FAO/UNEP/USSR
International Training Course
«TRAINING ACTIVITIES ON FOOD CONTAMINATION CONTROL
AND MONITORING WITH SPECIAL REFERENCE TO MYCOTOXINS»

E. I. GORELOVA

STORAGE PRACTICES IN RELATION TO VEGETABLE PRODUCTS AND PREVENTION OF THEIR CONTAMINATION WITH MYCOTOXINS IN DIFFERENT PARTS OF THE WORLD

Centre of International Projects, GKNT
Moscow, 1984

STORAGE PRACTICES IN RELATION TO VEGETABLE PRODUCTS AND PREVENTION OF THEIR CONTAMINATION WITH MYCOTOXINS IN DIFFERENT PARTS OF THE WORLD

E. I. Gorelova

Introduction

The storage of any vegetable object is the final stage of its production — a stage when all efforts and money expenditures on the growing, harvesting and post-harvesting treatment may be reduced to naught if wrong practices and methods of its storage are used.

The complexity of storage, for instance of grain and seed of different crops is that the mass of grain consists of several living components such as grain proper, weeds, microorganisms and not infrequently — pests. Under definite conditions their activity might lead not only to a deterioration in the quality of the vegetable materials but also render their use as food dangerous for health. Certain storage conditions favour the development of toxic and carcinogenic properties dangerous for human health in grain and products of its processing.

Large-scale studies have been undertaken in the past few years to establish contamination of various types of vegetable raw materials with mycotoxins, to study sources of such toxins, to ascertain areas of their spread and to work out measures to prevent this type of contamination. Even today the
1222 problem of mycotoxins is still pressing for practically all countries.

This is why the specialists engaged in protecting the health and life of man should be aware of causes of toxicity in the main types of vegeatble products and have a general idea about methods of storing and preventing this type of toxicity.

Main Storage Practices in Different Parts of the World

The application of some or other storage practices in relation to vegetable products are governed by specific features of the given type as a storage object, its qualitative state during harvesting and, finally, the development level of the facilities used for handling and storing this type of material.

One of the basic crops in South-East Asia is rice, in Central Europe — grain crops, mainly wheat, in North America -- wheat and maize, in Africa — peanuts, sorgo, grains.

Rice, wheat, and rye are all graincrops but they differ widely as objects of storage. These distinctions are decisive for the use of a definite storage pattern though crop treatment methods may be one and the same. Rice grain is physiologically more active than, say, wheat which is due to the conditions of growth of rice -- humid environment of cultivation. Besides this, rice has the ability for cracking which worsen its teonological properties during transportation or in case of sharp changes in temperature. When wheat is dried one has to take into consideration effects of temperature on the protein component. Temperature, however, is of smaller consequence for rye. Therefore, though one and the same storage practice — drying — is used for rice, rye and wheat, types of drying should be different.

The next aspect which determines a storage practice is the quality of vegetable material during harvesting. Wheat grown in Australia is harvested at a low humidity level and it does not require drying, cooling and conservation in terms of lowering its physiological activity. Grain harvested in West Europe has high humidity and requires immediate drying or temporary conservation by cold. Maize grain grown in the USA is often characterized by humidity reaching 30% and calls for urgent measures to bring it to a stable state. Sorgo cultivated in Sudan retains its properties only at a humidity below 12-12.5%, though it is harvested at a high humidity.

Finally the third aspect crucial for the proper choice of a storage practice is the state of facilities for treatment and storage of vegetable products. The state of facilities is determined by the availability of modern storages. For grain these are elevators where all processes are mechanized and automatized, where the air-tight construction of buildings makes it possible to conserve grain by cold or using neutral gases. Today, a medium-size elevator can handle more than 2,500 tons of grain, clean 8,500 tons, and dry 1,400 tons. Grain driers, grain cleaning machines of high capacity, conveying and handling appliances are also part of the technical facilities of an enterprise.

The development of these facilities in the Soviet Union proceeds at a high rate. Soviet specialists have built hundreds of elevators of various design and in different climatic zones, every year they put into operation both in the USSR and abroad tens of modern elevators. The Soviet Union has high capacity grain drying equipment, various types of refrigeration plants, it also uses neutral gases and installations of active ventilation.

Australia is a highly developed industrial-agrarian country holding the third place in terms of wheat export. Australia has a developed material and technical foundation, it mostly uses metal storages high capacity equipment and it applies neutral gases.

Storage facilities in the United States are represented in the main by elevators which comprise 80% of the total capacity of grain storages. This includes storing and production elevators those which service the brewery industry, malt and maize processing enterprises. The United States makes large use of artificial refrigeration and systems of active ventilation.

A number of countries, however, the developing countries, in the main, have poorly developed facilities for the storage of vegetative products. The Democratic Republic of Sudan — one of the largest African states which occupies 8.3% of the area of Africa is one of the largest suppliers of the seed of peanuts, castor-oil plants and sorgo grain.

Accroding to F.A.O. grain losses in storage in tropical Africa amount to 30% of the harvest. This is due to the conditions which favour the development of both insects and moulds. The Aspergillus genus which numbers more than 130 species prevails among all other species of mould fungi in the microflora of tropical products. Mould fungi of the Aspergillus genus produce mycotoxins which possess carcinogenic properties. In humid tropics mould fungi occur 30 times more often than in the temperature zone.

The main cause of grain losses of all crops is the lack of the necessary facilities for grain treatment and storing — facilities which would allow for the application of scientific achievements and advanced practices in the field of safe-keeping of grain.

Effect of Different Storage Practices on the Prevention of Active Microbiological Processes

Different practices of grain storage are based on ecological factors which determine the intensity of physiological, biological, and what is most important, microbiological processes in the mass of grain or some other vegetable product.

H u m i d i t y   a n d   d r y i n g

Grain humidity is crucial for the activation of microbiological processes and is the main cause of possible development of mycotoxins. It is known that whenever grain is dry and its humidity is below critical, microorganisms pose no serious danger for it. Critical humidity is the boundary of safe storage of grain. As for wheat, rye, oats, buckwheat and seed of cereal grasses the critical boundary lies within 14.5–15.5%, for seeds of legumes and of forage grasses from the beans family, clover, common vetch, alfalfa it is 15–16%. The critical humidity for sunflower seeds is 8–10%, for maize — 13–14%, and for peanuts — 8%.

The storage of grain and seed of different vegetable crops in a dry state is an indispensable condition for their safety and conveyance by various modes of transport over large dis-
1222 tances. One of the main practices of bringing grain and other vegetable objects into a stable state in storage is drying.

Drying of grain and seed as a technological process is applied in most countries where grain and seed of different crops are harvested at an increased humidity. From the point of view of the impact of thermal drying on the microflora of standard grain and seed it should be considered in the main, as a process which reduces its viability (see Table 1).

Table 1

Humidity, %		Temperature, °C		Microorganisms, thousands per 1 g of grain		Infection of internal microflora, %
before drying	after drying	heat carriers	grain	bacteria	fungi	
			Spring wheat			
	-	-	-	800.0	160.0	50
19.3 (start.)	14.0	200	40	12.0	5.6	40
	14.2	225	44	10.2	2.4	25
	13.5	250	49	8.0	2.0	20
	13.8	275	52	8.8	1.8	10
	14.1	300	56	6.0	0.8	None
	15.0	325	62	5.2	1.4	None
	13.4	350	65	4.0	1.0	None
			Millet			
23.7 (start.)	-	-	-	7200.0	40000.0	None
	13.6	210	35	460.0	14.0	None
			Barley			
23.6 (start.)	-	-	-	34.0	100.0	None
	14.2	330	40	6.0	4.8	20

However, overheating of grain during drying greatly reduces its stability during storage under unfavourable conditions. The comparative changes in the composition of microflora of overheated and unheated grain under similar conditions of storage are shown in Table 2.

Table 2

Grain	Germination, %	Microorganisms (thousands per 1 g of grain)			
		before storage		after storage	
		bacteria	fungi	bacteria	fungi
Grain (control)	97	6.0	none	12.2	3.2
Wheat overheated	0	0.9	none	34.0	200.0
Maize (control)	100	0.5	2	5.0	16.8
Maize overheated	0	0.2	none	140.0	480.0

Helkowski et al. (1981) also corroborate that viable wholesome grain of sereal crops are resistant to fungi, grain which is incapable of germination (for instance, following autoclaving) is most quickly infected with fungi.

A d m i x t u r e s   a n d   c l e a n i n g

The grain mass includes various admixtures of vegetable, animal and mineral origin. Admixtures get into grain during harvesting, trashing and conveying. The main sources of microorganisms are chipped, gnawed, or otherwise damaged grain and mineral admixtures. When the content of admixtures ranges from 3.0 to 4.0%, more than 60 per cent of the entire-microflora falls to the share of this admixture. The so-called "spoilt"
1222 grains the presence of which is associated with increased activity of microscopic fungi have special importance in terms

of the safety of grain and seed. According to L. A. Trisvyatovsky (1963) moulded grain contain from 30 to 150 times more microflora than wholesome grains.

The Food and Agricultural Organization (1979) recommends the separation of moulded material and, in general, the cleaning of seed and grain is an obligatory measure at grain storing facilities. Cleaning besides removing mineral admixtures and spoilt grain from the grain mass greatly diminishes its contamination with mycotoxins if this was the case. Thus, Goldblat (1971) indicated that during cleaning, a small volume of waste concentrates the main percentage of aflatoxins. Specifically he lists data showing that in the cleaning of peanuts with the initial rate of contamination being 25 ppb of aflatoxins the rate of contamination of the part of waste with aflatoxins comprised 350 ppb whereas the cleaned part contained but 5 ppb of aflatoxins.

Various machines are used to clean grain and seed. These include precleaners which clean grain from large and light admixtures, various separators and triers, the latter remove admixtures which differ by their length in relation to the main grain. To withdraw admixtures the size of which is similar of grain (mineral admixtures, seed of weeds) a grain sieve-winnowing machine and a pneumosorting stand are used. The FAO recommends (1979) to remove moulded peanuts by applying electronic equipment, manually or using both techniques.

Hence, cleaning should be considered as the first stage of struggle aimed at preventing or reducing the level of contamination of grain and seed with mycotoxins.

Temperature and conservation with cold

In many countries in certain years, the delivery of untreated and humid grain considerably surpasses the available drying capacity of facilities. In connection with the danger of mould fungi formation and also considering the susceptibility of all living components of the grain mass to diminished temperatures, it is necessary to temporarily conserve untreated or humid grain by reducing its temperature until the drying facilities would become available. This can be carried out by using special installations, the so-called installations of active ventilation designed for discharging cold atmospheric air into the mass of grain or seed.

Active ventilation is used widely in many countries: USSR, USA, UK, Belgium, the Netherlands, Austria, France, etc.

Active ventilation systems are rather simple but it is necessary to stringently observe the regimen of ventilation the necessary duration of ventilation and the supply of adequate volumes of air. Table 3 shows the changes in the microflora of the grain mass during active ventilation (Bratersky F. D., 1979).

It follows from these figures that inadequate supply of air from even facilitates moulds fungi formation. It happens that when the supply of air is insufficient, water vapours saturate the air, moiture condenses on the grain surface and this creates conditions for activation of microbiological processes and the potential growth of mycotoxins.

Table 3

Specific air supply, m^3/hour per 1 ton	Humidity, %		Microorganisms (thousands per 1 g of grain)			
	before venti- lating	after venti- lating	before venti- lating		after ventilating	
			bacteria	fungi	bacteria	fungi
30	15.9	15.0	120	2.1	200	4.8
30	21.8	21.1	770	4.0	1600	8.0
60	15.9	14.7	190	1.2	240	2.6
60	16.6	15.1	125	2.4	366	2.2
120	15.9	15.3	153	22.0	140	4.2
120	22.0	20.0	960	80.0	1000	38.0
240	15.3	14.2	66	2.2	18	none
240	20.5	18.8	198	60.0	40	8
360	22.4	18.7	180	44	70	10

Besides this, insufficient supply of air fails to ensure rapid drop of temperature which, in its turn, creates favourable conditions for the development of aerobic microflora due to good aeration. However, it is not always possible to apply natural atmospheric air. Even during night hours, it may be of high temperature. Therefore, the technique of applying artificial cold cold to cool grain is being increasingly applied. The technique of conserving grain by artificial cold has been first applied early in the 60s in such European countries as the FRG, UK, Belgium, and France. Somewhat later this technique became wide spread in America. Today it is widely used in several regions of the world.

To conserve shelled rice Japan uses air tight warehouses and refrigerating plants which guarantee round the year steady temperature in the range of 12-15°C. In Spain, rice with a humidity of 15-20% is cooled to 7-8°C without preliminary drying. In Australia, a technique was worked out of active aeration of grannaries with an artificial cooling of the supplied air. In the GDR where the bulk of the grain of a new harvest is of high humidity grain conservation by cooled air is a common practice. Silage elevators with a total capacity of about 300,000 tons are equipped there with refrigerating plants. According to published information, in Czechoslovakia grain cooling is viewed as more economical compared with drying. In the FRG more than 2 million tons of grain crops is annually conserved by cooling. Refrigerating plants are widely used in Italy, Brazil, Indonesia, Ghana, Kenya, Mali, the Philippines and some other countries. More frequently the technique of artificail refrigeration of grain is called upon to ensure temporary safe storage of untreated and humid grain to increase the seasonal capacity of available grain driers, to reduce the cost of grain drying and to maintain grain temperature at the best possible level. A number of Soviet scientists and scientists in other countries worked out approximate time periods of safe storage of grain and oil bearing crops subject to humidity and temperature.

Table 4

Humidity, %%	Storage time of rice-grain/days at t°C						
	25	20	15	10	5	0	-5
14	110	120	120	120	120	120	120
15	56	84	120	120	120	120	120
16	28	42	80	114	120	120	120
17	12	20	37	78	104	120	120
18	7	11	21	40	62	120	120
19	4	7	14	21	38	105	120
20	2	4	8	12	23	63	120
21	1	2	4	8	14	39	86
22	-	1	2	4	8	22	44
23	-	2	1	3	5	10	20

Table 4 (Melnik B. E., 1977) shows that cooling of grain, for instance, with a humidity of 18% exceeds considerably the critical value, safeguards it against active microbiological processes at +10°C for 40 days and at 0°C — for 120 days or more.

Mobile and stationary refrigerating plants with air or water cooling are applied for grain refrigeration. Daily capacity of such grain conserving installations ranges from 120 to 500 tons with a reduction of grain temperature by 20°C.

Other types of conservation

Various types of conservation of vegetable products are oriented towards the suppression of moulds activity during storage. Application of various organic acids and also the control of the gas composition in grain mass proved to be particularly effective, for instance, in case of grain preservation.

C h e m i c a l   c o n s e r v a t i o n

For the first time the technique of conserving humid grain by applying low molecular carbonic acids was used in Great Britain in 1965-1968. Today it is being applied in many countries. The following mixtures of low molecular carbonic acids based on the propyonic acid are used for the needs of conserving -- Propcorn (Britain), Luprizil (FRG), Kamstor (Canada), Sentry, Orto Grain (USA), etc. As a rule, forage grain with a humidity of 16-25% is conserved and this practice preserves the initial properties of grain for 12 months or more. It also precludes the necessity of drying grain and reduces the outlays for postharvesting treatment.

The dose of the applied conservant ranges from 0.5 to 2.5% and even more in relation to the grain mass subject to its type, humidity, temperature and the intended duration of storage. Addition of 0.3% propyonic acid to the grain mass kills most mould fungi and yists (up to 80%), and a greater dose (0.5-0.75%) kills microorganisms practically totally. At these concentrations of propyonic acid the bacterial microflora is largely suppressed. Propyonic acid also has an insecticide action. Thus, according to the data of Fink et al. (1975) it suppresses the activity of insects and ticks at a concentration of 0.5% to the grain mass, and kills them at a concentration of 1.0%.

One of the main reasons for large-scale application of propyonic acid for the needs of conserving, of grain and seed, specifically, is its safety for man and animals. This is corroborated by its active participation in the metabolism of vegetative and animal organisms. According to WHO propyonic acid is a food additive, the permissible dose for man being 10-20 mg per 1 kg of body weight daily. As of 1973 FAO/WHO officially allowed the application of propyonic acid as a food conservant.

N e u t r a l   g a s e s

The application of neutral gases as a technique of storing is based on air oxygen requirement of the live components of grain and other vegetable products. The absence of air oxygen suppresses activity of mould fungi and bacteria. Thus, during storage of untreated maize grain in a gas medium consisting of 1% oxygen, 10-12% CO_2, and nitrogen -- 87-89% one observes a drop in mould fungi activity compared to the control (Dorosheva E.N. et al., 1981). Whereas the control sample contained 11,700 (moulded fungi per thousand in 1 g of grain). After the passage of 15 days, the sample which was stored in a gaseous medium contained 370 and after the passage of 30 days, 17850 and 420, respectively. Large-scale research into the storage of wheat, rice, barley and sunflower seed has been conducted in Italy (Sheibal J., 1979) where the author also noted an inhibited formation of moulds, and primarily of Aspergillus and Penicillium if storage was conducted in a medium of technical nitrogen and complete absence of these moulds in a medium of pure nitrogen. There are gas generators in the Soviet Union which produce neutral gaseous media with a content of oxygen ranging from 0.5 to 20% and that of CO_2

ranging from 0 to 12-14%.

The application of neutral gases calls for air tight storages. The questions of air-tight storages are resolved in different regions differently. Those in areas with low temperatures one can store grain of high humidity in air-tight conditions, in warm tropics, for instance, in some parts of Africa and South-East Asia one can store grain in air-tight storages with a humidity not more than 13%. Higher humidity of grain under high temperatures would facilitate rapid formation of mould fungi.

Damage and its prevention

Grain is covered by a solid and thick envelope consisting primarily of cellulose and waxy substances which are resistant to the action of microorganisms. Therefore wholesome seed with undamaged hulls contain microflora practically only under surface. Mechanical damage of the envelope opens up the access for microscopic fungi to the nutritive substances of grain and facilitates their viability, the damage in the area of the kernel being parcticularly dangerous. The growth of those fungi is particularly rapid whenever there is free moisture in the grain, i.e. when humidity is above the critical level. An increase in the numbers of mould fungi on maize grain (humidity being 18%) depending on the nature of damage is shown in Table 5. The content of moulds in the control undamaged grain prior to storage is assumed as 100 per cent.

However, even in conditions when humidity is below the critical level, the surface of injured grain has a higher degree of insemination with microorganisms.

Table 5

Grain description	Number of mould fungi, %% at storage in days			
	7	10	12	14
Control grain (with no cracks, with injured hulls)	104	186	176	300
Grain with mechanically injured hulls in the area of the endosperm	116	192	-	456
Grain with mechanically inpured hulls in the area of the embryo	376	407	389	722
Cracked grain	600	5285	9593	8293

Ways of reducing damage to grain during storage include a reduction in the number of movement of grain and seed, reduction in the altitude of their falling, dumping of shock and friction against metal surfaces of working organs of machines, observance of the regimen of grain and seed drying, specifically of those which are inclined to crack formation in the process of drying (maize, rice, sunflower seed).

I n f e c t i o n a n d p e s t s c o n t r o l

Many storage moulds, including A. glaucus, A. niger, A. flavys, A. sydowi, A. ruber, A. terrens, P. janthinellum, P. purpurogenum, P. oxalium, P. cyclopim, and P. implicatum (Husain S.S. and other, 1968), were isolated from insects which habitate in grain mass. This proves convincingly that insects are vectors of fungi and thereby facilitate the spread of moulds in grain mass. Moreover, vital activity of insects and ticks engenders favourable conditions for the growth of mould fungi. Thus Agraval and others (1957) have shown that the infection of wheat with a humidity of 14.6–14.8% with the grain weevil led in five months, to an increase in the grain humidity up to 17.6–23.0%. Christensen and Hodson (1960) demonstrated that the weevils infection of grain led to an increase in grain humidity and a rapid increase in the number of mould fungi. Moreover, vital activity of insects and ticks is associated with the liberation of heat, moisture and heat are direct results of their activity which paves the way for the growth of mould fungi. On the other hand, pests damage grain, disrupting its hull. Such pests as Acaridae ticks and Pyralidae feed, primarily on kernels. The Tenebrioides Mauritanicus, Dermestidae, Tenebrionidae and Curculionidae feed on the endosperm. In any case, the grain hull is damaged. We observe a direct relationship between the damage caused to maize knobs by insects and the presence of aflatoxin in the maize grain (Lillehoj E., 1980). A definite correlation between pests damage to grain and the content of aflatoxins in them has been also found in South-East areas of the United States during a study of environmental impacts on contamination of maize grain with aflatoxins (Stoldt L. et al., 1981).

Prophylactic measures which include the observance of the sanitary regimen, a range of measures obstructing the entry of pests, the application of ecological factors are the decisive trend in the grain pests control. Unfortunately, extermination measures have to be resorted too frequently and one of the most important among those measures is chemical control. Considering the errors of the past (the application of DDT-based chemicals and their cumulative character) today we have to approach the chemical control from three positions. Firstly, the elaboration and introduction of organophosphorous pesticides, which with the passage of time are decomposed to non-toxic metabolites, secondly, the checking of every recommended pesticide for its factor of selective toxicity and, finally, the determination of the threshold of economic expediency of pest control. Current chemical means of protecting grain in storage include the application of malathion, phosphathion and its analogues (Rumethan, Phospholan, Delicia, methylbromide, etc.).

Increasing attention is being paid of late to the study of various pesticides effects upon the formation of moulds and mycotoxins.

There are reports stating that fumigants -- ethylene oxide, methyl bromide and others suppress the spread of moulds and the formation of mycotoxins(Naraximkhen K.S.et al.,1968,Regknechen A.N.et al.,1969).According to Rao and Kharin,(1972)the DDVP(organophosphorous insecticide)in some cases facilitates the prevention of aflatoxin production(at a dose of 20 mg/kg),in others -- to the contrary aflatoxins emerge even in greater numbers(at a dose under 20 mg/kg)or when the experiments were conducted not upon wheat but upon maize, rice or peanuts). However, th doses of DDVP which prevent the production of mycotoxins largely exceed the recommended norms for the control of grain pests. This is why the application of fumigants for the prevention of moulds should not be recommended for large-scale application.

The study of the life cycle of pests opened up the possibility of controlling their life. The absence of a regular temperature of the body and the dependence of the activity of pests upon ambient temperature offered the possibility of using this factor in the control of pests. The lower threshold of active existence of the main pests of grain and seed is on the level of 6–12°C, the upper –– 36–42°C. Thus the application of lower temperatures is not only an effective means of preventing the development of moulds, but also a reliable method of control of grain and seed pests.

The conservation of grain products and of other vegetative objects by means of neutral gases is also an effective means of controlling pests which need air oxygen for their life.

New trends in the control of grain and seed pests include the application of hormonal preparations and pheromones. The former, selectively disrupt physiological processes which are inherent in insects only, for instance, growth, development, multiplication, etc. Therefore, these compounds are safe for man and higher animals. Feromones are biologically active substances which are produced by animals and capable of affecting other organisms. Among them, noteworthy are attractants and repellants. The former attract insects by their odour which makes it possible to use them as traps for the highest accumulation of pests, largely reducing the area of treatment and the volume of the applied pesticide. The attractant, being mixed with a sterilizing preparation deprives males of their procreative ability, finally, attractants may be used for mechanical control only, destroying amassing pests.

Repellents are substances the smell of which frightens away pests, hence the treatment of tare before placing seed in it prevents seed against contamination.

One of the promising trends of pest control is radiation desinsection of grain. Radiation of infected grain by a flux of accelerated electrons at a dose of 20-30 krad entails the reduction of their life span and the loss of their ability for multiplication.

Effects of irradiation upon the life of mould fungi and their ability to mycotoxin production is also of definite interest. According to the data of the Joint Conference on Mycotoxins (Kenya, 1977) the radiation dose of 15-100 krad, recommended by the report of the FAO expert committee for the protection of grain against insects is insufficient for mould inhibition. Besides this it is reported that when grain is irradiated by a dose which kills moulds, the grain acquires a foreign aftertaste and odour.

Summing up this communication we may emphasize that of decisive importance in preventing the growth of mycotoxins in grain and seed during storage is humidity and temperature.

Зак. 1222 ПИК ВИНИТИ