Quality Deterioration of Cereal Grains Caused by Fungal Infection during Storage

— Corn and milo imported for feed —

By OSAMU TSURUTA

Stored-Product Microbiology Laboratory, National Food Research Institute
(Yatabe, Ibaraki, 305 Japan)

To keep the quality of harvested grains from deterioration for a long period, it is most desirable to maintain the storage temperature as low as possible,[3-6] in addition to appropriate methods of harvesting and handling. Therefore, in various countries, underground storage of grain has been attempted, partly aiming at controlling insect pests.[1,5] In Japan, heated-air drying facilities, in which drying is not disturbed by weather, and low-temperature warehouses (13–15°C, 73–75% relative humidity (RH)) are widely used, mainly for rice, the staple grain of the country. However, in many grain-producing countries, the grains are dried to water activity (Aw) levels below about 0.68, and then stored in silos and others to prevent hygroscopic moisture absorption, for maintaining grain quality. This means that the feed grains harvested, handled (including heated-air drying) and sometimes stored at normal temperature in grain-producing countries are imported to Japan.

Since feed grains in Japan depend largely on imported ones, in other words they support the livestock industry of Japan, the possibility of reserving the imported feed grains is discussed. However, the discussion concerns not quality but always quantity of grain to be reserved. Such a situation led the author to take up the present research.

The qualities of corn and milo imported for feed to Japan in large quantities vary with different cargo lots, as shown in Table 1. In Japan the imported grains are usually consumed within 1–2 months. However, there is a fear that the quality such as chemical component of some lots of grains may be deteriorated within this period. Therefore, feed grains to be reserved by long-term storage (in silos, etc.) should be sorted out from imported grains. As an index for the sorting, moisture content is useful, because it influences significantly the storability of grain. However, the use of fat acidity value (FAV), one of the indicators of quality deterioration of grain, is quite questionable, because the extent of fungal infection to grains is not necessarily associated with FAV. In fact, in some cases, FAV of grains considerably damaged by fungi was lower than that of grains damaged a little.

In view of such a background, results of corn studies on (1) relationship between FAV and species of fungi which invade grain, (2) mode of fungal invasion of corn kernels, and (3) storage conditions for corn and milo, both imported as feed grains will be

Table 1. The quality of imported corn and milo

Cereal	Producing country	Whole kernels (%)	Blighted kernels (%)	Germination percentage	Fat acidity value (KOH mg/100 g)
Corn	U.S.A.	74.2—90.8	1.1— 7.2	0.0—62.0	17.7—81.1
	Thailand	85.0—91.9	4.8—11.4	0.0—36.0	36.5—99.9
Milo	U.S.A.	90.2—95.9	0.0— 1.5	12.0—91.0	23.9—73.1
	Argentina	92.5—96.9	0.0— 1.4	15.0—83.0	21.5—54.8
	Australia	92.7—96.0	0.1— 0.5	43.0—98.0	19.0—28.4

presented in this paper.

Fat acidity value of grains infected with fungi[8)]

The changes in FAV were examined with corn and milo grains, which were conditioned to Aw 0.86 or 0.80 at 28°C and then inoculated with 7 species of common grain-infecting fungi (Tables 2 and 3).

Since FAV was measured when fungi grew slightly but on almost all kernels in a plot, and then when the fungal damage proceeded considerably, the date of sampling for FAV measurement may present the growing speed of each fungus.

These results showed that FAV were significantly different among grain samples which were similarly damaged by fungal growth in appearance; the effect of fungal infection on FAV was significantly different with different species of fungi. Consequently, it has a value to compare FAV among fungus-free grains, but it may be difficult to estimate the degree of quality deterioration of fungus-infected grains by this value.

Of the species tested in the experiment, *Aspergillus candidus, A. flavus* and *A. terreus* (which cause serious damage to grains, especially in tropical and subtropi-

Table 2. Changes in fat acidity value and moisture content of corn and milo kernels at a water activity of 0.86 inoculated with fungi and kept at 28°C

Fungus	Corn (Whole kernels)				Corn (Broken kernels)				Milo (Whole kernels)			
	Days needed for infection[a)]	Days of incubation	Fat acidity value[b)]	Moisture content (%)	Days needed for infection	Days of incubation	Fat acidity value	Moisture content (%)	Days needed for infection	Days of incubation	Fat acidity value	Moisture content (%)
A. flavus	6	8	42.5	16.6	6	8	85.2	16.0	6	8	45.4	16.3
		15	100.7	16.5		18	147.2	16.7		18	81.6	16.5
A. terreus	8	22	71.7	16.6	8	22	107.1	16.4	11	20	40.8	16.4
		29	118.4	16.6		82	130.3	16.5		32	49.6	16.0
A. candidus	4	7	42.2	16.9	3	10	166.4	17.2	5	7	45.9	15.7
		14	194.8	17.1		17	240.1	16.9		14	179.1	17.2
A. versicolor	8	18	64.8	16.5	7	11	82.9	16.6	7	18	36.0	16.6
		25	73.6	16.2		22	117.7	16.2		25	46.7	16.4
A. restrictus	5	10	32.7	16.5	4	6	60.4	16.5	4	10	34.0	16.2
		29	36.3	16.1						29	43.8	16.4
E. chevalieri	4	6	42.7	16.6	3	7	105.0	16.4	4	7	59.2	16.4
		12	193.5	16.7		14	258.4	17.5		26	124.4	16.2
E. amstelodami	4	6	48.4	16.8	3	5	63.8	16.4	3	6	47.5	16.6
		12	170.8	16.9		10	313.2	17.5		14	103.8	16.3
No inoculation	—	0	32.0	16.8	—	0	60.2	16.2	—	0	25.1	16.1
(Control)	—	14	34.5	17.0	—	12	60.1	17.0	—	14	26.1	16.3
	—	29	43.8	16.6	—	29	64.7	16.6	—	25	29.7	16.2

a) Days after inoculation when the fungal growth was observed by the naked eye.
b) Fat acidity value=KOH mg/100 g

Table 3. Changes in fat acidity value and moisture content of corn and milo kernels at a water activity of 0.8 inoculated with fungi and kept at 28°C

Fungus	Corn (Whole kernels)				Corn (Broken kernels)				Milo (Whole kernels)			
	Days needed for infection[a)]	Days of incubation	Fat acidity value[b)]	Moisture content (%)	Days needed for infection	Days of incubation	Fat acidity value	Moisture content (%)	Days needed for infection	Days of incubation	Fat acidity value	Moisture content (%)
A. restrictus	12	18	35.6	15.6	8				9	15	26.7	14.8
		71	50.4	15.7		59	80.5	14.8		59	32.5	15.8
E. chevalieri	12	35	43.5	15.1	8				9	21	34.9	15.0
		77	81.0	14.9		77	192.7	15.0		77	48.2	14.8
E. amstelodami	12	21	37.1	14.8	7				10	18	42.3	15.4
		70	86.2	15.2		70	193.3	15.3		71	147.5	15.3
No inoculation	—	23	35.5	15.1	—	0	60.2		—	23	26.6	15.0
(Control)	—	32	31.6	15.0	—				—	32	32.1	14.9
	—	78	49.3	15.4	—				—	78	82.9	15.0

a) Days after inoculation when the fungal growth was observed by the naked eye.
b) Fat acidity value=KOH mg/100 g

cal regions) and *Eurotium amstelodami* and *E. chevalieri* (which are world-widely known as storage fungi) showed strong effect on FAV of grain. On the other hand, *A. versicolor* showed a small and *A. restrictus* showed a very small effect, although these two species are also common storage fungi.

A paper,[10] examining the increase in fatty acid of peanut kernels inoculated with each of isolated fungi separately, reported that *A. tamarii* showed the greatest fat-deccomposing activity, *E. chevaerieli*, *E. repens*, *A. restrictus*, *E. rubrum* were intermediate, and *Penicillium citrinum* showed the smallest activity. In other experiment,[2] *A. terreus* was included into a group having a strong lipase activity. In our experiment above mentioned, *A. restrictus* showed an extremely low fat-decomposing activity. Whether this difference is due to difference in substrate or not remains to be solved.

Fungal damage, in respect of both external appearance and internal quality of grain, proceeded more rapidly with broken kernels than with whole ones. Changes in FAV, as an example showing differences in the rate of quality deterioration, are given in Fig. 1. These data demonstrated that the increase in FAV caused by fungal infection differed largely with different fungal species. Moreover, the result that broken kernels were infected more easily than whole ones supports a general view that the storability will decrease with increasing moisture content and percentage of broken or injured kernels in a given lot of grain.

The problem of aflatoxin contamination of corn caused by *A. flavus* is not discussed in this paper (See the paper[7]).

Mode of invasion of fungi to whole kernels of corn[9]

In order to know the mode of fungal invasion to corn kernels, healthy whole kernels were inoculated with spores of *Aspergillus candidus*, *A. flavus*, *A. restrictus*, *Eurotium chevalieri* and *Penicillium aurantiogriseum*, and examined under a scanning electron microscope.

The invasion to a kernel began mostly at the tip cap, but it occurred sometimes on the central part of

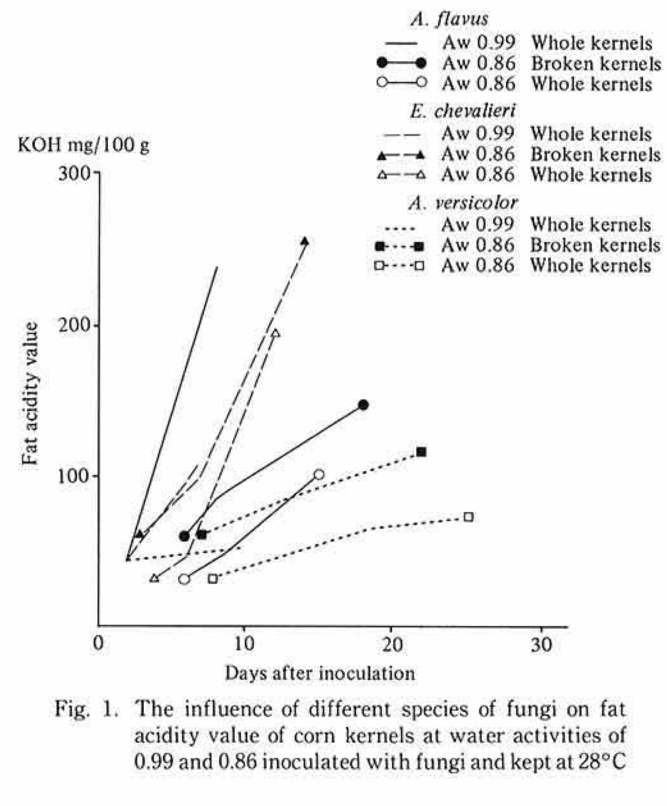

Fig. 1. The influence of different species of fungi on fat acidity value of corn kernels at water activities of 0.99 and 0.86 inoculated with fungi and kept at 28°C

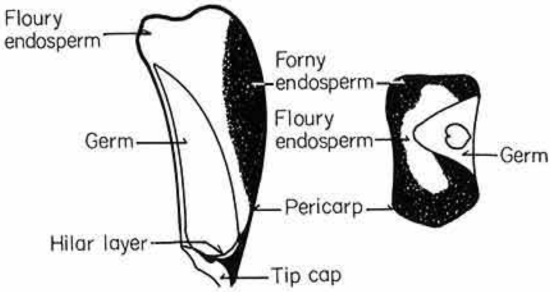

Fig. 2. Structure of the mature corn kernel (dent corn)

germ (Fig.2). In the latter case, however, although the fungus began to grow and produced abundant mycelia on the surface of germ, only a few hyphae penetrated the pericarp. As to the hyphal invasion of the germ, it was observed that *A. restrictus* was hardly able to enter into the hilar layer, while other species penetrated it and spread to the end of germ and to endosperm by creeping through the boundary between pericarp and germ. These results indicate that the fungal invasion through pericarp of corn kernels is difficult to occur, and that even when only a slight fungal infection is found by external appearance, the hyphae entered from the tip cap have often spread widely in the kernel.

The effect of storage conditions on fungal infection, fat acidity and moisture content of corn and milo

To know the effect of storage conditions expected in practice on the quality of grains, FAV and moisture content were examined over the period of 2 months with corn and milo (their qualities are shown in Table 4) stored under several conditions

Table 4. Fat acidity value and moisture content of kernels tested

Sample	Fat acidity value (KOH mg/100 g)	Moisture content (%)
Corn (USA)	29.1	13.9
Corn (Thailand)	45.5	12.5
Milo (Australia)	25.4	13.0
Milo (USA)	43.9	13.5

prepared by combining temperatures (28 or 18 °C) and RH (86–87%, 80–81%, 75–76%, and 68–70%) (Table 5). At 68% RH and 28 °C, no fungal infection was found even after 70 days, because this condition is near the limit for growth of the xero-tolerant storage fungi, although the temperature (28 °C) is optimal. The increase in FAV was also very small. At 75% RH, fungal infection occurred, but FAV increased only to a small extent due to slow fungal growth. On the other hand, at 80% RH and 86% RH, both fungal infection and growth were promoted, resulting in rapid deterioration of grain quality.

At 18 °C, both 88% and 81% RH caused fungal infection, but the infection and growth of fungi were considerably slower than those observed at the similar RH conditions at 28 °C. The increase in FAV was also very little in these cases.

From these results, the following estimation can be made in the viewpoint of moisture content of grains which are handled on the market: (1) When corn and milo, both with moisture contents of 14.1–14.3% (Aw below 0.75) are placed at 28 °C for about 15 days, fungal infection may begin. (2) When their moisture contents are lower than 12.8–13.5% (Aw 0.68), they can be kept without fungal infection, and hence their quality is preserved. (3) When low temperature condition (18 °C) is available, both corn and milo with moisture contents up to 14.4–14.8% (Aw 0.76) can be stored without fungal infection and quality deterioration. In other words, under low temperature storage conditions (13–15 °C and 73–75% RH) practically used in Japan, grains with Aw up to 0.75 can be stored for a long time without fungal infection and quality deterioration. Occurrence of insect pests can also be prevented.

Based on the above results and discussion, it can be said that it is desirable to dry harvested corn and milo more rapidly in higher temperature areas to Aw levels of about 0.75, and finally below 0.68–0.65, and then to store them in silos or similar facilities in order to prevent hygroscopic moisture absorption.

Judging from the aspect of moisture content, which is one of the major factors influencing the storability of grain, the following estimation may be possible: 1) 90% of cargo lots of corn from U.S.A., 76.5% of corn from Thailand, and 100% of milo from U.S.A., Argentina and Australia may maintain their quality for about 60 days, although slight fungal

Table 5. Comparison of influence of different temperature and relative humidity conditions on fungal infection, fat acidity value and moisture content of corn and milo kernels

Sample			Temperature and humidity							
			28°C				18°C			
			R.H. 86%	R.H. 80%	R.H. 75%	R.H. 68%	R.H. 88%	R.H. 81%	R.H. 76%	R.H. 70%
Corn (USA)	Days needed[a] for infection	Intact kernels	4—5	5—7	12—16	**[c]	12—13	15—23	*[b]	***[d]
		Broken kernels	3	5—7	10—13	**[c]	7—9	15—20	*[b]	***[d]
	Days of incubation		31	61	60	71	33	61	67	74
	Fat acidity value[e]		146.9	—	55.7	34.4	58.5	43.3	33.5	33.0
	Moisture content(%)		16.4	15.0	14.2	13.5	16.8	15.2	14.4	14.1
Corn (Thailand)	Days needed for infection	Intact kernels	5—7	13—16	28—34	**	25—26	49—57	*	***
		Damaged kernels	3—6	12	27—30	**	20—23	39—45	*	***
	Days of incubation		31	59	59	71	32	60	67	74
	Fat acidity value		185.9	75.8	74.1	54.0	58.0	50.0	—	49.7
	Moisture content(%)		17.0	15.1	14.2	12.8	16.3	15.0	14.5	13.6
Milo (Australia)	Days needed for infection	Intact kernels	5—6	7	12	**	16	20—23	*	***
	Days of incubation		31	60	60	71	32	61	67	74
	Fat acidity value		81.3	70.3	30.2	31.0	41.9	38.2	31.9	29.6
	Moisture content(%)		16.3	14.9	14.2	13.2	16.6	15.4	14.8	14.2
Milo (USA)	Days needed for infection	Intact kernels	3—6	5	11	**	9—12	15-21	*	***
	Days of incubation		31	60	60	71	32	61	67	74
	Fat acidity value		95.8	66.8	53.0	50.9	61.4	53.7	50.7	48.9
	Moisture content(%)		16.9	15.3	14.1	12.8	16.4	15.4	14.7	13.8

a) Days of incubation when the fungal growth was observed by the naked eye.
b) No fungal growth after 67 days
c) No fungal growth after 71 days
d) No fungal growth after 74 days
e) Fat acidity value=KOH mg/100 g

damage may occur, 2) 52.3% of corn from U.S.A., 47.1% of corn from Thailand, and 75% of milo from U.S.A., Argentina and Australia may be stored without any fungal damage for a considerably longer period (more than 2 months), if they are stored under conditions preventing the hygroscopic moisture absorption.

These results suggest that eligible cargo lots should be sorted out for a long-term storage (reserves) of corn and milo imported as feed grains.

References

1) Barre, H. J.: Country storage of grain. *In* Storage of cereal grain and their products. ed. Anderson, J. A. & Alcock, A. W. American association of cereal chemists St. Paul. Minnesota. 308–557 (1954).
2) Cornelius, J. A., Eggins, H. O. W. & Wallbridge, A.: Biodeterioration of palm oil constituents caused by fungi. *Int. Biodeterior. Bull.* **1**, 46 (1565).
3) Shibuya, N. et al.: Studies on deterioration of rice during storage. 1. Changes of brown rice and milled rice during storage. *Nippon Shokuhin Kogyo Gakkaishi,* **21**, 597–603 (1974) [In Japanese with English summary].
4) Sibuya, N., Iwasaki, T. & Chikubu, S.: Lipase activity in rice kernel. *Rep. Nat. Food Res. Inst.* **30**, 10–13 (1975) [In Japanese with English summary].
5) Tsuruta, O.: Storage of husked rice in cave — Changes in microflora of husked rice — *Rep. Nat. Food Res. Inst.* **28**, 10–18 (1972) [In Japanese with English summary].
6) Tsuruta, O. et al.: Long term storage of rice according to its form. *Rep. Nat. Food Res. Inst.* **32**. 11–12 (1977) [In Japanese with English summary].

7) Tsuruta, O., Sugimoto, T. & Minamisawa, M.: Mycotoxin production on contaminated maize by several fungal species. *Rep. Nat. Food Res. Inst.* **32**, 21–24 (1977) [In Japanese with English summary].

8) Tsuruta, O., Watanabe, S., & Saito, M.: The quality deterioration of fungus-infested cereals. 1. Changes in fat acidity of imported corn and milo for feed. *Rep. Nat. Food Res. Inst.* **33**, 57–64 (1978) [In Japanese with English summary].

9) Tsuruta, O., Gohara, S. & Saito, M.: Scanning electron microscopic observation of a fungal invasion of corn kernels. *Trans. mycol. Soc. Jpn.*, **22**, 121–126 (1981).

10) Ward, H. S. & Diener, U. L.: Biochemical changes in shelled peanuts caused by storage fungi. 1. Effects of *Aspergillus tamarii,* four species of *A. glaucus* group, and *Penicillium citrinum. Phytopathol.*, **51**, 244–250 (1961).

(Received for publication, August 18, 1986)