheless, the interaction mechanism between hexanal and Ca^{2+} signalling in inhibiting PLD expression remains unclear. Furthermore, hexanal treatment greatly reduced annexin expression and calcium accumulation during PLD expression in raspberries (El Kayal et al., 2017). It is suggested that annexin forms Ca^{2+} channels to transport Ca^{2+} across the plasma membrane, leading to the increase of cytoplasmic Ca^{2+} concentration, which triggers the expression of lipid membrane metabolism-related genes, including PLD (El Kayal et al., 2017). It shows the role of calcium signalling in the hexanal-induced membrane stabilisation mechanism by regulating the expression of genes related to cell walls and lipid membrane metabolisms.

BIOSAFETY OF HEXANAL

Numerous studies have ensured that hexanal usage in agricultural and food industries does not harm the environment and humans. *Trichogramma* spp. are biological control agents that protect some fruits against parasites (Mohan et al., 2017a). The growth of *Trichogramma* spp. (*Trichogramma chilonis* and *Trichogramma pretiosum*) was not affected by 0.02%–0.06% hexanal formulation treatment (Mohan et al., 2017a). A biosafety study of hexanal has been done on honey bees (*Apis cerana indica*, *Apis mellifera*, and *Apis florea*) (Mohan et al., 2017b). It is reported that 0.02%–0.06% hexanal formulation treatment was safe for all tested honey bee species (Mohan et al., 2017b). Green lacewing (*Chrysoperla zasrowi sillemi*) is an important biological control agent used to control the growth of a wide range of parasites such as aphids (*Myzus persicae*), coccids (*Coccoidea*), mealy bugs (*Pseudococcidae*), and mites (*Tetranychus urticae*) (I. J. Nair et al., 2020). The exposure of hexanal formulation (0.02%–0.06%) to green lacewing was harmless, with less than 30% mortality (Mohan et al., 2020). Similarly, hexanal treatment using the direct spray method on *Chrysoperla* grubs and 0.02% hexanal formulation on *Trichogramma japonicum* revealed that hexanal was not toxic to insects (Karthika et al., 2015).

Earthworms are known as a "farmer's friend" due to their activities in soil, which benefit both soil and crops (Gunasekaran et al., 2015). No ill effect was found on earthworm (*Eudrilus eugeniae*) exposed to hexanal formulation with concentration ranging from 0.02-0.06% (Gunasekaran et al., 2015). The toxicological studies of hexanal exposure to animals have also been reported (Cho et al., 2021). The exposure of hexanal vapour at 600, 1,000, and 1,500 ppm concentrations to the Fischer 344 rat strain showed potential pulmonary toxicity to the animals (Cho et al., 2021). The genes involved in the intracellular signalling cascade associated with pulmonary toxicity were identified by analysing the expressed mRNA (Cho et al., 2021). It suggested that

hexanal exposure could be toxic to humans at those concentrations. On the other hand, the study of hexanal toxicity in the human cell lines (HeLa, A549 and HepG2) has also been reported (Gunasekaran et al., 2015). Hexanal was found to be toxic to all tested cell lines (HeLa, A549, and HepG2) at a concentration of more than 2,000 ppm based on lactate hydrogenase (LDH) and tetrazolium (MTT) assay (Gunasekaran et al., 2015). The recommended spray concentration for pre- and post-harvest applications was below 0.04% (400 ppm), which is safe for human cell lines (Gunasekaran et al., 2015). Therefore, using hexanal to improve the shelf-life and quality of fruits is safe for the environment and human health.

HEXANAL APPLICATION IN ENHANCEMENT OF SHELF-LIFE OF FRUIT

Hexanal has been found to have antimicrobial properties and the ability to enhance the shelf-life of fruits (Khan & Ali, 2018; Song et al., 1996). Pre-harvest treatment of 1% hexanal followed by post-harvest treatment of 1 ppm 1-methylcyclopropene (1-MCP) on cherry (*Prunus avium*) enhanced its shelf-life and sensory attributes (Sharma et al., 2010). Guava (*Psidium guajava*) treated with 0.015% (v/v) of hexanal showed enhanced quality and shelf-life of up to four weeks of storage (Gill et al., 2016)—meanwhile, the treatment of 0.02% hexanal dip on mango var. Neelum (*Mangifera indica*) extended its shelf life (Jincy et al., 2017). The PLD activity and ethylene evolution rate were also significantly reduced (Jincy et al., 2017). The ethylene evolution rate increases as the fruit ripens. The reduction of PLD activity and ethylene evolution rate leads to delayed ripening, extending the mango var Neelum (*M. indica*) shelf-life (Jincy et al., 2017).

In addition, the activity of enzymes related to ripening and fruit softening, such as pectinmethlyesterase, catalase, peroxidase, and polygalacturonases in mango, was reduced after being treated with 0.02% hexanal using the direct-spray method (Preethi et al., 2021). Pre-harvest spray of 0.02% (v/v) hexanal was able to preserve the firmness of nectarines for at least 45 storage days at 2°C (Kumar et al., 2018). The onset of internal browning and mealiness was delayed for about eight days (Kumar et al., 2018). In addition, the expression of three PLD genes was significantly down-regulated (Kumar et al., 2018). The shelf-life of bananas was extended by spraying them with 2%–3% hexanal on days 15 and 30 pre-harvest and dipping them in hexanal post-harvest (Yumbya et al., 2018). However, pre-harvest 2%–3% hexanal spray showed better fruit preservation (Yumbya et al., 2018).

Antibacterial and Antifungal Properties of Hexanal

The ability of hexanal to prolong fruit shelf-life correlates with its antibacterial and antifungal properties, which are listed in Table 1. *Salmonella* Typhimurium is one of the major causes of foodborne illness (Hanning et al., 2009; He et al., 2021). It

is resistant to many antibiotics, such as ampicillin, chloramphenicol, streptomycin, tetracycline, and sulphonamides (D. V. T. Nair et al., 2018). The treatment of hexanal on fruit-based foods may be beneficial to prevent the infection due to its antibacterial activity while enhancing shelf-life. The exposure of 50 µl hexanal on *Salmonella* Typhimurium did not show any inhibitory effect for 30 min of exposure time. The inhibitory effect started to show from 45–75th min. No growth was observed for more than 90 min of hexanal exposure (Lamba, 2007). Moreover, hexanal inhibited pathogenic bacteria usually found in raw food, such as *Escherichia coli*, *Salmonella* Enteritidis, and *Listeria monocytogenes* (Lanciotti et al., 2004). It is reported that 150 ppm of hexanal caused significant inhibition of *Listeria monocytogenes* growth (Lanciotti et al., 2004). Hexanal (150 ppm) also inhibited the growth of *E. coli* and *Salmonella* Enteritidis at the lag phase inoculated at levels of 10^4–10^5 CFU/g (Lanciotti et al., 2004). Since hexanal is a hydrophobic molecule, it easily interacts with hydrophobic membranes and causes damage to the bacterial cell (Helander et al., 1997).

Table 1
Antibacterial and antifungal activities of different methods of hexanal treatment

Treatment method	Concentration	Microorganism	Reference
Hexanal vapour	50 µl/L	*Monilinia fructicola* and *Monilinia laxa*	Baggio et al. (2014)
Hexanal vapour	450 µl/L	*Botrytis cinerea*, *Monilinia fructicola*, *Sclerotinia sclerotiorum*, *Alternaria alternata*, and *Colletotrichum gloeosporioides*	Song et al. (2007)
Hexanal vapour	40 µmol/L	*Penicillium expansum*	Fan et al. (2006)
Hexanal dipping	0.02% (v/v)	*Lasiodiplodia theobromae*	Seethapathy et al. (2016)
Hexanal vapour	800 ppm	*Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*	Dhakshinamoorthy et al. (2020)
Hexanal vapour	5.02 µl/L	*Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*	Dhakshinamoorthy et al. (2020)
Haxanal vapour	150 ppm	*Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* Enteritidis	Lanciotti et al. (2004)
Hexanal containing broth	400 µl/L	*Aspergillus flavus*	Li et al. (2021)
Hexanal added to a trypticase soy agar plate	50 µl	*Salmonella* Typhimurium	Lamba (2007)
In vitro (disc-diffusion method) Hexanal dissolved in dimethyl sulfoxide	0.015–500 ppm	*Moraxella catarrhalis*, *Escherichia coli*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Salmonella* Enteritidis (no significant antibacterial activities against these bacteria)	Bisignano et al. (2001)

Meanwhile, a previous study showed that *in vitro* (disc-diffusion method) antibacterial activity of hexanal (0.015–500 ppm) dissolved in dimethyl sulfoxide (DMSO) was not significant against all tested bacterial strains such as *Moraxella catarrhalis*, *E. coli*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Salmonella* Enteritidis (Bisignano et al., 2001). It indicated that the effectiveness of hexanal as an antibacterial volatile compound depends on the treatment methods and the types of fungal or bacterial strains. Therefore, the study for optimisation of hexanal treatment is important to ensure its effectiveness in enhancing the shelf-life of fruit by killing the bacteria and fungus that cause fruit spoilage.

Monilinia fructicola and *Monilinia laxa* are plant pathogenic fungi that cause brown rot disease in fruits (Martini & Mari, 2014). It is reported that hexanal vapour treatment at a concentration of 50 µl/L reduced the development of brown rot disease on peaches (*Prunus persica*) (Baggio et al., 2014). The growth of *M. fructicola* and *M. laxa* in the peaches was also inhibited (Baggio et al., 2014). Similarly, the treatment of 450 µl/L of hexanal vapour on major post-harvest fungal pathogens such as *Botrytis cinerea*, *M. fructicola*, *Sclerotinia sclerotiorum*, *Alternaria alternata*, and *Colletotrichum gloeosporioides* for 24 hr inhibited the growth of all tested fungal pathogens (Song et al., 2007). The treatment of 900 µl/L hexanal vapour reduced the decay of raspberry (*Rubus idaeus*) and lesion development on peaches (*Prunus persica*) for at least two days at 20 and 7°C (Song et al., 2007).

The viability of *Penicillium expansum* in apples was reduced following 24 hr of 40 µmol/L hexanal vapour treatment (Fan et al., 2006). Similarly, the treatment of 0.02% hexanal dipping inhibited the growth of stem-end rot disease *Lasiodiplodia theobromae* in mango (Seethapathy et al., 2016). In growth, *C. gloeosporioides* and *L. theobromae in vitro* were inhibited after exposure to 800 ppm hexanal vapour (Dhakshinamoorthy et al., 2020). In addition, the exposure for 3 hr of 5.02 µl/L hexanal vapour treatment on bananas significantly reduced the infection of *C. gloeosporioides* and *L. theobromae* (Dhakshinamoorthy et al., 2020).

Hexanal also exhibited antifungal activity against *Aspergillus flavus* at a minimal inhibitory concentration of 0.4 µl/ml (S.-F. Li et al., 2021). It was found that several metabolites such as phosphatidylcholine, riboflavin, D-sorbitol, D-ribose, D-mannitol, L-malic acid, and deoxyinosine in *A. flavus* were significantly reduced (S.-F. Li et al., 2021). From the metabolite analyses, it was suggested that the inhibition of *A. flavus* growth by hexanal involved the membrane synthesis, adenosine triphosphate binding cassette (ABC) transport system, and tricarboxylic acid (TCA) cycle (S.-F. Li et al., 2021). It evidenced the potential of hexanal as an effective antifungal agent to prevent fruit spoilage. The study of the effect of vapour pressure on the antifungal activity of volatile compounds revealed that the antifungal activity was enhanced as the

vapour pressure increased using hexanal as a model molecule (Gardini et al., 1997). The increase in temperature led to the increase in vapour pressure, thus enhancing the antifungal activity of hexanal vapour against *Aspergillus niger* (Gardini et al., 1997).

CONCLUSION

Hexanal is a naturally biosynthesised compound from plants and is safe for the environment and humans. Hexanal has promising potential to be used in pre- or post-harvest technology to enhance the shelf-life and quality of fruits. Moreover, hexanal exhibits antibacterial and antifungal activities that could help prolong the shelf-life of fruits. However, the efficiency of hexanal treatment depends on several factors, such as formulation, method of treatment, and type of fruit. Hexanal is a potent PLD inhibitor, the key enzyme in lipid membrane deterioration. However, the inhibition mechanism of PLD by hexanal at the molecular level is still unclear. The expression analysis of PLD and other ripening-related genes using quantitative real-time PCR (qRT-PCR) could be used to understand the mechanism of PLD inhibition by hexanal. In addition, determining PLD crystal structure by X-ray crystallography could reveal the possible binding site of hexanal to PLD.

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