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Lipid Degradation During Seed Deterioration

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Seeds contain two major types of lipids: storage and functional. Storage lipids are primarily neutral triglycerides. Functional lipids can be grouped in several classes: phospholipids, glycolipids, sterols, sterol esters, sterol ester glucosides, etc., and are present in membranes, subcellular organelles, and other compartmentalized structures. Storage lipids, especially triglycerides, serve as reservoirs of energy to be mobilized by specific enzymes upon damage to the seed by bruising, disease, other stress factors, or by germination. When seeds are damaged by improper storage conditions or are exposed to certain microorganisms, lipid degradation reactions can occur. These reactions can be catalyzed by their own endogenous enzyme systems, or by enzymes from the microorganisms, depending upon the environment and/or the extent of the damage. Lipase and lipoxygenase are the two principal enzymes involved in degradation of lipids in seeds.

Hydrolysis of triglycerides is catalyzed by lipases, which are ubiquitous. Animal and plant lipases hydrolyze ester bonds in the 1 and 3 positions of the glycerol moity. Pancreatic lipase is an example of this type of enzyme. Lipases found in microbes can hydrolyze all three ester bonds; however, the middle position is generally hydrolyzed more slowly. In general, plant lipases have been studied less extensively than animal lipases. Most reports of lipase activity in seeds have been devoted to lipases of oilseeds because activation of these enzymes can adversely affect economic utilization of the oil. Among these adverse effects are changes in flavor of food products, increased acidity of the oil, and release of unsaturated fatty acids that are oxidized by the activation of another enzyme, lipoxygenase. This enzyme catalyzes the formation of polyunsaturated fatty acid hydroperoxides by adding oxygen at their double bonds. Thus, these two enzymes catalyze the major changes that occur in the lipids of deteriorating seeds. This report briefly describes some properties of these enzymes.

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Lipases

Oilseeds are rich sources of triacylglycerides. In the germinating seed these lipids are important sources of energy. Lipases catalyze the breakdown and metabolism of these triglycerides to glycerol and fatty acids which can then be oxidized to provide energy for the newly emerging plant. Despite economic importance of the oil from soybeans, cottonseed, peanuts, corn, safflower, and tung seeds, most research in oilseeds has been done on the acid lipase of the castor bean, an industrial oilseed that contains an active lipase in the ungerminated seed. There are a few reports on lipases in other industrial oilseeds (eg, soybean, cottonseed, corn, safflower, coconut, sesame, and peanut) (3).

The acid lipase of the dormant castor bean has been studied extensively by Ory and associates, as reviewed by Ory (9). The enzyme rapidly hydrolyzes its endogenous substrate, castor oil, in vivo (11,19) and in vitro (12). The pH optimum of the lipase is 4.0–4.2 with a macerate of the seed acting on endogenous castor oil as substrate (12) and is 4.5 with a partially purified enzyme preparation and cottonseed oil as substrate (13). In a pathological situation, such as damaged or diseased seeds in storage, this enzyme would likely be active. Acid lipase activity in castor beans germinated for 4 days also exhibits a pH 4.5 optimum, confirming that highest activity is between pH 4.0 and 5.0. The pH optimum does not vary between 25 and 40 C and only about 20% of its activity is lost after 30 min at 60–75 C (13). The enzyme stability at rather high temperatures would be conducive for rapid deterioration of the oil during storage of this oilseed.

The effects of metal ions and organic inhibitors on castor bean lipase activity were tested. Only sulfhydryl group reactants inhibited the acid lipase at very low concentrations. Mercuric ion and *p*-chloromercuribenzoic acid, potent sulfhydryl inhibitors, blocked 65–100% of the activity (12). Calcium, copper, and lead inhibited the enzymes slightly, but only at high concentrations. The acid lipase is, therefore, a sulfhydryl-sensitive enzyme with cysteine in its active site.

Ory et al (13) showed that the acid lipase rapidly hydrolyzed all triglycerides from C-4 and C-18 chain lengths, but not fatty esters such as butylricinoleate, thus confirming that this enzyme is a true

lipase, not an esterase. With synthetic triglycerides having oleic and palmitic acids alternately in the 2 and 1,3 positions as substrates, results of analysis by gas chromatography suggested immediate cleavage of 1- and 3-position fatty acids and cleavage at the 2-position fatty acids after 7–10 min (10). Other results suggested that hydrolysis of the C-2 fatty acids may occur after isomerization to the C-1 (or C-3) position. Borgstrom and Ory (2) later employed radioactive-labelled substrates to study reaction intermediates and end products within the first 20 min of the reaction and suggested that isomerization of 1,2-diolein to 1,3-diolein may be catalyzed by castor lipase or an isomerase. Subsequently, Noma and Borgstrom (8) used $^{14}C/^{3}H$-doubly labelled substrates in various combinations and examined the intermediates and end products. They confirmed the rapid, complete hydrolysis of triglyceride and formation of 1,3-diglyceride from 1,2-diglyceride and concluded that the reaction proceeded, not by direct isomerization of the 1,2-diglyceride and 2-monoglyceride, but by cleavage of the 2-position fatty acids and reacylation of the 1 and 3 positions. It is, therefore, apparent that if seed lipase activity is initiated in oilseeds due to disease, stress, or germination, the degradation of triglycerides will go completely to fatty acids and glycerol.

A neutral castor bean lipase was reported by Yamada (26). St. Angelo and Altschul (19) examined lipase activity in the dormant and germinating castor beans for 8 days and were unable to detect neutral lipase activity. A reinvestigation of the castor bean neutral lipase was undertaken by Ory and St. Angelo (11). Castor beans were carefully dusted with fungistats prior to germination to prevent possible microbial lipase activity, but all other conditions of Yamada were employed. No neutral lipase activity was detected. This inability to detect neutral lipase activity in castor beans suggested that this lipase may have been derived from some other source, possibly microbial.

In 1978, DeLucca et al (5) reported the isolation and identification of lipolytic microorganisms found on rough rice grown in Louisiana and Arkansas. Approximately 10% of the total bacteria isolated were lipolytic, nonsaccharolytic, alkali-producing pseudomonads. All molds showed various amounts of lipase activity as determined by the size of the lipolytic zones. Thus, microbial lipases, such as identified in their study, could act on the rice and ultimately produce off-flavors or cause rancidity during prolonged storage.

In other studies on microbial lipases, Dirks et al (6) compared the lipase of wheat germ to those found in *Aspergillus* and *Pencillium*. Their results suggested that fungal lipases may be responsible for the observed fatty acid hydrolysis in cereal grains. They also devised a method for differentiating between the mold lipase and those found in wheat germ by using inhibitors. In general, most of the microbial lipases appear to be serine enzymes that are inhibited by diisopropyl fluorophosphate (DFP) (3). This is in contrast to castor bean lipase, a sulfhydryl enzyme, which is not inhibited by DPF, but is inhibited by mercurial compounds that bind SH groups (9).

Ramakrishnan and Banerjee (16) found that fungal lipases grown on oilseeds were more active than the endogenous seed lipases. They compared lipases from castor seeds and showed that the fungal lipases were most active at pH 6.2–7.2. The castor bean lipase has an optimum pH at 4.2 (12). Activity of the fungal lipase increased with age of the seedling to a certain point before decreasing, whereas that of the lipase from these infested seeds decreased steadily.

Lipoxygenases

Lipoxygenase (E.C.1.13.1.13) is found in most fruits, vegetables, and oilseeds; however, the most active enzyme is found in soybeans. Lipoxygenase is specific in that it reacts only with *cis, cis*-1,4-pentadiene structures, such as linoleic, linolenic, or arachidonic acids and it degrades either the free acid, the triglyceride, or methyl or ethyl esters. The primary products are optically active *cis-trans*-conjugated hydroperoxides (14). These hydroperoxides are formed via a free radical mechanism, and either decompose or are further oxidized to secondary products, such as alcohols, acids, ketones, and/or aldehydes, which can adversely affect nutritive value, flavor, and quality of foods. Free radicals generated by these oxidative mechanisms have also been implicated in reactions that can lead to pathological changes in animal and human tissues (18).

Primary interest in lipoxygenase has been on its effects on sensory characteristics of foods and the mechanism of action; its physiological function remains a mystery. Recent reports (1,27) suggest that the enzyme may be involved in the synthesis of plant hormones; particularly, of prostaglandinlike compounds. Also, lipid oxidation reactions are associated with seed aging and death (7).

In soybeans, lipoxygenase can be isolated from a soluble fraction, has a molecular weight of approximately 100,000, contains a single polypeptide chain, and has one atom of iron per mole. There are several isozyme forms whose activities have pH optima ranging from 6 to 9. When linoleic acid is the substrate, the enzyme forms predominantly the 13-hydroperoxide rather than the 9-isomer. Chan et al (4) showed that the individual positional isomers of methyl linoleate can decompose into hexanal, methyl, octanoate, 2,4-decadienal isomers, and methyl 9-oxononanoate. St. Angelo et al (22) separated and identified eight products (pentane, pentanal, 1-pentanol, hexanal, 2-heptanone, 2-pentylfuran, and two decadienal isomers) from a peanut lipoxygenase/linoleic acid mixture by using direct gas chromatography/mass spectrometry. All of these compounds have been implicated in flavor problems of vegetable food products (15).

St. Angelo and Ory (23) partially purified peanut lipoxygenase by ammonium sulfate fractionation. With linoleic acid as the substrate, its pH optimum was 6.2. It appeared to be unstable, even when stored below freezing. Approximately 30% of the activity was destroyed by incubating the enzyme for 30 min at 36 C; all activity was lost at temperatures above 40 C. Sanders et al (17) isolated three isozymes from raw peanuts. Two had pH optima of 6.2 and the third had a pH optimum of 8.3. Surprisingly, the molecular weight of each isozyme was the same, 73,000. Depending upon the conditions of the assay and the presence or absence of Tween-20, calcium acts as an activator or inhibitor of lipoxygenase.

Tappel (25) reviewed the inhibitory effects of polyphenolic antioxidants, including tocopherols, nordihydroguaiaretic acid, propyl gallate, hydroquinone, and α-naphthol, on plant lipoxygenase. Other inhibitors include a catechol-like tannin from peanut skins, and erucic acid, an unsaturated fatty acid with 22 carbons and a *cis* double bond at the C-13 position (21). Several other long-chain *cis* monounsaturated fatty acids also inhibit soybean lipoxygenase (24). Cyanide does not affect the activity of the enzyme (20).

Conclusion

The major enzyme systems in lipid degradation in deteriorating seeds are lipases and lipoxygenases. Very few seeds have an active lipase in the quiescent seed. Lipases may arise through the activation of preexisting materials, de novo synthesis, or through production by microorganisms. In each of these, the lipase mechanisms are very similar and the end result is the same—an increase in free fatty acids. The microbial lipases hydrolyze all three positions on a triglyceride quite rapidly. In contrast, seed lipases hydrolyze the 1 and 3 positions rapidly, but hydrolyze the 2 position much more slowly. Selective inhibitors may prove useful in determining the biological origin of lipases.

Lipoxygenases oxidize polyunsaturated fatty acids and esters to hydroperoxides, which degrade to ketones, aldehydes, acids, and other low-molecular-weight compounds. Several of these compounds cause off-flavors and odors in stored seeds. These compounds can also react with proteins, amino acids, and vitamins, further lowering seed quality. In addition to reacting with polyunsaturated fatty acids to form hydroperoxides, lipoxygenase has been implicated in the synthesis of plant hormones, such as prostoglandinlike compounds, and may play an important function in seed aging. Although the enzyme is involved in many reactions, its biological role is unknown.

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