

**MYCOTOXINS IN FOOD (1) CEREAL GRAINS AND THEIR PRODUCTS**

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Fungal poisons have been known for many years but they have not been considered a major factor in human health problems until almost the last two decades. Epidemics of dry gangrene and various derangements of man was common feature in Europe from the 11th through the 16th centuries. A disease called holy fire was actually ergotism and was caused by a related group of alkaloids from the sclerotia of a mould *Claviceps puepurea*. The source of the ergot which contained the toxin was infested rye, which was ground into flour, baked and consumed by the affected persons (Forgacs and Carl – 1962).

Despite such historic beginnings the Scientists remained distinterested in the mycotoxins until the 1960s when antibiotics were discovered. From microbial point of view, Pencillin which is a metabolite of several mould species, is a mycotoxin (Balay – 1960).

Approximately at the same time, World War II brought to our attention three drama-tic mycotoxicoses in Japan and Russia (Balay – 1960, Forgacs and Carl, 1962 Campbell 1974). The first Japanese case was referred to as the “yellow rice disease” and oadsed a number of deaths. It was associated with the invasion of the rice by a number of moulds including *Pencillium islandicum*, *Penicillium citrinum* and *Penicillium citreoviride*. The second mycotoxicoses was the “alimentary toxic aleukia” (ATA) of man. Rice stored under poor conditions was imported into Japan in large quantities. The toxic agents such as citrinin, citroviridin, luteoskyrin, and islandotoxin were isolated and characterised but their natural occurrence in rice has not yet been demonstrated. The third mycotoxicosis was in Russia where rye harvest was delayed due to labour shortage during the war. *Fusarium sportrichoides*, growing on the over wintered grain, elaborated a potent toxin that caused necrotic ulceration on the fingers, lips and oral mucosa of the consumers eating bread from the contaminated grain. In this case also, a number of deaths were reported (Balay 1960). The toxic agent was probably one of the trichothecenes.

In 1952, an acute fatal hemorrhagic hepatitis of swine and cattle eating mouldy corn was observed in U.S.A. (Sippel, Burnside and Atwood – 1954). In 1958, a number of hunting dogs died from acute hepatitis after consuming canned food containing mouldy corn (Wogan – 1965). These two mycotoxicoses were eventually related to ailatoxin and rubratoxin produced by *Aspergillus flaws* and *Penicillium rubrum* respectively.

Aflatoxins designate a group of mycotoxins produced by fungi in the flaws - parasiticus group of the genus *Aspergillus* (Diener and Davis 1969). These ubiquitous fungi can invade and produce aflatoxins on a seemingly endless variety of food and feed stuffs including cereals consumed by humans and animals (Scott – 1978). Although aflatoxin B1, B2, G1 and G2 are the four major metabolites of these fungi, antitoxin B1 is

predominant and is considered to be the principal toxic element. Aflatoxins are primarily hepatotoxins, potent carcinogens in trout (Halver – 1969) and rats (Wogen et al – 1974). A growing volume of epidemiological evidence showing a high correlation between the incidence of human hepatomas and aflatoxin consumption clearly indicates the carcinogenic potential of aflatoxin to man (Wilson – 1978).

In an outbreak of hepatitis in Western India in 1974, 400 people were affected, of whom over 100 died (Krishna – machari et al 1975). This outbreak was associated with the consumption of mouldy corn containing large quantities of aflatoxin.

The toxicity, carcinogenicity, ubiquity and documented outbreaks in swine (Loose-more and Harding 1961), cattle (Loose more and Markson 1961), Poultry (Hamelton – 1971) and humans (Krishnamachari – 1975) emphasizes the threat from mycotoxins to both animal and human health.

Out of plant products, cereal grains are the most important because they represent the base of foods and feeds. The mycotoxin problem in cereals is not restricted to any geographic or climatic region. In general, mycotoxins, are more of a problem in the tropics than in the temperate zone, but no region of the world can be considered mycotoxin free because of the movement of cereals from one part of the globe to another (Hasseltine – 1974). Toxins are produced on cereals both in the field and during storage. They involve both the grain and the whole plant. The genera of fungi most commonly involved are *Aspergillus*, *Fusarium*, *Penicillium* and *Claviceps*. Mycotoxins known to occur naturally in cereals are presented in Table – I (Strzelecki – 1979). Scott (1965), in his review of mycotoxins in stored grains and their products listed wheat, wheat flour, spaghetti, corn, rice, sorghum, oats, rye, barley, malt sprout, breakfast cereals and all major grain staples.

Campbell and Leonard (1974) reported more than 100 ppm of mycotoxins in maize and rice from South Africa. From Uganda, the same workers reported 135, 152 and 26 µg/kg (ppb) mycotoxins on maize, sorghum and millets respectively. They further added that examination of prepared rice for family meal in Thailand contained 71 to 600 µg/kg of mycotoxins. In a survey of U.S. grain sorghum for zearalenone, ochratoxin and aflatoxin in 1975-76 crops, Shotwell et al (1980a) reported zearalenone in 28% samples at 200 – 6900 µg/g (PPM) and aflatoxin from 6.54 µg/g (PPM) of Sorghum. No ochratoxin was reported up to 160 PPB of aflatoxin in four samples of millets collected from Kano market in wet season. All these values are above the 20 ppb maximum limit of U.S. Food and Drug Administration.

Shotwell et al (1980b) investigated aflatoxins in kernels, cobs and husks from infected field and reported that kernels of 84% of plants contained upto 300 µg/kg while cobs of the same plants were reported to contain 1 – 260 µg of aflatoxin per kg. Shotwell et al (1980c) analysed 923 corn samples for aflatoxin contamination. Only 0.3% samples were reported containing more than 20 ppb of aflatoxin. No zearalenone was detected in 47 samples analysed. Lillehoj et al (1980) investigated the interaction between hybrids, field environments planting date and *Aspergillus* Flaws infection of developing kernels. Significantly higher levels of aflatoxins 7300 ppb were reported in inoculated ears than the controls.

**Table 1. Mycotoxins Found in Cereals**

S. No.	Name of Toxin	Name of Mould	S. No.	Name of Toxin	Name of Mould
1	Aflatoxin	Aspergillus Flavus Aspergillus prarsiticus	7.	Penicillic Acid	Penicillium puberulum Penicillium cyclopium
2	Ochratoxin A & B	Aspergillus ochraceus Penicillium viridicatum	8.	Kojic Acid	Aspergillus flavus Aspergillus Oryzae
	Zearalenone	Fusarium graminearum Gibberella zeae	9.	Tanuzonic Acid	Piricularia oryzae
4	T2 – Toxin	Fusarium tricinctum	10.	Sterigmatocystin	Aspergillus nidulans Aspergillus versicolor
5.	Citrinin	Penicillium citrinum Aspergillus niveus	11.	Rubratoxin	Penicillium rubrum
6	Patulin	Penicillium patulum Penicillium expansum	12.	Ergot	Claviceps species

Reference: Stzelecki (1979)

According to Pantovic (1981) a large number of imported cereals into Yugoslavia including wheat, maize, rice, barley and beans were analysed for mycotoxin contamination. Out of the 242 cereal grains examined, 51.3% were reported to contain aflatoxin B1 and G1. Zearalenone was detected in 50% of the corn samples examined at a concentration level of 0.25 to 26 ppm (250 – 2600 ppb). However, no ochratoxin was reported in any sample. Szehtotka et al (1982a) analysed 406 samples of mouldy cereal grains for ochratoxin A. They reported that 6 samples of barley and 8 samples of wheat contained from 20 – 130 pg/kg and 20 – 100 pg/kg of ochratoxin A respectively. The same workers (1982b) investigated a number of mycotoxins in cereals during 1975 – 1978 and found about 140 pg/kg of ochratoxin A in wheat, barley and rye.

Aflatoxin B1 content in 15 rice varieties colonized by *Aspergillus* flaws ranged from 0.457 pg/kg. Each value was reported to be above the tolerance level of 20 ppb of total aflatoxin set by the U.S. Food and Drug Administration. The grain was incubated at 20.30°C and 27% moisture. Most of the aflatoxin was reported in the bran polish of the kernel and very little in the milled rice (Una and Juliano - 1982).

*Factors affecting mycotoxins production:*

The discovery that aflatoxins are potent hepatotoxic metabolites of *Aspergillus* flaws (Sargent et al – 1961) has spurred extensive research on the factors affecting the production of this toxin and other mycotoxins on feed and food. Van Warmelo et al (1968) investigated the development of aflatoxin in maize samples stored for 12 months and naturally infected with *Aspergillus* *nevus* at 17 to 19% moisture content and incubated at 20, 25 and 30°C for four weeks. They reported that aflatoxins accumulated only in those samples with a moisture content of 18-19% incubated at 25-30°C. Trenk and Hartman (1970) reported aflatoxin content in maize samples at different moisture levels stored at different temperatures. These workers reported aflatoxin B1 and B2 developed at 17.5% moisture content stored at 24°C. According to Sorensen et al (1977) optimum temperatures for the production of aflatoxin B1 was 28 to 32°C and for aflatoxin G1 28°C. No aflatoxin was reported at temperature below 8°C.

Chang and Markakis (1981) inoculated two varieties of barley, one huskless and the other with husk with *Aspergillus* *parasiticus* and incubated at 25°C at moisture levels 10-37% up to 50 days. It was reported that the huskless variety had 946 pg/kg of aflatoxin as against 2684 pg/kg in the case of variety with husk after 50 days of incubation at 25% moisture. They further added that in the event of aflatoxin contamination moisture content of 16% or above is hazardous in the storage of barley at temperature near 25°C. The effect of temperature cycling on the relative production of aflatoxin B1 and G1 by *Aspergillus* *parasiticus* was studied by Lin, Ayres and Koehler (1980). It was reported that the cycling of temperature between 33 and 15°C favoured aflatoxin B1 accumulation whereas cycling between 25 and 15°C favoured aflatoxin G1 production. Cultures subjected to temperature cycling between 33-25°C at various time intervals changed the relative production of aflatoxin B1 and G1 drastically.

In contrast to aflatoxin, the physiology of production of the various fusarial toxins is radically different in that they are stimulated by cool temperature or by exposure to a low temperature for part of their development. Joffe (1971) has shown that *Fusarium* *poae*, *F. sporotrichoides* and *Cladosporium* *fragi* preferred low temperature from — 7°C to 25°C for growth and toxin production. He further reported that alternate freezing and thawing for 9-15 days was the best for this purpose. According to Burmeister (1971) *Fusarium* *tricinctum* produced maximum toxin at 15°C on white corn grits and declining quantities at higher temperatures, yielding no toxin at 32°C.

Sherwood and Peberdy (1972b) found that high quantities of zearalenone could be produced by *Fusarium* *graminearum* experimentally in wheat, barley and oats at

moisture contents 23 to 37% at 25°C. The production of toxin was reported to increase linearly from 4 to 5000 ppm over the range 14.5 to 54% moisture content. The same workers further reported (1974) that optimal production occurred on wheat with a moisture content of 37% when held for four weeks followed by six weeks at 12°C. When the temperature was kept at 25°C, however, toxin yields were low being seldom higher than 100 µg/g (100 ppm) even though mycelial growth was rapid. According to Christenson (1973), the critical moisture content for mould growth could vary widely from one food stuff to another and was estimated to be 14.5% for sorghum, and 12.5 to 13.5% for wheat and maize.

*Effect of Processing on the fate of Mycotoxin:*

Chelkowski et al (1981) investigated the concentration of Ochratoxin A in commercial samples of wheat and barley and the changes in its concentration as a result of various processing operations. It was reported that neither dry nor wet cleaning had major effect on ochratoxin concentration. Milling trials showed ochratoxin concentration to be approximately equal in the flour and in the bran. Pearl barley was reported to have a relatively low Ochratoxin concentration while bran removed had higher concentration. It was further reported that normal processing methods do not give adequate detoxification of ochratoxin contaminated grain. The effect of wet milling of maize on the distribution of zearalenone was investigated by Bennet et al (1978). Most of the zearalenone was reported in the feed products (fiber, germ and gluten) and none in the starch. The germ fraction from which edible oil is extracted contained 9-11% of the total zearalenone. The fiber fraction was reported to contain 15-19% and the gluten fraction contained 49.50% of the total zearalenone. Bennet et al (1976) also studied the effect of dry milling of maize and reported high concentration of zearalenone in the germ and feed fractions. The fate of zearalenone (T2 toxin) was also, studied by Collins (1981) in the wet milling of corn. The major part of the toxin (70%) was found in the soluble fraction and 26% was reported to be evenly distributed between the germ, gluten and fibre. The remaining 4% was found in the starch. Toxin was not destroyed by steeping of corn. Contaminated corn was produced for this study by growing *Fusarium tricinctum* on dent corn. Scott and Lawrence (1980) reported 62 µg/kg ergocristine as a major alkaloid in commercial wheat and rye flour.

The fate of aflatoxin B1 in the baking was investigated by Reiss (1978). He added crystalline toxin to the dough at the rate of 10.8 and 5.4 µg/g (10.8 and 5.4 ppm) and reported 10-20% of the toxin in the final dough. It was further reported that subsequent baking at 120°C for 30 min. did not reduce the amount of the toxin. The same worker (1981a) reported that *Aspergillus flavus* and *A. parasiticus* grew and produced aflatoxin B1 and G1 on various types of bread. *Aspergillus parasiticus* was reported also, to grow better and produce more aflatoxin M1 on cake. The same worker (1981b) investigated the development of ochratoxin A in a variety of bread with wheat germ, whole meal bread with linseed and whole meal rye bread. It was reported that the growth of *Aspergillus ochraceus* was enhanced by linseed and wheat germ and that appreciable quantities of

ochratoxin A were recorded. Maximum growth of the mould was reported at acid pH and 20°C. However, optimum temperature for synthesis of ochratoxin A was reported to be 15-20°C. A maximum concentration of 0.8 µg/g (0.8 ppm) of ochratoxin A was reported and it was concluded that hazardous levels of the toxin may be formed in the whole meal wheat bread.

Buflot (1982) studied the behaviour of aflatoxin M1 during the various stages of biscuit processing using two full cream milk powders. One milk powder was naturally contaminated and contained 10 µg/kg of aflatoxin M1 while the second milk powder was artificially contaminated with 50 µg/kg of the same toxin. After mixing, laminating, cutting and baking for 6 minutes at 120°C, the residual toxin was analysed. In both cases negligible amount (0.05 and 0.2 µg/kg or 0.05 and 0.2 ppb) were found in the biscuits.

Several chemical and physical treatments were studied by Bennet, Showell and Hasseltine (1980) as possible methods for destroying zearalenone in corn. An ammoniation process which significantly lowers aflatoxin levels was reported to have no effect on zearalenone contamination in yellow corn. Also, treatments with propionic acid, acetic acid, hydrochloric acid, sodium bicarbonate and hydrogen peroxide failed to reduce toxin levels. High temperature treatment at 150°C was reported also to be ineffective. However, formaldehyde in vapour forms was reported to destroy significant quantities of zearalenone.

Chang and Markakis (1982) investigated the effect of irradiation on aflatoxin in barley containing different moisture levels. The barley samples were inoculated with *Aspergillus parasiticus* and stored at 25°C and 85% relative humidity. The samples were irradiated with gamma radiation up to 400 Krads. A reduction of total aflatoxin was reported from 819 to 308 µg/kg barley at 400 Krads irradiation dose. It was further reported that under most of the conditions studied irradiation reduced aflatoxin production in barley samples. The most striking effect, however, was reported to be exerted by moisture level and this is the factor which should be controlled in practice in order to minimize aflatoxin contamination.

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