MICROBIOLOGY OF CONTROLLED ATMOSPHERE STORAGE OF GRAINS--AN OVERVIEW

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INTRODUCTION

Work has been underway for more than 25 years on the use of modified atmospheres to reduce microbiological spoilage of stored grains. Present technology makes such storage feasible, and worldwide food requirements may make it essential. As an overview, this paper will present information first on the basic considerations that must be addressed in controlled atmosphere storage. Next, the influence of both intrinsic and extrinsic factors on the stability of stored grain and associated microbiological activity will be reviewed. Finally, the interactions of these fundamental parameters as they relate to controlling undesirable microorganisms on grain will be assessed.

BASIC CONSIDERATIONS

The rationale for use of commodity-modified atmosphere, modified atmosphere or controlled atmosphere storage is based on several key considerations. Storage capabilities are increased when spoilage is reduced and when safety is ensured and changes in the grain are minimal. The undesirable activities of biological agents such as insects, mites, rodents, and birds as well as microorganisms must be controlled. Undesirable chemical reactions also must be minimized. Preservation can be further extended through reduced moisture, low temperature, and chemical protectants.

The microbiological advantages of controlled atmosphere storage include the inhibition of aerobic fungi, elimination of mycotoxin production, conservation of desirable quality factors in the grain, and manipulation of economic advantages from extended storage (Christensen, 1978).

The microbiological disadvantages of controlled atmosphere storage include elimination of microorganisms that compete under aerobic conditions, development of populations of certain aerobic microorganisms before the atmosphere is sufficiently modified, requirements for expensive gases and complex technical facilities and capabilities, and generation of adverse quality factors (Christensen, 1978).

The following is a list of many of the major considerations that influence the effectiveness of controlled atmosphere storage: (1) microbial load, (2) type of grain, (3) climate of storage facility, (4) moisture or drying requirement, (5) potential variation in storage conditions, (6) type of controlled atmosphere, and (7) technical capabilities.

A primary and major consideration in the application of controlled atmosphere storage of grain is the types and numbers of microorganisms that play a role in determining storage stability of grains. The "storage fungi" include *Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Rhizopus*, *Chaetomium*, *Scopulariopsis*, *Paecilomyces*, *Neurospora*, *Nigrospora*, *Sporendonema*, (Wallemia) (Hyde, 1974; Wallace, 1975; Christensen & Meronuck, 1976). The "field fungi" include *Alternaria*, *Cladosporium*, *Helminthosporium*, *Fusarium*, and numerous other genera. Yeasts that have been considered influential under controlled atmosphere conditions include *Candida* and *Hansenula* (Hyde, 1974; Wallace 1975; Christensen & Meronuck, 1976). Bacteria that must be considered in the evaluation of controlled atmosphere include such genera as *Lactobacillus*, *Clostridium* and *Bacillus*. General groups such as the coliforms are also frequently evaluated. Furthermore, specific functional groups of bacteria that constitute the thermophiles or the psychrotrophs are, in general, strategic in their role in high moisture grains (Hobbs & Greene, 1976; Bothast, 1974).

The principal types of grain that must be considered when one evaluates the microbiology of controlled atmosphere storage include corn (maize), wheat, rye, sorghum, durham, sunflower, rice, barley, millet, oats, and soybeans (Hobbs & Greene, 1976; Christensen & Kaufmann, 1977; Christensen, 1975).

The primary physical parameters, both intrinsic and extrinsic factors, that influence the microbial activity on grain include the moisture or water activity (a_w) of the grain, the temperature of storage, the atmosphere surrounding the grain during storage, and duration of the storage period.

WATER ACTIVITY

Moisture content or equilibrium relative humidity or water activity (a_w) have been key parameters that have been used extensively to predict grain storage stability irrespective of atmosphere. The role of moisture is related to the variations of grains, the limitations of naturally occurring biological substances, the influence of harvesting and early storage, the requirements for viability and product performance considerations, the uniformity within a given unit of grain, the effects of temperature on relative humidity, and the individual microbial responses to various water activity levels.

Christensen and Kaufmann (1977) have previously identified key fungi that are found at various relative humidities and related equilibrium moisture content of common grains. Table 1 restates earlier summaries.

TABLE 1

Equilibrium moisture content of common grains at relative humidities of 65-95%+; fungi found at each level (Christensen & Kaufmann, 1977).

% R.H.	Moisture Content	Fungi
65-70	12.5 - 13.5	*Aspergillus halophilicus*
70-75	14.5 - 15.0	*A. restrictus, A. glaucus, Sporendonema*
75-80	15.0 - 15.5	Above + *A. candidus, A. ochraceus, A. versicolor*
80-85	18.0 - 18.5	Above + *A. flavus,* few species of *Penicillium*
85-90	19.0 - 20.0	Above + several species of *Penicillium*
95-100	22.0 - 24.0	All advanced decay fungi, yeasts, and bacteria.

These observations are directly related to the minimum a_w that supports growth of these various fungi at optimum growth temperatures from 26°C to 30°C (Table 2).

TABLE 2

Minimum water activity (a_w) for growth at optimum temperature (Christensen & Kaufmann, 1974).

Aspergillus halophilicus	0.68
A. restrictus	0.70
Sporendonema (Wallemia)	0.70
A. glaucus	0.73
A. candidus, A. ochraceus	0.80
A. flavus	0.85
Penicillium (depending on sp.)	0.80 to 0.90

A recent publication by Northolt (1979) summarized the minimum a_w levels required for fungal growth and mycotoxin production on several agricultural products. In some cases, the organism was part of the natural flora present on the grain. In other cases, the organism was present in combination with competitive flora on the grain, or it was present as a pure culture. The literature reviewed by Northolt is summarized in part in the adapted information presented in Table 3. The influence of incubation temperature, as well as product and competition from other microorganisms, is readily evident. Direct comparisons are difficult because of the variations in the test parameters. Nevertheless, it appears that reduced competition results in the ability of the organisms to produce mycotoxins at lower a_w than would otherwise be possible.

TABLE 3

Minimum water activity (a_w) for growth and mycotoxin production (Northolt, 1979).

Product	Fungus	°C Temp.	Growth	Mycotoxin
Natural Flora				
Barley	*Aspergillus sp.*	20	0.80	-
	Penicillium sp.	20	0.87	-
	Aspergillus flavus	19	0.88	-
	Aspergillus flavus	24	-	0.92
Oats	-	25	0.75	-
Sorghum	-	30	0.8	-
Competitive Flora				
Barley	*Penicillium viridicatum*	12	0.85	0.85
	Aspergillus flavus	35	0.77	-
	Aspergillus flavus	15	0.84	-
Wheat	*Aspergillus ochraceus*	20	0.75	-
	Aspergillus parasiticus	30	0.70	0.80
	Aspergillus flavus	25	0.85	0.85
Pure Culture				
Corn	*Penicillium expansum*	19	0.91	-
	Aspergillus ochraceus	19	0.76	-
	Aspergillus ochraceus	25	0.88	-
	Penicillium viridicatum	19	0.85	-
Wheat	*Aspergillus flavus*	20	-	0.80
Rice	*Aspergillus flavus*	30	-	0.76
	Aspergillus ochraceus	25	0.86	-

Northolt studied the minimum water activity for growth and mycotoxin production by *A. flavus*, *P. expansum*, *A. ochraceus*, and *A. parasiticus*. A comparison of Northolt's data with those found in the literature shows some dramatic differences in stated minimum water activities. These differences are summarized in Table 4 that was adapted from Northolt (1979).

TABLE 4

Significant differences between minimum a_w - Literature vs. Northolt (Northolt, 1979).

Product	Fungus	Growth	Mycotoxin
Corn	*Aspergillus flavus*	-	+0.09
	Penicillium expansum	+0.07	-
	Aspergillus ochraceus	-0.07	-
	Aspergillus ochraceus	+0.05	-
Wheat	*Aspergillus ochraceus*	-0.08	-
Rice	*Aspergillus parasiticus*	-0.10	-0.03
	Aspergillus flavus	-	-0.07

+ indicates literature is higher than Northolt
- indicates the reverse

TEMPERATURE

Storage temperature is a well established parameter that influences microbial activity as well as other characteristics of grain. The ambient temperature may fluctuate with climatic change or it may be partially controlled through construction or mechanical devices. Under specific conditions it may be controlled very precisely for experimental determinations. Reduced temperatures generally reduce chemical reactions, and growth is an example of a response that is slowed by reducing temperatures. Although many chemical reactions continue to accelerate at elevated temperatures, growth above optimum levels generally is somewhat reduced to a maximal level at which growth ceases. Temperature, as an influential parameter, is affected by action of various organisms, including microorganisms that may be metabolically active in association with the grain; or the temperature may be influenced by the climate or by the facility used for storage. The fluctuation or uniformity of the temperature of storage can dramatically influence subsequent response by microorganisms or the grain itself.

Christensen & Kaufmann (1974) reported the minimum, optimum, and maximum temperatures for growth of common storage fungi on grains. Table 5 presents a summary of this information.

TABLE 5
Minimum, optimum, and maximum temperatures (°C) for growth of common storage fungi on grains (Christensen & Kaufmann, 1974).

	Minimum	Optimum	Maximum
Aspergillus restrictus	5-10	30-35	40-45
A. glaucus	0- 5	30-35	40-45
A. candidus	10-15	45-50	50-55
A. flavus	10-15	40-45	45-50
Penicillium	-5- 0	20-25	35-40

Recently Northolt (1979) summarized findings in the literature for a variety of agricultural products. His summary of the literature is adapted in Table 6. It is readily evident that reduction in temperature alone is insufficient to control growth and mycotoxin production by various fungi on grain.

TABLE 6

Minimum temperature (°C) for growth or mycotoxin production on grain (Northolt, 1979).

Product	Fungus	Growth	Mycotoxin
Barley	*P. cyclopium*	<1	<1
Corn	*P. cyclopium*	<1	<1
	P. martensii	ND	1
	A. ochraceus	-	10
Wheat	*A. ochraceus*	-	10
	P. viridicatum	<5	<5
Rice	*A. flavus*	-	11
	P. cyclopium	<1	<1
Sorghum	*P. cyclopium*	<1	<1

ATMOSPHERE

The storage parameter primary to our efforts here is the atmosphere around the stored grain. Evaluation of the effects of atmosphere on retarding deterioration of various food products has been extensive during the last 25 years. The atmosphere surrounding products of one type or another can be influenced or changed through various means which subsequently lead to the respective identifying titles of controlled atmospheres, modified atmospheres, and commodity-modified atmospheres (Brecht, 1980). In commodity-modified atmosphere, respiration and basic metabolism of the components of the system generally reduce available O_2 and increase CO_2. In the case of modified atmosphere, CO_2, O_2 and N_2 are introduced as an atmosphere over the product at specific concentrations. This is generally a singular event of initial change and should take into consideration anticipated product requirements and subsequent commodity-modified changes. Modified atmosphere is not as dynamic as the commodity-modified atmosphere but is of obvious greater expense. Controlled atmosphere, in its most fundamental definition, is the precise constant maintenance of selected gases such as CO_2, O_2, and N_2 at specific pressures or under partial vacuum. The controlled atmosphere system, as defined in this fashion, is considerably more expensive than the other alternatives and is also technically demanding. This precise response to commodity activity results in a less dynamic system than the others that were described.

Airtight storage is an excellent example of commodity-modified atmosphere storage. It is dynamic and, if functioning as desired, would ideally demonstrate a reduction in O_2 to 0.5-1.0% and if at all possible down to 0.2% O_2. Simultaneously, increases in CO_2 up to 50% would be observed. Airtight storage is a method where the atmosphere is self-developed and dependent upon the indigenous activity of the organisms and grain system. Airtight storage has utilized a variety of containers to accomplish appropriate changes in atmosphere, including the traditional pit, tank, bin, and silo (Hyde, 1974).

Atmosphere has a dramatic influence on metabolic activities of fungi. Toxin production by *Penicillium* or *Aspergillus* can be controlled by elevated CO_2 levels. Data presented in Table 7 show that penicillic acid production or aflatoxin production in laboratory media can be inhibited by specific atmospheres containing 60 or 90% CO_2.

TABLE 7
Atmospheric inhibition of toxin production in laboratory medium.

%	[1]Penicillic Acid (at 20°C or 40°C)	[2]Aflatoxin (at 28°C)
O_2	20	10
CO_2	60	90
N_2	20	-

[1] Lillehoj, Milburn, Ciegler, 1972
[2] Shih & Marth, 1973

The influence of atmosphere on growth or toxin production on grain is also well documented. Studies on corn or wheat have shown that reduced O_2 or increased CO_2 levels will result in the inhibition of growth or toxin production at optimum or suboptimum growth temperatures. (Table 8.)

TABLE 8
Modified atmosphere inhibition of growth or toxin production on grain.

%	[1]Corn	[2]Corn		[3]Corn		[4]Wheat
O_2	0.5	0.3	0.5	20	20	0 - 4.5
CO_2	13.5	0.0	13.5	40	60	0 0
N_2	84.8	99.7	84.8	40	20	100 - 95.5

[1] Wilson & Jay, 1975 (27°C)
[2] Wilson, Huang & Jay, 1975 (27°C)
[3] Lillehoj, Milburn, Ciegler, 1972 (5° & 10° C)
[4] Shejbal, 1979 (22-32° C)

INTERACTION

In most cases the reports in the literature or the specific observations made in the field have reported temperature or atmosphere or relative humidity (a_w) effects on growth or toxin production by fungi on stored grain. Rarely has there been a concerted effort to evaluate the interactions amongst these various storage parameters. Bottomley, Christensen & Geddes (1950) conducted a comprehensive study on corn and, with complete statistical evaluation, determined that: the influence of temperature was statistically significant; the influence of atmosphere was statistically significant; and the influence of humidity was statistically highly significant. When they measured the interaction, they observed that the interaction of temperature and atmosphere was not statistically significant nor was the interaction of temperature and humidity. However, the interaction of atmosphere and humidity was statistically highly significant. Three-way interactions displayed no statistical significance.

The influence of the interaction of atmosphere and temperature on the production of penicillic acid is readily evident in Fig. 1 which has been adapted from Lillehoj, Milburn & Ciegler (1972). As the amount of CO_2 in the atmosphere was increased, the production of penicillic acid at temperatures below optimum was reduced or totally inhibited. This figure dramatically demonstrates an atmosphere-temperature interaction.

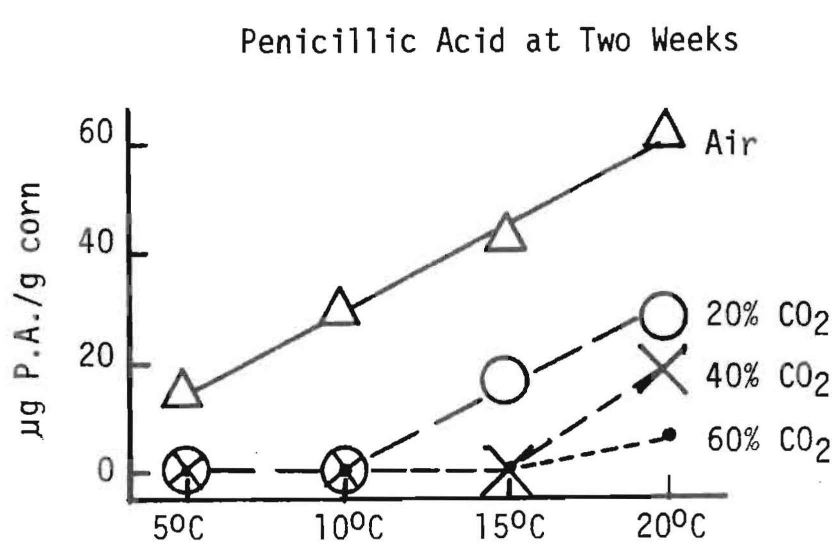

Fig. 1. Influence of temperature and atmosphere on penicillic acid production (Lillehoj, Milburn & Ciegler; 1972).

Interaction between temperature and a_w on growth or penicillic acid production was also evident in data reported by Northolt, Van Egmond, and Paulsch (1979). Reduction in a_w not only eliminated penicillic acid production at a variety of growth temperatures, but in general influenced growth directly as a dramatic interaction (Fig. 2).

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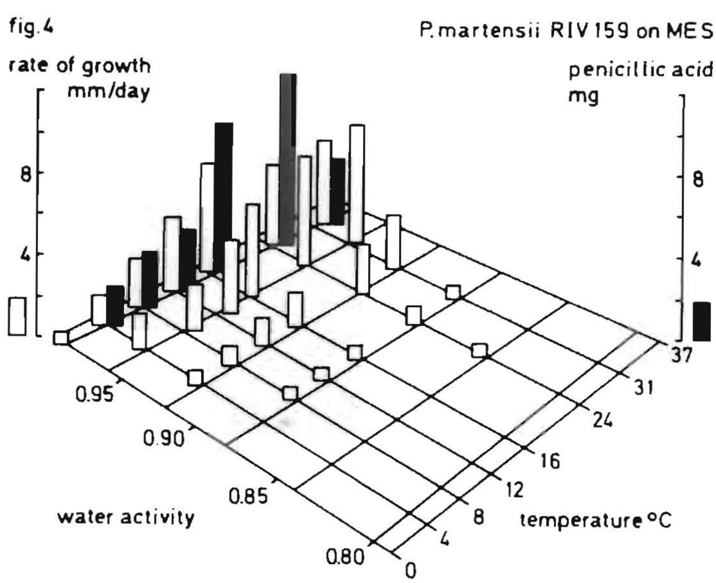

Fig. 2. Interaction of influence of water activity and temperature on growth and toxin production (Northolt, Van Egmond, and Paulsch; 1979).

The interaction of temperature and atmosphere on spore germination by *Penicillium martensii*, the same organism studied in the penicillic acid production, also has been documented by Lillehoj, Milburn, and Ciegler (1972). Key data are reproduced in Fig. 3. These data show that increased levels of CO_2 dramatically narrow the range of temperatures at which spore germination was observed and reduce the mean percent germination as well.

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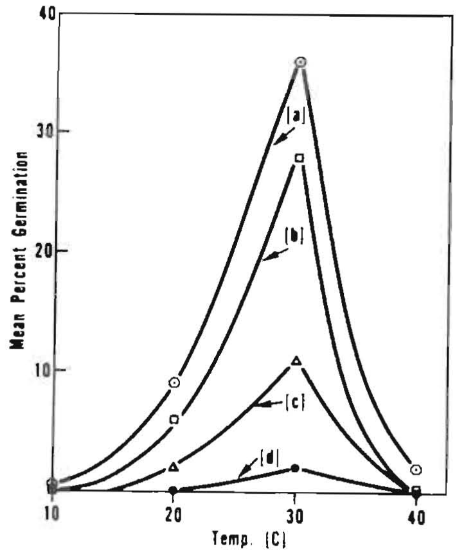

FIG. 2. *Mean germination of P. martensii spores after 16 hr at 30 C. Gases employed: (a) air; (b) 20% CO_2, 20% O_2, 60% N_2; (c) 40% CO_2, 20% O_2, 40% N_2; (d) 60% CO_2, 20% O_2, 20% N_2.*

Fig. 3. Interaction of temperature and atmosphere on spore germination (Lillehoj, Milburn, and Ciegler; 1972).

It should not, therefore, be unexpected that one could observe under appropriate conditions, a three-way interaction among humidity, temperature, and atmosphere. Interrelating the data of Northolt et al. (1979) and Lillehoj et al. (1972) one should be able to speculate that increasing the CO_2 levels associated with *P. martensii* would have an overall interaction and antagonistic effect on the amount of growth, most likely narrowing the range of maximum and minimum temperatures at which growth would be observed, increasing the minimum a_w at which growth would occur, and reducing the overall amount of growth observed at any level of water activity. Using this combination of existing data as a stimulus, it seems readily evident that there is a need for optimization among these three-way interactions between humidity, temperature and atmosphere.

From the standpoint of the type of grain, the microbiology associated with that grain, the entomology, the energy consumption requirements, the product quality and the overall economics of the situation; an optimal interaction or several interactions could be developed that would lead to a practical, feasible, and effective storage procedure.

As an overview, we have attempted to analyze much of the information that has been available in the literature and to stimulate your imagination in projecting possible interactions from those data. We look forward to the following presentations on oxygen depletion by Pelhate; on the effects of nitrogen storage by Di Maggio; on wet grain storage by Richard-Molard, Cahaghier, and Poisson; and finally, the influence of nitrogen on moist wheat by Serafini, Fabbri, Shejbal, Fanelli, Di Maggio, and Rambelli which should prove enlightening and contribute considerably more data to stimulate technological progress.

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