CHAPTER 8

FUNGAL DETERIORATION AND RELATED PHENOMENA IN CEREALS, LEGUMES AND OILSEEDS

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ABSTRACT

Fungi are a major cause of postharvest deterioration of cereals, legumes and oilseeds. Concern over fungal invasion of these grains has intensified over the last 17 yr because mycotoxins (toxic metabolites produced by filamentous fungi) are being detected in some commodities. Apparently, the harvesting of more and more grain at moisture levels too high for safe storage has contributed to the mycotoxin problem. The fungi that invade grains are generally grouped into two categories: (1) field fungi which include species of Alternaria, Cladosporium, Fusarium, and Helminthosporium; and (2) storage fungi which are predominantly species of Aspergillus and Penicillium. This division is based primarily upon moisture requirements. In addition to moisture, other factors such as temperature, O2–CO2 atmosphere, aeration, pH, and grain condition interact to affect fungal growth. Fungal deterioration of grain is a dynamic ecological process which often involves a succession of microorganisms, the breakdown of organic matter to yield CO2 and H2O, and the generation of heat. Nutrients are lost because of changes in carbohydrates, protein, lipids and vitamins. Germinability is lost and aesthetic changes occur which include discoloration, caking and abnormal odors. Also, mycotoxins such as aflatoxin, ochratoxin, citrinin, zearalenone, T-2 and vomitoxin may be produced and are capable of eliciting a toxic response when ingested. Usually these deteriorative changes in grains are prevented by reducing the moisture content to a level too low for fungi to grow. High-temperature drying has been feasible in the past, but as fuel becomes more expensive and less available, alternate methods to control fungal deterioration will be required. Low-temperature drying, solar drying, chilling, controlled atmosphere storage, chemical preservatives and various combinations of these control methods have application.
INTRODUCTION

A major problem of agricultural production is loss of grain during and after harvest. Microorganisms, insects, and rodents contribute greatly to these postharvest losses. A commonly quoted estimate provided by the FAO for worldwide losses for all cereals, leguminous seeds, and oilseeds is 10% (Janicki and Green 1976). Christensen and Kaufmann (1974) state that fungi are the major cause of spoilage in stored grains and seeds in the technologically advanced countries, because insects and rodents are effectively controlled. Over the past 3 yr, the Agricultural Stabilization and Conservation Service (1976) reported that mold deterioration of the containers occurred in 25% of the shipments of Corn Soya Milk (CSM) under the Food for Peace Program. This spoilage resulted in losses estimated at 3% of the total amount of CSM shipped.

Consequently, it is well established that fungi destroy food and feed. However, the basic problem remains of implementing effective measures to reduce fungal losses.

This review presents a general discussion of the kinds of molds, factors, and processes involved in fungal deterioration of plant material. The effects of fungal invasion of grains on nutritional quality, aesthetic value, and mycotoxins are discussed. Finally, attention is focused on control methods to reduce losses and conserve resources.

FUNGI AND GRAIN

The microflora of cereals (including oilseeds) is made up of a wide variety of fungi, bacteria and actinomycetes. These microorganisms are the same as those found in soil, air, and on or in living or dead plants and animals (Semeniuk 1954). Christensen and Kaufmann (1969) reported that more than 150 species of fungi have been isolated from within seeds or kernels. Fungi are generally ubiquitous and omnivorous (Wallace 1973). However, the exact mechanism and time of fungal invasion of seeds is unclear. Christensen (1957) grouped fungi that invade cereals into two categories: (1) field fungi and (2) storage fungi. This division is not taxonomically valid but is based primarily upon moisture requirements. Field fungi attack developing and mature seeds which contain at least 20% moisture or are at an equilibrium relative humidity (RH) of 90–100%; storage fungi are usually encountered when grain is stored after harvest at moisture levels of 13–20% or in equilibrium with RH of 70–90%. The major field fungi are species of Alternaria, Cladosporium, Fusarium, and Helminthosporium, although species of Curvularia, Stemphylium, Epicoc-
cum, and Nigrospora infect seed at or near harvest and are included in this group. These fungi may discolor the grain, weaken or kill the embryo, or cause seedling blight, scab, or other disease. A few species of Fusarium and Alternaria may produce toxins in the invaded grain (Christensen et al. 1968). Nevertheless, species of Alternaria, Helminthosporium, and Cladosporium commonly occur in freshly harvested seeds.

Storage fungi are predominantly species of Aspergillus and Penicillium. "Species" of Aspergillus are not always well defined and are sometimes referred to as "groups." The major storage fungi consist of five or six groups of Aspergillus, plus several species of Penicillium which are common until deterioration is well advanced (Christensen and Kaufmann 1974). Certain other species of Penicillium are considered field fungi (Mislivec and Tuite 1970). Wallace (1973) lists 26 species of Aspergillus and 66 species of Penicillium which have been isolated from stored grain and grain products.

Over the past 20 yr, Christensen and Kaufmann (1974) have tested thousands of samples of cereal grains from commercial bins in the United States, Mexico, South America, and several European countries. Two groups consistently associated with beginning or incipient deterioration have been A. restrictus and A. glaucus. In grain where the equilibrium RH is less than 78–80%, these are the only species that can grow. However, in grain with an equilibrium RH above 80%, an ecological succession usually occurs; A. restrictus and A. glaucus appear first and may be followed by A. candidus, A. flavus, A. ochracus, A. versicolor, and Penicillium spp. Each species has a rather sharp lower limit of moisture equilibrium, below which it cannot grow. Table 8.1 reflects the minimum equilibrium RH at which different common storage fungi can grow.

In contrast to the Aspergilli, the Penicillia require more moisture and vary little in color, ranging through shades of blue to green and grey. The Penicillia are frequently referred to as "blue" or "green" molds or Penicillium spp. because they are difficult to identify (Wallace 1973).

Other molds included in the storage fungi category are Absidia, Mucor, Rhizopus, Chaetomium, Scopulariopsis, Paecilomyces, and Neurospora. Absidia, Mucor, and Rhizopus are generally associated with spoilage under moist conditions because they require a minimum RH of 88% for growth; consequently, they are not usually initiators of grain deterioration in storage (Wallace 1973).

For the most part, the above categories are accurate; however, exceptions exist. A. flavus can invade in the field and Fusarium can continue to decay grain in storage if the moisture is high enough (Lillehoj et al. 1975A, 1976; Caldwell and Tuite 1974).
TABLE 8.1
APPROXIMATE MINIMUM EQUILIBRIUM RELATIVE HUMIDITY FOR GROWTH OF COMMON STORAGE FUNGI

<table>
<thead>
<tr>
<th>Mold</th>
<th>RH Limit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus halophilicus</td>
<td>68</td>
<td>Christensen and Kaufmann (1974)</td>
</tr>
<tr>
<td>A. restrictus group</td>
<td>70</td>
<td>Christensen and Kaufmann (1974)</td>
</tr>
<tr>
<td>A. glaucus group</td>
<td>73</td>
<td>Christensen and Kaufmann (1974)</td>
</tr>
<tr>
<td>A. chevalieri</td>
<td>71</td>
<td>Ayerst (1969)</td>
</tr>
<tr>
<td>A. repens</td>
<td>71</td>
<td>Ayerst (1969)</td>
</tr>
<tr>
<td>A. candidus group</td>
<td>80</td>
<td>Christensen and Kaufmann (1974)</td>
</tr>
<tr>
<td>A. candidus</td>
<td>75</td>
<td>Ayerst (1969)</td>
</tr>
<tr>
<td>A. ochraceus group</td>
<td>80</td>
<td>Christensen and Kaufmann (1974)</td>
</tr>
<tr>
<td>A. flavus group</td>
<td>85</td>
<td>Christensen and Kaufmann (1974)</td>
</tr>
<tr>
<td>A. flavus</td>
<td>78</td>
<td>Ayerst (1969)</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>78</td>
<td>Ayerst (1969)</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>82</td>
<td>Ayerst (1969)</td>
</tr>
<tr>
<td>Penicillium, depending on species</td>
<td>80-90</td>
<td>Christensen and Kaufmann (1974)</td>
</tr>
<tr>
<td>P. cyclopium</td>
<td>82</td>
<td>Ayerst (1969)</td>
</tr>
<tr>
<td>P. martensii</td>
<td>79</td>
<td>Ayerst (1969)</td>
</tr>
<tr>
<td>P. islandicum</td>
<td>83</td>
<td>Ayerst (1969)</td>
</tr>
</tbody>
</table>

FACTORS INFLUENCING FUNGAL GROWTH AND DETERIORATION OF GRAIN

The principal factors which control fungal growth and deterioration are moisture, temperature, atmosphere, aeration, pH and condition of the grain. All of these factors interact as deterioration progresses, but moisture and temperature are probably most important.

Moisture

If the moisture content is maintained at a sufficiently low level, grain can be stored for many years with little deterioration even under otherwise unfavorable storage conditions (Pomeranz 1974). However, modern farming practices and harvesting techniques yield grain at moisture levels too high for safe storage. This grain must be dried, protected by airtight storage, or preserved if fungal growth is to be prevented.

The most useful measure of the availability of water to fungi is the ratio of the vapor pressure of the water in the substrate to that of pure water at the same temperature and pressure. This figure is referred to as water activity (a w) or when it is expressed as a percentage, as the equilibrium RH. Pomeranz (1974) reports that an RH of 75% is about minimum for the
germination of most mold spores at ordinary temperatures and that the equilibrium moisture content of different grains (Table 8.2) at 75% RH may vary markedly because of differences in composition. Consequently, the critical moisture level for a particular grain is the percentage moisture when the seed is in equilibrium with an atmospheric RH of approximately 75%. The equilibrium RH of grain is more important than the moisture content for controlling fungal deterioration.

Despite the fact that grain may be uniform and within what is normally considered a safe moisture limit at the outset of storage, fungal deterioration may still result because of excessive moisture. Differences in temperature between different portions of a grain bulk can result in a rapid transfer of moisture from warmer to cooler regions. RH of interstitial air in stored grain tends to remain in equilibrium with moisture in the grain. At any RH, the actual amount of water vapor per cubic foot of air increases with rising temperature. Thus, when warm air reaches a cool region, it gives up moisture to the grain to maintain equilibrium. This moisture interchange usually takes place in the vapor phase; but with extreme temperature differences, the warm air may be cooled below the dew point and water will condense on the surface of the grain or bin. Christensen and Drescher (1954) reported a range in moisture contents from a low of 10% to a high of 18% in a bin of wheat in which the moisture content of a representative sample was 13.2%. Ramstad and Geddes (1942) found soybeans with a moisture content of 28% in a bin where the average moisture content of the beans was 15%. Numerous other examples of moisture transfer are in the literature (Johnson 1957; Holman 1950; Christensen

<table>
<thead>
<tr>
<th>Grain</th>
<th>Moisture Content¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td>15.3</td>
</tr>
<tr>
<td>Rye</td>
<td>14.9</td>
</tr>
<tr>
<td>Wheat</td>
<td>14.6</td>
</tr>
<tr>
<td>Barley</td>
<td>14.4</td>
</tr>
<tr>
<td>Corn</td>
<td>14.4</td>
</tr>
<tr>
<td>Rice</td>
<td>14.4</td>
</tr>
<tr>
<td>Oats</td>
<td>13.9</td>
</tr>
<tr>
<td>Soybeans</td>
<td>13.5²</td>
</tr>
<tr>
<td>Cottonseed</td>
<td>11.4</td>
</tr>
<tr>
<td>Sunflower seed</td>
<td>10.5²</td>
</tr>
<tr>
<td>Peanuts</td>
<td>10.5</td>
</tr>
<tr>
<td>Flaxseed</td>
<td>10.0</td>
</tr>
</tbody>
</table>

¹Adapted from data of Milner and Geddes (1954).
²Adapted from Christensen (1972).
Consequently, the actual moisture content of the grain in localized areas of the bin is important rather than the moisture content of the grain when loaded into the bin.

**Temperature**

Growth of fungi is accelerated by an increase in temperature until such factors as thermal inactivation of enzymes, exhaustion of substrate, oxygen or moisture depletion, or accumulation of carbon dioxide become limiting. The interrelationships of these factors are so complex that determination of minimum, optimum, and maximum temperatures for growth of fungi is approximate. Nevertheless, Christensen and Kaufmann (1974) have summarized the temperature limits for growth of common storage fungi (Table 8.3).

There is a balance between safe moisture content and safe temperature in the grain bulk. Within limits, low temperature can be substituted for low moisture in prolonging the storage of grain. Burrell (1974B) reported that *A. glaucus*, several species of *Penicillium, Cladosporium, Fusarium*, and *Mucor*, and some yeasts grow at −5° C to −8° C, and in some instances sporulate at temperatures below freezing. Also, fungal toxins can be produced on moldy grain at low temperature (Joffe 1965; Kurtzman and Ciegler 1970). Nevertheless, at a given moisture content, grain can be stored longer at lower temperatures. Fig. 8.1 from the work of Steele et al. (1969) gives an example of the length of time that corn can be stored under various conditions of temperature and moisture before fungi destroy 0.5% dry matter. However, extrapolation of the data of Steele et al. (1969) to field recommendations is risky because of the specific conditions (aeration, RH, and mechanical damage) under which their experiments were conducted.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Temperature for Growth, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td><em>Aspergillus restrictus</em></td>
<td>5−10</td>
</tr>
<tr>
<td><em>A. glaucus</em></td>
<td>0−5</td>
</tr>
<tr>
<td><em>A. candidus</em></td>
<td>10−15</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>10−15</td>
</tr>
<tr>
<td><em>Penicillium</em></td>
<td>−5−0</td>
</tr>
</tbody>
</table>

1 From Christensen and Kaufmann (1974).
Atmosphere

Generally, it is accepted that fungi are aerobic microorganisms and that oxygen depletion or carbon dioxide build-up limits their activity. Detroy et al. (1971) state that reducing oxygen from 5 to 1% dramatically inhibits growth of *A. flavus* and aflatoxin formation, while Christensen and Kaufmann (1974) reflect that a carbon dioxide concentration above 14% is...
detrimental to mold growth. Nevertheless, some fungi can grow at low oxygen concentrations. Tabak and Cooke (1968) tested a number of fungi, including *Fusarium*, under conditions “as anaerobic as could be maintained in the laboratory” and all fungi grew to some extent. Peterson *et al.* (1956) reported that growth of storage fungi was reduced with decreasing oxygen concentration, but some growth occurred down to about 0.2% oxygen. Below a minimum oxygen level, fungal growth will cease, but this does not mean that the fungus is killed.

**Aeration**

Aeration of grain consists of blowing or drawing ambient air at a low rate through the grain mass. This practice almost seems self-defeating because a favorable O2–CO2 environment is ensured for fungal growth. However, the main function of aeration is to establish and maintain a moderately low and uniform temperature throughout the bulk. Burrell (1974A) reported that fungal growth rarely occurs during storage periods up to 8 months in bulks of cooled grain at 5–10°C if the moisture content is below about 18%. Also, Burrell (1974A, B) states that no change in fungal counts occurred during aerated storage of wheat and barley up to 18% moisture. However, it is clear that aeration as a means for slowing fungal attack is only effective in cool climates where the grain is not over 18% moisture content. In warmer climates, fairly rapid drying to 14% moisture or less is necessary to prevent fungal deterioration.

**The Grain and Its Condition**

Substrate is a determining factor in fungal deterioration. Most grains, leguminous seeds, and oilseeds support mold growth. Nutritional investigations have shown that simple carbon and nitrogen sources, plus selected mineral salts, provide the necessary nutrients for growth of most fungi (Detroy *et al.* 1971). Most molds can grow over a wide pH range (2–8.5) but an acid pH usually favors growth (Frazier 1967). However, species of *Scopulariopsis* grow best under alkaline conditions (Bothast *et al.* 1975B).

Condition or soundness of grain can be critical in establishing fungal invasion. Condition of grain is influenced by the environment during growth and maturation, by the degree of fungal invasion, by the maturity at harvest, by methods of harvesting, and by handling prior to and after storage, e.g., during drying and during international shipment, stress cracks and mechanical damage or breakage predispose grain to fungal invasion (Thompson and Foster 1963; Steele *et al.* 1969). Also, varietal differences may influence fungal attack. Softer types of wheat respire more rapidly than harder types at similar moisture levels and tempera-
tures (Pomeranz 1974). With so many interacting factors affecting fungal growth, it is clear why it is difficult to establish an absolute maximum moisture limit for the safe storage of grain.

**RESPIRATION AND HEATING**

When fungal deterioration of grains occurs, energy is required. This energy is normally produced in the presence of oxygen by the respiratory process. Respiration involves the breakdown of organic matter (carbohydrate-hexose) to yield carbon dioxide, water, and the generation of heat. However, in the absence of oxygen, the process is less complete (less CO₂, H₂O, and heat) and other compounds such as acetic acid or ethyl alcohol are produced. The direct effects of respiration on grain are loss in dry matter, gain in moisture content, increase in CO₂ in the intergranular air, and a rise in temperature of the grain (Milner and Geddes 1954).

Early research on respiration of moist grain did not distinguish between respiration of the grain itself and respiration of the microflora on and within the grain (Bailey and Gurjar 1918). However, later work by Ramstad and Geddes (1942) and Milner and Geddes (1945) showed that fungi are primarily responsible for respiration and heating of moist soybeans. Hummel *et al.* (1954) found in a study with mold-free wheat that respiration at moisture contents of 15–31% and a temperature of 35°C was low and constant. Consequently, the evidence shows that the respiratory activity of seed or grain itself usually plays only a minor role, whereas fungi are often solely responsible for the initial heating of moist grains and moist plant material.

The succession of microorganisms during the heating of moist (30% moisture) agricultural materials has been studied extensively (Carlyle and Norman 1941; Norman *et al.* 1941; Wedberg and Rettger 1941; Gregory *et al.* 1963; Gray *et al.* 1971). At the start of the process, the indigenous mesophilic flora (molds, yeasts, bacteria-growing best between 20–40°C) multiply and the temperature rises. At approximately 40°C, the activity of the mesophiles diminishes and degradation continues by thermophiles. At 60°C, the thermophilic fungi die and the process is kept going by certain spore-forming bacteria and thermophilic actinomycetes to maximum of 70°C. Thereafter, heating cannot be attributed to microorganisms but to chemical processes (Milner and Geddes 1946; Milner *et al.* 1947; Currie and Festenstein 1971).

These investigations are not directly applicable to stored grain because respiration and heating of grain begin at lower moisture levels (*i.e.*, a moisture content in equilibrium with a relative humidity of 70–75%)
where only certain molds can grow. Christensen and Kaufmann (1974) effectively described the succession of fungi during the heating of grain: "Either some of the grain is moist enough when stored, or through moisture transfer later acquires a high enough moisture content, so that *Aspergillus restrictus* and *A. glaucus* can grow. *A. restrictus* grows so slowly that it probably does not increase either the temperature or moisture content of the grain appreciably. *A. glaucus*, however, if growing rapidly, can increase the temperature of the grain at least to 30–40°C. This results in some increase in moisture content in the grain where the fungus is growing, and a greater increase in the grain just above. Once the moisture content of the grain exceeds 15.0–15.5%, *A. candidus* can grow; and given optimal conditions, it can increase the moisture content and temperature of the grain rapidly. Once the moisture content of the grain reaches that in equilibrium with a relative humidity of 85% (18.5% moisture in the cereal seeds), *A. flavus* can grow. *A. candidus* and *A. flavus* together can increase the temperature of the grain to 55°C and hold it there for weeks. Depending on whether the metabolic and distillation water from the activities of these fungi is carried off or whether it accumulates in the grain, the heating may gradually subside, or may pass into the next stage in which thermophilic bacteria plus perhaps a variety of thermophilic fungi may be involved."

Milner and Geddes (1946) made simultaneous measurements of seed viability, mold infection, chemical changes, respiratory exchange, and temperature increase in aerated soybeans containing 22.8% moisture under near adiabatic conditions. Fig. 8.2 shows the course of temperature increase, CO₂ evolution, and fungal infection. The first heating stage is a result of fungal metabolism and ends when the thermal death range (50–55°C) of certain molds (predominantly *A. glaucus* and *A. flavus*) is reached. The second heating stage may initially involve thermotolerant fungi (*A. fumigatus*, *Mucor pusillus*, etc.), but as the temperature increases the molds and seeds are killed. The rapid heating and CO₂ production after 14 days is due to chemical oxidation which follows death of microorganisms.

As more high-moisture grain is harvested and alternatives to costly high-temperature drying are adapted, the heating process in grain may be modified. For example, in our studies on preservation of high-moisture corn (Bothast *et al.* 1975A), the temperature of 1,500 bu (38 metric tons) of ammonia-treated corn (27% moisture content) increased from 25–60°C (Fig. 8.3). Chemical heating was probably responsible for the initial rise in temperature, but subsequent heating was by bacterial respiration. Apparently, fungi contributed little to the heating of this grain.
FIG. 8.2. THE COURSE OF SPONTANEOUS ADIABATIC HEATING, RESPIRATION, AND FUNGAL INFECTION EXHIBITED BY ILLINI SOYBEANS, UNDER ADIABATIC CONDITIONS, AT 22.8% MOISTURE. THE AERATION WAS DOUBLED ON THE SEVENTH DAY

NUTRITIVE CHANGES

Fungal activity can cause changes during storage of grain and grain products that are detrimental to nutritive value (Zeleny 1954). Specifically, nutrients are lost because of changes in carbohydrates, protein, lipids, and vitamins (Semeniuk 1954). Pomeranz (1974) has reviewed some of these changes.
Carbohydrates

Conditions that favor fungal activity lead to carbohydrate decomposition. Sugars are consumed and converted into $\text{CO}_2$ and $\text{H}_2\text{O}$. At moisture levels of approximately 15%, grain loses both starch and sugar and the dry weight decreases.

Ramstad and Geddes (1942) found a marked increase in reducing sugars in soybeans stored at more than 15% moisture. The increase was followed by an equally significant decrease in nonreducing sugars. Milner and Geddes (1946) demonstrated that sugars in stored soybeans disappear during the biological phase of heating, but that reducing substances increase when the heating has advanced to chemical oxidation. Bottomley et al. (1950, 1952) reported a marked disappearance of nonreducing sugars in corn stored at high moisture levels. The nonreducing sugars are converted...
into reducing sugars by the action of invertase or similar enzymes, which are produced by molds and by amylase which is normally present in corn.

Fig. 8.4 shows the relationship between the number of viable mold spores in the corn and the content of nonreducing sugars.

Glass et al. (1959) studied aerobic and anaerobic storage of wheat in the laboratory. When damp wheat was stored in air, extensive mold growth occurred and the increase in reducing sugars was only about one-fourth as great as the decrease in nonreducing sugars. The difference in sugars was attributed to metabolism by molds.

In an atmosphere of nitrogen, where mold growth was prevented, marked changes still occurred in the sugars. The decrease in nonreducing sugars was almost exactly compensated for by the increase in reducing sugars.
sugars. Lynch et al. (1962) stored 20% moisture wheat under various atmospheres for 8 wk at 30°C. In the sample stored in air, reducing sugars remained unchanged while sucrose decreased (Table 8.4). In wheat stored under nitrogen or CO₂, maltose remained unchanged, whereas fructose and glucose each increased threefold and galactose four to fivefold. The increase in galactose reveals that sucrose is not the only nonreducing sugar hydrolyzed during storage. It appears that the reducing sugars produced from the breakdown of nonreducing sugars in air are used by fungi.

In our studies (Lancaster and Bothast 1976) on preservation of high-moisture corn, *Scopulariopsis brevicaulis* invaded ammonia-treated corn and altered the sugar composition markedly. Sucrose decreased from 2.4% to 0.6% while reducing sugars increased from 0.21% to 0.77%.

**Protein**

The total protein content of grain as calculated from its nitrogen content is generally assumed to be constant during storage. However, as fungal deterioration advances and carbohydrate is used in the respiratory processes, protein increases mathematically. Daftary et al. (1970) demonstrated this by finding that the protein content (determined by the Kjeldahl method) was slightly, but consistently, higher in flours from mold-damaged samples than in corresponding flours from sound wheat.

Proteolytic enzymes produced by fungi can modify the proteins in grains by hydrolyzing them into polypeptides and amino acids. Subsequently fungi can convert these materials into fungal protein which can be nutri-

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**TABLE 8.4**

**CHANGES IN THE MONO- AND DISACCHARIDES OF WHEAT STORED UNDER VARIOUS ATMOSPHERES FOR 8 WK AT 30°C AND 20% MOISTURE**

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Control</th>
<th>Stored in Air</th>
<th>Stored in Nitrogen</th>
<th>Stored in Carbon Dioxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>6</td>
<td>5</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Glucose</td>
<td>8</td>
<td>3</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Galactose</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>54</td>
<td>21</td>
<td>39</td>
<td>36</td>
</tr>
<tr>
<td>Maltose</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Total reducing sugars, as maltose</td>
<td>41</td>
<td>41</td>
<td>117</td>
<td>117</td>
</tr>
<tr>
<td>Total nonreducing sugars, as sucrose</td>
<td>190</td>
<td>43</td>
<td>100</td>
<td>115</td>
</tr>
</tbody>
</table>

1Data of Lynch et al. (1962).
tionally beneficial to animals. Although these effects are only significant at advanced stages of deterioration, several investigators have reported qualitative transformations of free amino acids. DeVay (1952) observed changes in concentration of gamma-aminobutyric acid in hard red spring wheat stored at 19.5% moisture. Linko and Milner (1959) showed a considerable change in the composition of free amino acids of wheat which had been wetted.

Bothast et al. (1975B) reported that lysine, methionine, and proline increased in proportion to total amino acids while glutamic acid, leucine, alanine, and phenylalanine decreased when S. brevicaulis was grown on ammonia-treated corn. In contrast, Trolle and Pedersen (1971) reported that the lysine content of barley decreased during storage damage. Kao and Robinson (1972) investigated the changes in amino acid content of molded wheat. Arginine, cysteine, lysine, and histididine decreased while methionine increased.

Lipids

Because most molds have a high lipolytic activity, fats and oils in grain are readily broken down into free fatty acids and partial glycerides during the fungal deterioration of grains. These changes are greatly accelerated when moisture and temperature are favorable for fungal growth (Goodman and Christensen 1952; Loeb and Mayne 1952). Nagel and Semeniuk (1947) grew pure cultures of nine fungi on steam-sterilized corn containing 32% moisture and found that all fungi increased the free fatty acid content of the corn. Christensen and Dorworth (1966) reported that invasion of soybeans by storage fungi was accompanied by increases in free fatty acids. Consequently, the free fatty acid content of grain has been used as an index for estimating grain deterioration (Zeleny 1954).

Pomeranz et al. (1956) reported a 20% reduction in free lipids of wheat when the mold count increased a thousandfold. Daftary and Pomeranz (1965) studied changes in lipids of soft and hard wheat stored at moistures and temperatures conducive to mold growth. A decrease of 40% in total lipid content was accompanied by an increase in mold count from 1,000 to about 2,000,000 per gram. Nonpolar lipids decreased about 25% and damaged wheat contained only one-third as much polar lipids as did sound wheat. Also, grain deterioration was accompanied by rapid disappearance of glycolipids and phospholipids. The breakdown of polar lipids was more rapid and extensive than formation of free fatty acids or disappearance of triglycerides. Subsequently, Daftary et al. (1970) investigated the effects of temperature (23, 30 and 37° C) on the composition of wheat flours stored for 16 weeks at 18% moisture. Mold counts increased up to 10,000-fold.
Aspergillus niger, A. candidus, and A. vesicolor were predominant. Free lipids and the polar components of bound lipids decreased during storage.

Ramstad and Geddes (1942) stored soybeans in excess of 15% moisture in glass jars for 1 yr. All samples were moldy and showed decreased iodine numbers. Zeleny (1954) concluded that the oil from damaged soybeans is likely to be of inferior quality. Krober and Collins (1948) reported that the free fatty acid increase in damaged soybeans gives rise to high refinery losses. In response to this problem, List et al. (1977) developed methods to improve the quality of oil from damaged beans by increasing the deodorization temperature and subsequently removing high molecular weight flavor bearing compounds.

Recently we (Bothast et al., unpublished data) studied the effect of specific fungi on flavorful carbonyl compounds in soybeans. Species of Aspergillus, Penicillium, Mucor, Rhizopus, and Candida increased the total carbonyl and the monocarbonyl content of crude oil during controlled fermentations (Table 8.5).

Vitamins

Cereal grains and their products are important sources of vitamins in food and feed (Zeleny 1954). Generally cereal grains are good sources of thiamine, niacin, pyridoxine, inositol, biotin, pantothenic acid and vitamin E. Vitamin A activity of yellow corn, although low and unstable, is

<table>
<thead>
<tr>
<th>Culture</th>
<th>Total Carbonyls</th>
<th>Total Monocarbonyls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus chevalieri</td>
<td>8.89</td>
<td>1.85</td>
</tr>
<tr>
<td>A. amstelodami</td>
<td>11.12</td>
<td>1.79</td>
</tr>
<tr>
<td>A. niger</td>
<td>2.70</td>
<td>1.57</td>
</tr>
<tr>
<td>A. flavus</td>
<td>5.65</td>
<td>3.62</td>
</tr>
<tr>
<td>A. candidus</td>
<td>5.51</td>
<td>4.90</td>
</tr>
<tr>
<td>Penicillium meleagrinum</td>
<td>5.57</td>
<td>4.25</td>
</tr>
<tr>
<td>Rhizopus oligosporous</td>
<td>7.18</td>
<td>3.87</td>
</tr>
<tr>
<td>Mucor pusillus</td>
<td>8.17</td>
<td>1.87</td>
</tr>
<tr>
<td>Candida lipolytica</td>
<td>3.85</td>
<td>1.53</td>
</tr>
<tr>
<td>Phycomyces blakesleeanus</td>
<td>4.48</td>
<td>2.02</td>
</tr>
<tr>
<td>Autoclaved control</td>
<td>2.71</td>
<td>0.90</td>
</tr>
<tr>
<td>Nonautoclaved control</td>
<td>3.00</td>
<td>1.31</td>
</tr>
</tbody>
</table>

1Unpublished data of Bothast et al.
2Fermentation was conducted at 28°C for 12 days.
3Mean values of two trials.
important in animal feeds and may also be of significance in human nutrition. No other grain has appreciable vitamin A activity (Pomeranz 1974). Consequently, losses in vitamin content that occur during storage are of considerable practical importance.

Bayfield and O'Donnell (1945) showed that wheat lost approximately 30% of its thiamine in a 5-month storage period at 17% moisture. Kao and Robinson (1972) reported near 50% losses in thiamine in molded wheat. In contrast, this same wheat increased in riboflavin, vitamin B₆, vitamin B₁₂, and pantothenic acid. For the most part, it is believed that the B vitamins, with the possible exception of pantothenic acid, are rather stable and are not readily destroyed under normal storage conditions.

Trolle and Pedersen (1971) demonstrated that the tocopherol content of barley decreased after storage damage, e.g., 30.5 γ/g in mold damaged barley compared to 54.5–81.0 γ/g in sound barley.

Minerals

Except under unusual conditions, the mineral content of grain or its products does not change during storage (Zeleny 1954). However, it is possible for the percentage of total mineral matter in grain, as determined by ash content, to increase as a result of the loss of other constituents such as carbohydrate. This type of change may be measured in grain that has undergone extensive fungal deterioration. An example of this was cited by Zeleny (1954):

"A sample of barley was taken from an excavation in Asia Minor and claimed to be from 3,000 to 5,000 years old. Its ash content on a moisture-free basis was 17.2% as compared to 3% for normal barley ash. The barley was black in color, very light in weight, and had obviously lost much of its organic substance."

GERMINATION AND AESTHETIC CHANGES

Numerous reports (Dorworth and Christensen 1968; Qasem and Christensen 1960; Christensen 1955; Armolik et al. 1956; Papavizas and Christensen 1957; Christensen 1962; Fields and King 1962; and Lopez and Christensen 1967) confirm that invasion of seeds by storage fungi can result in loss of germinability.

Briefly, samples of soybeans, wheat, barley, corn, sorghum and peas stored at moisture contents and temperatures favorable for the growth of storage fungi, but kept free of fungi, retained a germinability of 95–100% for a few months. On the other hand, similar samples invaded with storage fungi were reduced to zero or near zero germinability. Most of the storage
fungi invade the embryo of the seed preferentially as shown in Fig. 8.5. Thus, it is not surprising that germinability is reduced. An example of the effect of storage fungi on germination of wheat is illustrated in Fig. 8.6.

Aesthetic changes that occur when grain deteriorates in storage include discoloration, caking, and abnormal odors. With increasing fungal invasion, grain loses its natural luster and becomes rather dull and lifeless in appearance. General appearance alone is considered a quality factor in the routine inspection and grading of barley, oats, grain sorghums, and soybeans. According to Christensen (1955), Papavizas and Christensen (1957), and Schroeder and Sorenson (1961), it is highly probable that

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**FIG. 8.5.** *Aspergillus flavus* GROWING FROM THE EMBRYO OF A SURFACE-STERILIZED CORN KERNEL ON MALT AGAR
invasion of the germ or embryo of seeds by storage fungi is a major cause of discoloration. Damaged kernels can be identified by the brown to black color of the germ. Wheat damaged in this manner is frequently referred to as "sick wheat" (Pomeranz 1974).

Abnormal odors, such as musty or sour odors, and caking are associated with grain that has reached a fairly advanced stage of deterioration. Sour odors may be produced by bacterial fermentation, whereas fungi are usually responsible for the musty odors, which may carry over into products made from deteriorated grain.

**MYCOTOXINS**

Toxic metabolites (mycotoxins) produced by filamentous fungi in grains are capable of producing a toxic response (mycotoxicosis) when ingested. Despite the fact that numerous poisonings from the consumption of moldy grains were reported from 1826 through the mid 1900’s (Mayer 1953; Bilay 1960; Dounin 1926; Joffe 1971; Forgacs 1972), mycotoxin problems were
not fully recognized until it was discovered that aflatoxins were responsible for the deaths of a large number of turkey poults in England in 1960 and that the toxins were potent carcinogens in laboratory animals (Lancaster et al. 1961). Since 1960, hundreds of scientific reports and reviews have been published on mycotoxins (Goldblatt 1969; Lillehoj et al. 1970; Ciegler et al. 1971; Kadis et al. 1971, 1972; Purchase 1971; Rodricks 1976).

Cereal grains, peanuts and cottonseed may be contaminated with mycotoxins (Detroy et al. 1971). Mycotoxin production can occur in the field or during harvest, processing, storage and shipment. However, only a few mycotoxins have definitely been implicated in mycotoxicoses (Ciegler 1975). Although the diagnosed cases are usually more acute and dramatic, the most important mycotoxicoses probably involve subacute doses; e.g., livestock exhibit poor weight gains and lowered feed efficiency; humans may contract hepatomas and suffer from degeneration of the hematopoietic system. I will briefly summarize the major mycotoxins implicated in natural outbreaks of mycotoxicoses from grains (Table 8.6).

Aflatoxin

The aflatoxins (B₁, B₂, M₁, M₂, G₁ and G₂) are the most studied mycotoxins. They appear to constitute a contamination problem primarily in peanuts and peanut products, cottonseed meal, and corn; however, many other grains have also been reported to be contaminated (Ciegler 1975). The occurrence of aflatoxins is usually associated with poor storage conditions, although more and more evidence indicates that these compounds are also produced in the field.

Lillehoj et al. (1975A) determined that 2.5% of 5,000 test ears of field corn from southeast Missouri and east Central Illinois contained aflatoxin B₁ at levels exceeding 20 µg/kg. In a subsequent study of field corn in northeastern South Carolina, Lillehoj et al. (1975B) demonstrated a 49% incidence of aflatoxin in samples collected from 184 fields. Aflatoxin B₁ levels were less than 80 µg/kg in 80% of the samples containing detectable aflatoxin. Lillehoj et al. (1977) detected extensive A. flavus infection at harvest in 214 Iowa corn samples but only four contained 20 µg/kg or more aflatoxin B₁. According to comprehensive studies by Shotwell et al. (1969A, B) on samples mainly from the corn belt, only nine out of 1,368 samples of wheat, grain sorghum and oats, and 35 out of 1,311 corn samples contained small amounts of aflatoxins (up to 19 µg/kg). A second survey of U.S. corn for aflatoxin showed a similarly low incidence of aflatoxins at levels up to 25 µg/kg (Shotwell et al. 1970). Most of the contamination was in the lower grades.
<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Producing Fungi</th>
<th>Animals Affected</th>
<th>Biological Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td><em>Aspergillus flavus,</em> <em>A. parasiticus</em></td>
<td>Mammals, fish, birds</td>
<td>Hepatotoxin, cancer</td>
<td>Detroy <em>et al.</em> (1971)</td>
</tr>
<tr>
<td></td>
<td><em>A. ochraceous</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrinin</td>
<td><em>P. viridicatum</em></td>
<td>Swine</td>
<td>Nephrotoxin</td>
<td>Ciegler <em>et al.</em> (1971)</td>
</tr>
<tr>
<td></td>
<td><em>P. citrinum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zearalenone</td>
<td><em>Fusarium graminearum</em> (Gibberella zeae)</td>
<td>Swine</td>
<td>Volvovaginitis, abortion, enlarged mammary glands</td>
<td>Christensen <em>et al.</em> (1965)</td>
</tr>
<tr>
<td>or F-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichothecces</td>
<td><em>F. tricinctum</em></td>
<td>Cattle</td>
<td>Dermal necrosis, hemorrhage</td>
<td>Hsu <em>et al.</em> (1972)</td>
</tr>
<tr>
<td>T-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomitoxin</td>
<td><em>F. graminearum</em></td>
<td>Swine, man</td>
<td>Refusal vomiting</td>
<td>Vesonder <em>et al.</em> (1973, 1976)</td>
</tr>
</tbody>
</table>
In 1969 and 1970, Shotwell et al. (1973) expanded their survey to southern corn of all grades except U.S. No. 1. Incidence of contamination was near 30% and ranged from 5 µg/kg to 308 µg/kg. Only 16% of the positive samples contained more than 100 µg/kg.

In general, incidence and levels of aflatoxins in grains and grain products appear to be low (Scott 1973).

In addition to being a potent toxin, aflatoxin B₁ is the most carcinogenic mold metabolite known. Liver damage is the usual symptom. Wogan (1968) reported that a dietary level of 15 ppb aflatoxin B₁ was sufficient to cause 100% incidence of liver tumors in male rats after 68 wk. Susceptibility of domestic animals to aflatoxin varies widely (Keyl and Booth 1971). Pigs can tolerate up to 233 ppb. No toxic effects were observed at levels of 300 ppb or lower in beef steers fed for 4.5 months. In dairy cows, weekly intakes of 67–200 mg of aflatoxin B₁ produced 70–154 ppb aflatoxin M₁ in lyophilized milk. No adverse effects were discernible in broilers fed from 1 day to 8 wk of age a ration containing 400 ppb aflatoxin. Sheep were resistant for 3 yr to 17,500 ppb aflatoxin B₁ in the feed, while liver tumors were found in ducklings fed a diet containing 30 ppb aflatoxin (Scott 1973).

There is no established toxic dose for humans; but circumstantial evidence from Southeast Asia, India, and Africa, plus a suspect case in Germany, indicates that aflatoxins have been involved in human deaths, particularly among children (Ciegler 1975).

Ochratoxin A

Merwe et al. (1965) first isolated ochratoxin A in South Africa from maize on which Aspergillus ochraceus had grown. Since then the toxin has been experimentally produced by this fungus and Penicillium viridicatum on a number of substrates (Scott 1973). Shotwell et al. (1969C) detected the first natural occurrence of ochratoxin A in corn, but since then it has been found in wheat, oats, barley, peanuts, white beans, and mixed feed grain from Canada (Scott et al. 1972) and in coffee (Levi et al. 1974). However, the incidence of ochratoxin A in U.S. corn was low (Shotwell et al. 1970).

Ochratoxin A is a highly toxic metabolite that produces both liver and kidney damage. The oral LD₅₀ in male rats is 22 mg/kg (Purchase and Theron 1968). Chronic toxicity studies carried out with laying hens from 14 through 52 wk of age showed that up to 4 ppm ochratoxin A in the diet caused high mortality, depressed growth, delayed sexual maturity, low egg production, and poor egg shell quality (Choudhury et al. 1970). Krogh et al. (1974) studied the effect of ochratoxin in swine at levels of 200, 1,000 and 4,000 µg/kg of feed for about 4 months. These levels correspond to levels found commonly in animal feeds in Denmark. Changes in kidney structure was a characteristic symptom, and ochratoxin A was detected
in the kidney, liver, adipose, and muscular tissue of the experimental animals.

Citrinin

Citrinin was originally isolated from *Penicillium citrinum* by Hetherington and Raistrick (1931). Citrinin was considered responsible for the yellow color of rice imported into Japan from Thailand around 1951 (Scott 1973). However, the occurrence of citrinin as a contaminant of feedstuffs has been associated with *P. viridicatum* and always as a co-contaminant with ochratoxin. Scott *et al.* (1972) found citrinin at 0.07–80 mg/kg levels in 13 of 18 samples which also contained ochratoxin. Citrinin caused kidney damage in experimental animals (Krogh *et al.* 1970), comparable to that caused by feeding barley contaminated with *P. viridicatum*. Thus, it is possible that both ochratoxin A and citrinin are involved in the mycotoxicosis which has affected up to 7% of the pigs in Denmark (Krogh 1969). Hesseltine (1976) reported that citrinin occurs naturally in wheat, rye, barley, and oats in both Canada and Denmark.

Zearalenone or F-2

Various reports since 1928 indicate that the estrogenic syndrome in swine is associated with consumption of moldy corn (Ciegler 1975). Subsequently, Stob *et al.* (1962) isolated an anabolic uterotropic compound from corn infected with *Gibberella zeae* and partially characterized the toxin. Later, Christensen *et al.* (1965) and Mirocha *et al.* (1967) isolated the estrogenic substance and labeled it F-2. Urry *et al.* (1966) determined the structure of this compound and gave it the name, zearalenone.

The estrogenic syndrome is one of the best understood mycotoxicosis because zearalenone or F-2 has been found naturally in feeds in sufficient amounts to cause the disease. In swine, the estrogenic syndrome involves development of a swollen vulvae in the females, shrunken testes in young males, enlarged mammary glands in the young of both sexes, and breeding and abortions in females (Mirocha *et al.* 1968). The authors demonstrated experimentally that 1 mg of F-2 fed daily to gilts weighting 27 kg produced swollen vulvae within 5 days and in some cases atrophy of the ovaries after 8 days. Usually, levels of 1–5 ppm zearalenone in the feed are enough to induce these physiological responses in swine. At lower levels, zearalenone and various derivatives actually stimulate growth in farm animals (Hesseltine 1976).

The occurrence of zearalenone is related to low temperatures and the invasion of grain by various species of *Fusarium*, particularly *F. graminearum* or *F. roseum* (perfect stage, *G. zeae*). These organisms invade developing corn at silking stage in periods of heavy rainfall and
proliferate on mature grains that have not dried because of wet weather at harvest or on grains that are stored wet (Tuite et al. 1974; Caldwell and Tuite 1970, 1974).

In two general surveys of U.S. corn for zearalenone, Shotwell et al. (1970, 1971) found the mycotoxin in about 1% of the samples examined at an average level of 625 µg/kg (range 450–800 µg/kg). Subsequently, the FDA (Eppley et al. 1974) conducted a concentrated survey in an area where there was evidence of F. roseum damage. Zearalenone was found in 17% of 223 samples assayed, at an average level of 0.9 mg/kg (range 0.1–5.0 mg/kg). Zearalenone has also been found in feed grains from Finland, Denmark, France, England, Mexico, and Yugoslavia (Stoloff 1976; Hessel­tine 1976).

**Trichothecenes**

The 12,13-epoxy-Δ⁸-trichothecenes have been implicated in a variety of mycotoxicoses involving both humans and animals on a large scale; diseases include alimentary toxic aleukia, stachybotryotoxicosis, moldy corn toxicosis, and the refusal-vomition phenomenon (Ciegler 1975).

In the only natural isolation reported, Hsu et al. (1972) identified T-2 toxin as the cause of a lethal toxicosis in Wisconsin dairy cattle. These cattle had consumed feed containing 60% corn molded with F. tricinctum. The cows had extensive hemorrhaging on the serosal surface of all internal viscera, typical of previously reported cases of moldy corn poisoning (Smalley 1973).

Reports of vomiting in animals and humans caused by the consumption of moldy wheat, barley, and flour go back beyond the early 1900’s (Ciegler 1975; Hessel­tine 1976). Curtin and Tuite (1966) demonstrated that extracts of corn naturally infected with G. zeae caused emesis in pigs and also possibly caused refusal. Both effects have been observed naturally in the midwestern U.S. However, the causative agent eluded detection until 1973 when Vesonder et al. (1973) isolated a new trichothecene, vomitoxin, from corn infected in the field with F. graminearum. From barley invaded with Fusaria, Yoshizawa and Morooka (1973) isolated deoxynivalenol which is structurally the same as vomitoxin. Subsequently, Vesonder et al. (1976) demonstrated that vomitoxin was responsible for refusal. Vomitoxin does not appear to cause hemorrhaging and is less potent in causing dermal necrosis than is T-2 toxin.

**CONTROL OF FUNGAL DETERIORATION IN GRAIN**

During storage of grain, the primary aim is to prevent deterioration in quality. Generally, this is done by reducing the moisture content (drying)
to a level too low for fungi to grow. However, reduced temperatures in combination with low moisture are even more effective for preventing fungal deterioration. A low, uniform moisture content coupled with a low, uniform temperature (aeration) reduces the possibility of moisture transfer within the bulk and adds to the storage life of grain (Christensen and Kaufmann 1974). Other control methods include limiting the O₂ content or increasing the CO₂ content of the atmosphere, chilling, treating with chemicals, and combinations of these methods. In addition, time is an important consideration because fungal deterioration of stored grain is a dynamic process and control becomes more complicated as length of storage increases.

High-temperature drying to prevent deterioration has closely paralleled the growth of mechanical harvesting (combining) of high-moisture grain (Foster 1973). However, as fuel and electrical power for operating driers become less available and more expensive, alternate processes will be required. Fortunately, there appear to be some alternatives. Shove (1973) demonstrated that a 7° C temperature rise is sufficient to dry shelled corn to 13–15% moisture in Illinois from late October through the middle of December. The data indicate that the energy requirement for low-temperature drying was considerably less than for normal drying procedures. Nevertheless, there are risks involved in this type of drying. When unheated air is blown upward through the grain from a perforated floor, the drying front moves upward more slowly than with heated air. If the drying front reaches the top and all grain is dried before any spoils, the process is a success. A bad effect of drying grain with unheated air is that fungal deterioration may occur because of failure to quickly reach a low enough moisture level.

Application of solar energy to low-temperature grain drying is currently receiving much attention and appears to be feasible (Foster and Peart 1976). However, its availability depends on weather. Several alternate systems, differing mostly in the design of collectors, are available for drying farm products with solar energy.

Refrigerated or chilled storage of grain is another alternative, but this requires considerable energy, and the risks are appreciable (Burrell 1974B). Ensilation or storage of high-moisture grain in air-tight systems involves organic acid-producing fermentations and is exemplified by the Harvestore®. This system is successfully being used for animal feeds (Hyde 1974).

Still another alternative for minimizing fungal losses in storage and

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¹The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.
energy use in drying is the preservation of high-moisture grain with chemicals (Sauer and Burroughs 1974). Propionic acid is widely used in Europe to prevent mold and bacterial activity on damp grain in open storage. Feeding trials with cattle, pigs, sheep, and poultry were satisfactory (Hutson 1968). In the U.S., this procedure is also being used and several companies are marketing propionic acid and combinations of acetic, propionic, and formaldehyde for treating high-moisture grain to be fed on-farm. The feedlot performance of beef and dairy cattle consuming acid-treated grain has been excellent (Lane 1972; Perry 1972; Wilson 1972).

A novel approach for the storage of high-moisture grain, which I feel has considerable potential for reducing storage losses at a low cost, involves the introduction of a small amount of gaseous or liquid preservative into grain to control microbial growth during ambient air drying. In our initial test (Nofsinger et al. 1977) intermittent application of gaseous ammonia to 560 bu of high-moisture corn permitted low flow (1.8 m³/min/1,000 kg) ambient drying (Fig. 8.7). Moisture content was reduced from 23.3% to

![Diagram of gaseous ammonia-employed in ammonia-supplemented ambient temperature drying of high-moisture corn](image)
17.7% in 56 days and mold growth was effectively controlled throughout 6 months' storage. Feedlot steers gained fast (2.86 lb/day) and efficiently (6.57 lb feed/lb gain) on a ration formulated with this corn. Currently, larger scale tests using ammonia, formaldehyde, and methylene-bis-propionate to supplement ambient air drying of high-moisture corn are encouraging from both a cost and an energy conservation viewpoint.

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