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VOLATILE COMPOUNDS RELEASED FROM SEEDS - A REVIEW

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Dry seeds evolve volatile compounds as secondary products during storage and germination due to auto-oxidation of lipid and other metabolic processes. These volatiles have toxic effect to the adjacent high vigour seeds and they include the compounds of major categories such as aldehydes, hydrocarbons, alcohols and ketones. This paper is aimed to discuss about the auto-oxidation process, emission of volatiles, detection methods and their effects on seed vigour. The volatiles released by other plant parts may successfully be used for trapping the insect pests and managing the diseases.

Keywords : Alcohols; Aldehydes; Bioassay; Free fatty acids; Hydrocarbons; Hydroperoxides; Lipid peroxidation; Secondary products; Seed vigour; Volatiles.

Introduction

Seed is the most vital, basic and critical input in agriculture for increasing and sustaining the agricultural productions. The seeds are living things and as such must be handled with utmost care whether they are intended primarily for sowing or as a genetic reserve. These seeds should be genetically and physically pure, physically sound and physiologically active. The reduction in shelf life of seed due to natural ageing is associated with loss in viability, rate of respiration and increase in membrane permeability. Ageing of seed begins from the moment it attains physiological maturity and continues till the death of seed. During ageing many cytological and biochemical events bound to occur at cellular level leading to deterioration. Thus ageing does not cause momentary loss of viability due to instant damage, for example stress due to temperature or mechanical impact on seed coat etc. Insect predation is also not considered as a cause for ageing. The fungal infection, being more progressive in the process of deteriortion may be considered a part of the ageing process[1].

The increased accumulation of free fatty acids in seed is a important symptom of active process of ageing. Auto-oxidation of lipid at high temperature or at high oxygen content during storage of seeds also leads to ageing called accelerated ageing[1,2]. In oil seeds degradation of fat due to fungal infection is a major event leading to breakdown of lipids into free fatty acids. This is associated with rancidity of oil and has been reproted as early as in 1958. Rancidity of seed oil was a cause for declining germinability, proposed in pine seeds[3] and later, it was proved that the process of lipid auto-oxidation, perhaps the most cited cause of seed deteriortion[4].

Degradation of fat in to fatty acid and lipid auto-oxidation becomes a serious problem if seeds are stored for a long period with very low moisture content of 4 to 6%. Seeds stored with high moisture content (10-12%) invites pathogen infection at the earliest possible during storage, effecting generation of more heat due to active respiration and finally leading to loss in vigour and viability. Hence the optimal seed moisture content for prolonging the longevity, lies between the level of high moisture content at which fungi destroy viability (12-14%) and the low moisture content (4-6%) at which lipid auto-oxidation takes palce[5].

Lipid peroxidation

In stored seeds, the reserve fatty acid hydrocarbon chains spontaneously oxidized in the presence of oxygen and produces highly reactive free radical intermediates such as hydroperoxides and a wide variety of secondary products through hydroperoxide decomposition. The rate of this reaction is greatly accelerated by the class of enzymes called lipoxygenases[6]. The free radicals detached during this reaction are highly reactive, self-propagating and ubiquitous in their cycle. A number of secondary pathways are initiated, as a result, damage to cellular components are obvious. The breakdown products of these reactions

(for example, free fatty acids, aldehydes, conjugated dienes, schiff's bases) are biologically active and become toxic to the cell membrane if allowed to accumulate. Several of these breakdown products are able to cross link with proteins, enzymes and DNA[7]. Antioxidants quench this sequence by scavenging free radicals thereby breaking the chain reaction cycle[8].

Hydroperoxides

Hydroperoxides are nonvolatile stable compounds accumulated in the seed due to auto-oxidation process of lipid. In soybean seed the linolenic acid is oxidized to various oxygenated products such as di-enone-, hydroxy-, epoxy-, epoxyhydroxy- and epoxyhydro- peroxy fatty acids[9]. These hydroperoxide compounds and oxyygenated fatty acids are sufficiently stable to be isolated and had been detected in tissue[10]. Further, decomposition of these hydroperoxides yields smaller secondary products and more free radicals under both *in vitro* and *in vivo* conditions. These secondary products in nature are more volatile compounds, which is the subject of interest in this discussion.

Volatile compounds from plant system

Volatiles are highly reactive groups released from all parts of the plant such as leaves[11-13]; stem buds[14]; roots[15]; fruits[16-18]; seeds[19-23] and germinating seedlings[24-26]. In 1959, Wilks[27] detected small quantities of carbon monoxide and aldehydes from several plants, including algae and found that the carbon monoxide produced was in the presence of light than in dark or in the absence of oxygen. Similarly, Went[28] opined that condensed macromolecules of terpenes cause the blue hazes seen over vegetation during sunny days. The aromatic shrub particularly the plants of the genus *Salvia* releases large amounts of volatile terpenes[29].

Aldehyde group particularly C_6-aldehydes are responsible for a major characteristic odour of green leaves in plants including vegetables and fruits. These aldehydes are produced from C_{18} - unsaturated fatty acids containing a *cis-1, cis-4*- pentadiene moiety such as linoleic and linolenic acid[11]. Similarly, C_6-aldehyde formation is reported in tomato fruits[16] and bean leaves[30]. Hayata *et al.*[17] isolated 181 volatile compounds at the mature green stage, 221 at the turning stage, 240 at the pink stage and 367 at the red stage in tomato fruits. Of all the compounds identified, *cis-3*-hexenal was the most potent odorant formed in the highest concentration responsible for flavour in tomato.

Ethylene one among the volatile compounds produced by plants especially associated during wounding and infection. For example, cut carnation[31] and sweet potato root tissue[32] evolved enhanced ethylene when damaged or suffered from fungi infection. Craker[33]. isolated the ethylene compound after - ozone injury in tomato, tobacco and bean.

The studies show that the volatiles released from plants are also used as host-location signals by foraging parasitoids, which are natural enemies of insect herbivores[12]. More so these volatile compounds have strong fungicidal action, the allyl isothiocyanate compound from seeds of *Brassica spp.* has controlling effect on *Rhizoctonia* damping-off of cabbage[34]. The amount and number of identifiable volatile compounds may differ in leaves, stems, flowers, seeds and roots[15]. Wang *et al*[13]. suggested that the changes in number of volatile compounds might be due to changes in fatty acids.

Volatile compounds from seeds

During storage, imbibition and germination, many metabolic changes occur in seeds. Some of the metabolic products released in this process are volatiles. The volatile compounds may include hydrocarbons, aldehydes, ketones, and alcohols[7, 35, 36]. The amounts of volatiles released per seed are proportional to the amount of storage substances in them[36], seed moisture[35] and period or temperature of seed storage atmosphere[23, 35]. The emissions of volatiles are dependent on the degree of tissue hydration and it has been suggested that active metabolism be important in propagating oxidative stress[37]. Stotzky and Schenck[26] observed that the larger seeds evolve greater quantities than smaller seed, but on a unit weight basis, smaller seeds evolve more. They also demonstrated that dry, non-germinating or killed seeds evolve little or no volatiles. Thus indicating active metabolism is essential for the production of volatiles in seeds.

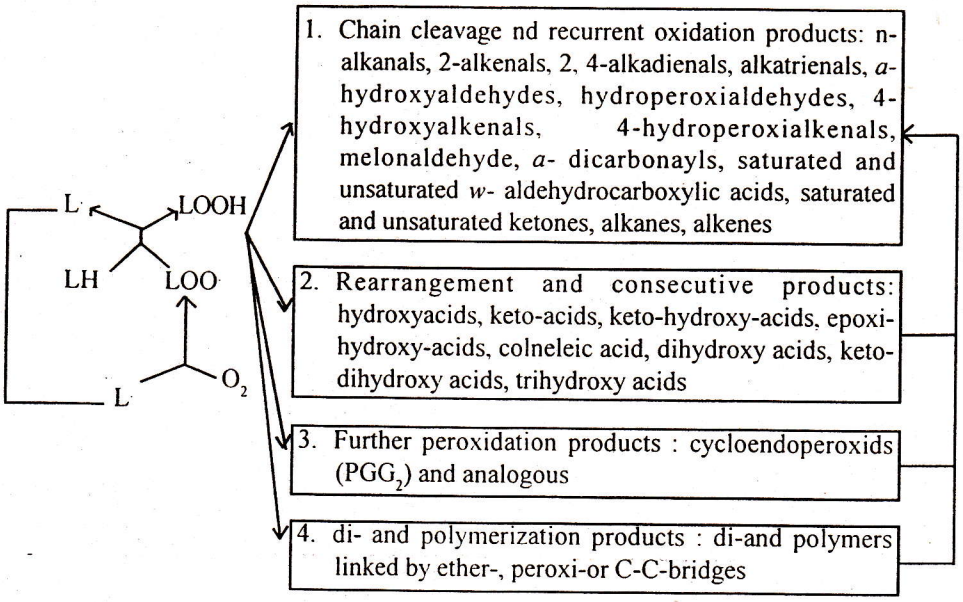


Fig 1. Schematic diagram of the aldehyde classes and other products derived from lipid peroxidation.

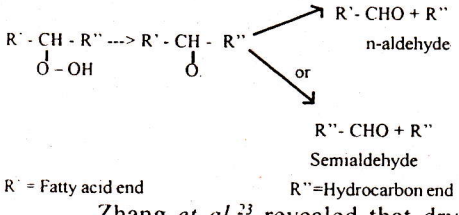
Volatile aldehyde compounds (VACs), hydrocarbons and alcohols

As discussed earlier, the most frequently identified seed volatiles are acetaldehyde, ethanol, propylene, ethylene and hexanal[22, 23, 37, 38]. Ethylene may be of hormonal origin but in can also be evolved in small amounts during lipid peroxidation[7]. Esterbauer[39] has provided a schematic diagram of the products derived during the lipid peroxidation process (Fig. 1) and indicated that aldehydes are formed by chain cleavage reactions from both the fatty acid monohydroperoxides and the secondary substances.

Amoung all the compounds evolved, aldehyde group is the major one. The lipid peroxidation induced respiratory reduction would lead to anaerobic metabolism and ultimately ethanol production and possible escape of acetaldehyde. Aldehyde and acetaldehyde has been reported to be produced in seeds in conjunction with a reduction in respiratory competence[22]. Stotzky and Schenck[26] found that seeds of many species evolve oxidizable volatile aliphatic aldehydes, of which, formaldehyde comprise only a small percentage of the total aldehydes. Melondialdehyde, a product of lipid peroxidation has shown an increase in non-viable soybean axes with an increase in corbonyl compounds from germinating aged seeds[20]. Bailey *et al.*[40] also found various other volatile compounds such as isovaleraldehyde, isobutyraldehyde, propionaldehyde, acetaldehyde, methanol, methyl acetate and diacetyl evolved in cocoa bean seeds.

C_6-aldehydes are major compounds formed from C_{18}- aldehyde formation from C_{18}-unsaturated fatty acids. These compounds include n-hexanal, *cis*-3-hexenal and *trans*-2-hexenal which is produced from linoleic and linolenic acids via their 13-hydroperoxides by the enzymes lipoxygenase and hydroperoxide lyase[25]. An equation indicating the dismutation of hydroperoxide into aldehydes and hydrocarbons has been given in Fig 2[37].

Fig 2. Dismutation of hydroperoxide into aldehydes and hydrocarbons.



Zhang *et al.*[23] revealed that dry

seeds evolve at least 24 kinds of volatile compounds during their storge. They have identified 11 kinds of volatiles in carrot seeds including propylene, methanol, acetaldehyde, butene, butane, ethanol, isopropanol, isobutyraldehyde, acetic acid and ethyl acetate stored at 23°C for one month. In fenugreek seeds there are 175 components of volatiles detected, of which the carbonyl compounds (hexanal, 2-methyl-2-butenal, 3-octen-2-one, trans-cis-and trans-trans -3,5 - octadien - 2-one), sesquiterpene hydrocarbons (δ– elemene, γ-cadinene and α- muurolene), alcohols (pentanol, hexanol, 2-methyl-2buten-1-ol, 1-octen-3-ol), heterocycle compounds (3-hydroxy - 4, 5- dimethyl -2 (5H) - furanone (sotolon), dihydro-5-pentyl-2 (3H) - furanone (γ - nonalactone), dihydro-5-ethyl-2 (3H) - furanone (γ - caprolactone) and other furan compounds are the prominents (21). Similarly, β-phellandrene, α-pinene, α-phellandrene and α- myrcene in *Levisticum officinale* seeds[15] and allyl isothiocyanate in *Brassica* spp. seeds[34] have been reported. Afsharypuor and Suleimany[41] isolated volatile components such as 4-methyl thiobutyl isothiocyanate (55.6%), 3-butenyl isothiocyanate (27.6%), allyl isothiocyanate (5.4%), 5-methyl thiopentanitrile (3.4%), 3-methyl thiopropyliso thiocyanate (2.6%) and 2-phenyl ethyl isothocyanate (2.4%) in the volatile oil obtained from *Brassica oleracea* var. *gongylodes* seeds. Abundant production of butan was reported in seeds of *Cleome spinosa* and *Cyperus alternifolius*[23]. Koch and Obendorf[42] reported that production of methanol in developing soybean seeds has been thought to be due to the activity of pectin methylesterase.

Origin of the volatiles in seeds

It is most important to determine the origin of the volatiles evolved in ageing seeds, imbibed seeds and germinating seedlings, particularly as several compounds are produced as a result of both metabolic uncoupling and lipid peroxidation[7]. Tappel[43] believed that free radical reaction and lipid peroxidation are the two processes that are in disruption of cell membranes. The oxidative attack is initiated randomly at some of the poly-unsaturted fatty acids located in the plasma membrane. These sites would enlarge via the free radical chain reaction into polar bridges across the hydrophobic barrier of the membrane leading to an increse in permeability[44]. The impairment of glycolysis and the tricarboxylic acid cycle influence the production of reduced nucleotides and damage electron transport mechanisms thereby affecting oxidative phosphorylation. Mitochondria might be the primary source of electrons leading to the production of free radicals[45] and it exhibits the age related ultra structural damage[37]. Mitochondria are also extremely vulnerble to oxidative stress and contain high levels of fatty acids that are the substrates for lipid peroxidation. Mitochondria is the active site of electron transport reactions and their terminal electron acceptor is molecular oxygen. If uncontrolled, mitochondiral reactions produce activated oxygen species $^1O_2$ and oxy-free radicals[5]. Thus, membrane oxidation could interfere directly with respiration at least in three ways such as causing changes in viscosity or polarity of the inner membrane, interferences with mitochondrial assembly during imbibition nd co-oxidation of critical enzymes and co-factors[37].

Volatiles and seed vigour

In general, the most cited cause of seed deterioration during storage is lipid peroxidation[4]. As in the earlier discussions, during lipid peroxidation polyunsaturated fatty acids present in the seed are converted to free radicals. Auto-oxidation of lipids will yield the hydroperoxides or lipoxygenase enzyme to act on free radicals and produces the hydroperoxide compounds. Then these compounds follow a series of reactions leading to the formation of more free radicals, hydroperoxides, volatile aldehydes, alcohols and ketones. Free radicals formed during lipid peroxidation have potential to create deteriorative changes in the cellular membranes resulting in the leakage of intra-cellular compounds and subsequent loss in vigour and viability

of seed[46, 47]. The mechanisms that have been suggested for loss of vigour are impairment of membrane function, mitochondrial disfunction, inhibition of protein synthesis, damage to enzyme systems, damage to nuclear material including DNA, RNA and chromosomes and biochemical change to nuclear material including DNA, RNA and chromosomes and biochemical changes resulting in lower levels of ATP[48-50].

Many tests have been suggested for determining seed vigour but only few are broadly accepted[51]. Harman and Mattick[4] measured both quantities of unsaturated fatty acids and production of volatile aldehyde compounds during germination of pea seeds and found that with increased ageing a decrease in concentrations of unsaturated fatty acids; and increase in production of volatile aldehyde compounds. They also suggested that these changes are more closely parallel in decreasing seed vigour than seed viability. Thus, release of volatile compounds may be taken as a highly sensitive indication of active phase of physiological ageing and potential loss in vigour of seed[19].

Many researchers have demonstrated a relationship between volatile compounds released and seed vigour in natural and accelerated aged seeds[20, 22, 35, 37, 38, 52]. Most seed evolve volatile compounds until three to four days of imbibition[26] and the peak production occurs during first and second days[26, 37]. In soybean low vigour seed produces larger number of volatile aldehydes and alcohols during germination than high vigour seeds20,22,38. Volatile aldehyde production is up to 30-fold higher in aged than in unaged pea seeds. This was 3 to 7 fold greater in poor quality soybean seed lots than the one with high quality[20]. Taylor *et al.*[53] suggested the use of volatile compounds as biochemical markers for estimation of seed quality and these volatiles are produced metabolically even in dry seeds[23].

The light - dark regime apeared around seed have little effect on volatile emission. But temperature has profound effect; for example in rice and barley, seeds stored at - 10°C had evolved no volatiles, but elevation of temperature to 80°C for 20 min caused emission of certain volatiles from both the seeds, suggesting that volatiles are either not released or trapped with in seed itself at subzero temperatures[23].

Bioassay of volatile compounds

A novel and inexpensive method of bioassay has been developed to demonstrate the gaseous emanation of volatiles released from deteriorating seeds[52]. In this method, germinating bioassay seed placed in a airtight gas jar is exposed to the gaseous emanations of germinating stock seeds having different vigour levels. The deteriorated or low vigour seeds of stock material produce larger quantities of volatile growth inhibiting substances during germination. The germination of bioassay seeds gets affected due to these volatiles. The quantities of volatiles released are measured by measuring the rate of reduction in germination of bioass ay seed lot[20, 52, 54-56]. The important components of these gaseous emanations are volatile aldehydes[20,52].

Methods of volatiles detection

The volatile compounds evolved by seeds can be detected by various methods which include active or passive trapping and concentration techniques[20, 37-39, 57], gas chromatography[22, 23], gas liquid chromatography[19, 25], gas chromatography - mass spectrometry[21, 23, 41, 58], gas chromatography - FID and gas chromatography - alfactometry[15], high performance liquid chromatography and thin layer chromatography[39, 59], and bioassay[52, 55].

Future thrust

In general deteriorating seed releases more volatile compounds either through lipid peroxidation or auto-oxidation process and they are toxic to the nearby healthy vigorous seeds. To avoid this damage, as a precautionary measures it is necessary to prevent / slowdown the auto-oxidation process initiated in seeds soon after the attainment of maturity and

subsequent harvest by treating them with specific antioxidants. The recent studies had shown that volatile compounds produced in a number of seed species also have the beneficial effect on protecting the phytosystem by way of pest and disease control. Hence much importance has to be given for further studies in the areas of detection of newer volatile compounds emanated from various plant parts, dry as well as germinating seeds. This will pave the way for identifying suitable seed treating chemicals for the control of seed deterioration as well for synthesizing pest attractant (pheromones) that are considered more important under biological system of pest control in crop plants.

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