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PRESENT STATUS OF MYCOTOXIN STUDIES IN KOREA

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=ABSTRACT=

Mycotoxins are a group of toxicants giving a risk potential to human health in connection with the daily food intake. Food commodities once contaminated with mycotoxins can not be detoxified by any economic means and prevention was suggested as the only measure.

In order to minimize the economic loss and health hazard posed by mycotoxins and toxicoses, systematic and toxicological studies on the subject should be undertaken. Most reports in Korea were concentrated on the mycological studies of relatively easy techniques and the confirmation or quantitation of mycotoxins was rarely done.

Research topics to be undertaken in future may be exemplifid below:

(1) Establishing assay methods for individual or multi-residue of mycotoxins
(2) Monitoring of mycotoxins for suspicious food or feed samples in Korea
(3) Epidemiological survey of mycotoxicoses
(4) Etiological survey of disease outbreaks associated with mycotoxins
(5) Accumulation of testing method and data on the toxicity of mycotoxins
(6) Legal regulation to control mycotoxins and development of their detoxification / elimination methods

Key Words: *Mycotoxin in Korea, Food Contamination, Epidemiology of mycotoxicosis,*

INTRODUCTION

Intensive studies on mycotoxins and mycotoxicoses have been undertaken in recent years, but many aspects still remain to be solved. The subject of mycotoxin problems called out much attention of many scientists in the fields of toxicology, preventive medicine and food production since the formation of mycotoxins represents a potential economic threat to agriculture and a direct impact on human health.

In particular, Koreans consume a large amount of cereals and fermented foods

as other Asian nations do and they are vulnerable to hazard potential by exposure to various mycotoxins. After the aflatoxin became a problem of world-wide concern, scientific studies on mycotoxins were undertaken by Korean investigators in different fields. Several review papers on mycotoxins were published in this country (Chun & Kim, 1980; Koh et al., 1972a; Lee et al., 1971b; Lee, 1978; Lee, 1983; Roh et al., 1979; Roh & Park, 1982).

This paper is to review the practical problems and current status of scientific studies as related to mycotoxins and mycotoxicoses and further to suggest any needed fields of investigation in future for this country.

PRACTICAL PROBLEMS OF MYCOTOXINS

The world concern of mycotoxins may be grouped into following three aspects.

1. Aflatoxin and aflatoxicoses

Aflatoxin discovered from the outbreak of turkey X-disease in England in 1960 causd a new recognition on the problem of mycotoxins. In particular, as the aflatoxin was proved to be a potent carcinogen by animal experiments and epidemiological studies, the problem called out a world-wide attention and legal regulation of mycotoxins in food commodities was made in many countries.

The detection of aflatoxin in the trade of farm products is becoming a common practice in many countries. However, Korea did not establish a tolerance for aflatoxin as yet for foods whereas that for animal feeds was tentatively set to 100 ppm in 1982.

The practical problems in Korea in connection with aflatoxin contamination were the presence of aflatoxin in the animal feed (peanut oilcake) imported form Southeast Asia in the range of 200-300 ppm in the late 1970's and the return of exported peanut products form Japan by the presence of aflatoxin in 1981.

2. Fusarium toxins and toxicoses

The first mycotoxicosis in the world was the outbreak of alimentary toxic aleukia which occurred in Russia in 1940's by the consumption of grains contaminated with *Fusarium* molds. Toxins produced by *Fusarium* species are known to be diverse and their toxicoses should receive much attention in connection with recent problems of fungal contamination in animal feeds.

It is sometimes reported for the unknown death of domestic animals, particularly cattles, in the rural area of Korea. Any etiological survey for such poisoning has not been undertaken and the doubt disappears soon after the news is merely reported in the mass communication media. It is very suspicious that such animal death might be due to the mycotoxicosis by *Fusarium* contamination (or probably pesticide residues) of farm products.

3. Yellowed rice toxins and toxicoses

The third is the yellowed rice toxins produced by contamination with *Penicillium* species of rice grains in storage. These belong to a group of mycotoxins produced

by artificial contamination of molds isolated from deteriorated rice and any toxicosis did not occur in the field.

As it is recognized that the incidence frequency of liver disease (cirrhosis and cancer) among Orientals is several hundred times higher than Americans, a part of the cause is likely due to the mycotoxins (luteoskyrin and islanditoxin) produced by *Penicillium* sp. contaminated in the rice.

Since it is reported that rice grains produced in Korea are sometimes contaminated with *Penicillium islandicum* and deteriorated in quantity storage, some attention should be paid on this aspect. As a practical situation 30,000 tons of rice grains exported to Japan in 1960 had to be inspected for the absence of toxin-producing *Penicillium* species and contaminated lots in certain storehouses had to be rejected. On the other hand, rice grains imported from Japan in 1970's were not examined by Korean side. This aspect should be reconsidered by appropriate health authorities of Korea.

INVESTIGATIONS UNDERTAKEN IN KOREA

It is said that the momentum for attracting a keen attention on mycotoxins including aflatoxin in Korea begins from the survey report by Crane et all. (1970) in Jeonju presbyterean Medical Center. According to their report on a clinical survey for cancer incidence of Koreans, stomach cancer comprised 32.4% out of 3,330 cancer patients seen in Korea from 1962 to 1968. On the basis of dietary survey for 170 patients and 170 normal persons, patients with stomach cancer showed a higher intake level of soy paste (2.2 times) than persons without stomach cancer as shown in Table 1. Meju, the starting material of soy sauce and paste made by natural inoculation of microorganisms on soybean substrate was sent to the Northern Regional Reserch Laboratory of USDA and found to contain *Aspergillus flavus-oryzae*. They, therefore, suggested that the high incidence of stomach cancer among Koreans is likely due to the ingestion of aflatoxin from soy paste (Seel, 1969).

Table 1. Stomach cancer and dietary intake by Koreans (Unit: G/Day/Person)

Diet	Without Cancer	Stomach Cancer	Intake Ratio
Kimchi	88.5	121.2	1:1.4
Soy Paste	25.6	55.9	1:2.2
Soy Sauce	5.4	14.2	1:2.6
Pepper Sauce	8.6	5.2	1:0.6
Alcoholics	416	284	1:0.7

Calculated from Crane et al. (1970).

They did not attempt to detect any aflatoxin from the soy paste. In considering the fact that aflatoxin causes liver cancer, their attempt to link stomach cancer with aflatoxin-containing foods sounds odd. It seems rather reasonable to point out the higher intake levels of soy sauce (2.6 times) and *kimchi* (1.4 times) by patients with stomach cancer than normal persons as seen in their survey.

Their report was, however, cited without objection at the time when the carcinogenicity of aflatoxin was widely accepted in the world. This was followed by several investigations in Korea for the presence of aflatoxins and toxigenic fungi in common foods consumed by Korean people.

Reports published in Korea in connection with mycotoxins are summarized in Table 2. The papers were classified into three categories of mycological studies, detection of mycotoxins and toxicity/elimination studies.

Table 2. Number of papers on mycotoxin studies in Korea

Period	Mycological Study	Mycotoxin Detection	Toxicity & Elimination	Total
1960-69	2	2	1	4
1970-79	18	5	15	37
1980-	7	2	1	9
Total	27	9	17	50

1. Aflatoxin and aflatoxicoses

In Korea, mycological studies of mycotoxin-producing fungi were undertaken for the most part as compared with other fields. The reasons may be assumed as follows:

(1) Fungal contamination of foods or feeds is visible and causes an economic loss as well as hygienic problems in trade of the commodities. This called out the interest of investigators in different fields.
(2) There are many microbiologists in diverse fields and less equipment and supplies are needed for microbiological studies.
(3) Mycotoxin problems were relatively a new research subject at the time and mycological experiments were easy of access to the topics.

Mycological studies consist of experiments for the mycofloral survey of domestic food commodities, isolation of mycotoxin-producing fungi of already known species

Table 3. Mycological studies on mycotoxin producers in Korea

Year	Researcher	Results
1960	Cho	Mycological inspection of Yellowed rice for export
1969	Chung & Kwon	Isolation of *A. flavus* from fermented soy foods
1970	Lee et al.	Isolation of *A. flavus* from cereals and fermented foods
1971	Lee et al.	Isolation of *A. flavus* from fermented soy foods and its aflatoxin formation
1971-3	Lew & Koh Koh et al.	Isolation of molds from cereals and thier aflatoxin formation
1973	Chun	Ochratoxin formation by *A. sulfureus*
1972-4	Cho et al.	Microflora of yellowed rice
1975	Lee et al.	Isolation of aflatoxin producer from rice and meju
1974-8	Lee et al. Kim & Lee	Aflatoxin formation by *A. flavus* Luteoskyrin formation by *P. islandicum*
1979-81	Suh	Effect of culture conditions and additives on aflatoxin formation by *A. flavus*
1980	Joo & Kwon	Aflatoxin formation by *A. Flavus* & *P. citrinum* in dried persimmon
1982	Chang & Markakis	Aflatoxin formation by *A. parasiticus* in barley
1982	Mheen et al.	Isolation of *A. flavus* from rice
1982	Lee & Park	Isolation of mycotoxin-producers from animal feeds
1984	Cho & Anderson	Biosynthetic pathway of luteoskyrin by *P. islandicum*

and conditions of aflatoxin formation in the laboratory. Reports on mycological studies are summarized in Table 3 according to year and research group.

In Korea, rice is a staple food and has been subjected to deterioration during its storage. In connection with yellowd rice, grains for export to Japan were inspected for the absence of toxin-producing *Penicillium* species (Cho, 1960). The results showed that rice grains from Jeonnam area were contaminated with *P. islandicum* and *P. citrinum* and the lots showing 0.3-1% of yellowed rice grains were found in 3 storehouses among inspected.

Chung & Kwon (1969) isolated *Aspergillus flavus* from 35 samples of fermented soy foods and claimed to produce aflatoxins B_1, B_2, G_1 & G_2 in Czapeck liquid medium. Lee et al. (1970) showd that the detection frequency of *A. flavus* from 228 samples of cereals and fermented foods was very high. Lee et al.(1971a) isolated 15 strains of *A. flavus* from fermented soy foods and showed that 3 isolates produced fluorescent substances with similar R_f values on TLC plate but UV spectra different from authentic aflatoxins. They concluded that the isolated *A. flavus* did not produce aflatoxin.

Lew & Koh (1971; Koh et al., 1972b; Koh et al., 1972c; Koh et al., 1972d; Koh et al., 1973a; Koh et al., 1973b) undertook a series of studies on the mycoflora of Korean foods and their toxicity. The detection frequency of molds in cereals and rice cake is shown in Table 4, according to genus groups. Upon screening the producibility of aflatoxin in liquid culture by 58 isolates of the Genus *Aspergillus*, 3 strains excreted aflatoxin-like substances upon R_f values on TLC plate.

Table 4. Detection frequency of dominant and toxic fungi in deteriorated rice

Species	Detection Frequency (% Rice Grain)	Mycotoxin Formation
Asp. oryzae	14.5	−
Asp. amstelodami	13.4	−
Asp. candidus	12.6	−
Asp. repens	10.8	−
Asp. chevalieri	8.1	−
Asp. versicolor	5.9	−
Asp. fumigatus	4.2	+
Asp. ruber	3.7	−
Pen. islandicum	3.1	+
Asp. montevidensis	3.0	−
Asp. flavus	1.7	+
Asp. ochraceus	0.6	+
Pen. citrinum	0.6	+

From Cho et al. :(1972); Kim & Cho (1974)

Cho et al. (1972; Kim & Cho, 1974) collected 89 samples of deteriorated rice from whole area of Korea and classified them into 7 types. From these, microorganisms which were considered infected into the grains were isolated and identified. Table 5 shows the list of dominant species and toxigenic fungi they isolated. It was suggested that the detection frequency of mycotoxin-producing fungi is generally low in domestic rice but the high frequency of *P. islandicum* in imported rice may cause a problem.

Chun (1973) inoculated 2 strains of *A. sulfureus* to rice and confirmed the formation of ochratoxin A, B and C upon TLC. Lee et al. (1975a) obtained 37 isolates of *A. flavus*, 48 isolates of *A.* sp., 12 isolates of *Penicillium* sp. and 105 isolates of other molds and

Table 5. Occurrence of fungi in cereals and rice cake (%)

Genus	Cereals (1971)	Cereals (1972)	Rice Cake (1972)
Penicillium	15.0	24.4	32.2
Aspergillus	16.2	20.5	30.1
Alternaria	10.5	13.4	2.1
Mucor	11.3	9.2	9.1
Fusarium	4.0	6.4	–
Rhizopus	5.9	5.3	12.5
Cladosporium	10.5	4.3	0.7
Others	18.4	8.7	7.8
Unidentified	8.2	7.8	5.5
Total Isolates	353	283	143

From Lew & Koh (1971); Koh et al. (1972b; 1972c)

showed that only one isolate of *Penicillium* sp. produced aflatoxin B_1 in Sabouraud medium upon TLC.

The author and coworkers (Lee & Lee, 1974; Lee et al., 1975b; Lee et al., 1975c) investigated the producibility of aflatoxin by 7 strains of *A. flavus* isolated from deteriorated rice and found that all strains produced aflatoxin B_1 the most and no G_1. The most potent strain was *A. flavus* var. *columnaris* which produced 7.8 ppm of aflatoxin B_1 in rice, only about 1/90 of that by the well-known strains of *A. flavus* ATCC 15517 or *A. parasiticus* NRRL 2999. Upon examination of risk potential by contamination of this strain on stored grains, aflatoxin production tended to decrease when the strain grows together with other fungi whereas its production drastically increased when existing alone. It was, therefore, warned that aflatoxin contamination becomes possible if *A. flavus* proliferates under specific conditions of rice storage.

There are two types of mycotoxins, hydrophilic islanditoxin and lipophilic luteoskyrin in yellowed rice and the producibility of luteoskyrin by *P. islandicum* isolated from deteriorated rice was examined (Kim & Lee, 1978). The result showed that luteoskyrin was formed up to 40 mg/g rice and its formation will reach the assumed tolerance level of 3.5 ppm before the mycelial growth of the fungus could be recognized with naked eyes. It was, therefore, concluded that the contamination of rice with *P. islandicum* may cause the risk of mycotoxicosis.

Suh (1979a; 1979b) studied the effect of culture conditions such as pH, moisture and temperature upon the aflatoxin formation by *A. flavus* in combined culture with *Bacillus subtilis*. She (Suh, 1981) also studied the effect of various additives to show that ginseng extract and saponin retarded the aflatoxin formation without regard to fungal growth and accelerated the aflatoxin degradation in liquid culture. It was, therefore, suggested that ginseng extractives may exhibit the detoxifying effect of aflatoxin in foods, in connection with their anticarcinogenic activity.

Joo & Kwon (1980) investigated the microfloral change during the storage of dried persimmon and found its contamination in the decreasing order of *Aspergillus Escherichia Mucor Alternaria Penicillium*. In liquid culture of 6 isolates, *A. flavus* formed aflatoxin B_1-like and *P. citrinum*, aflatoxin G_1-like substances, upon TLC and UV spectrum. Chang & Markakis (1982) inoculated *A. parasiticus* NRRL 2999 to barley grains and followed the aflatoxin formation. The results showed that the formation level and isomer ratios varied depending on the incubation temperature and moisture content.

Mheen et al. (1982a; 1982b) isolated and identified 27 strains of fungi from stored rice grains and observed the detection frequency of major fungi as shown in Table 6. *A. flavus* was found the most but no mention was made for the mycotoxin problem. Lee & Park (1982) examined the occurrence of toxigenic fungi in 72 samples of composite animal feeds in trade and found mycotoxin producers such as *A. flavus, A. candidus* and *A. ochraceus,* even in a low density of growth. No aflatoxin was found in the feed samples and artificially deteriorated samples by allowing further growth of existing fungi reached a total aflatoxin level of 12 ppb. They concluded that no worry is necessary for aflatoxin contammination in animal feeds.

Table 6. Occurrence of fungi in stored Korean rice

Sample	Fungal Species	% Frequency
Paddy	Aspergillus flavus	80
	Aspergillus candidus	33
	Aspergilus versicolor	10
	Aspergillus steckii	7
Brown Rice	Aspergillus ruber	73
	Aspergillus sydowii	13
	Aspergillus versicolor	7
	Penicillium chrysogenum	7
	Nigrospora spherica	3
	Rhizopus nigricans	3

From Mheen et al. (1982b)

Recently, Cho & Anderson (1984) studied the biosynthetic pathway of luteoskyrin in *P. islandicum* by means of radiotracer techniques and found the route of emodin anthrone → emodin → skyrin.

2. Detection of Mycotoxins

As the aflatoxin contamination called out a world-wide attention, Korean scientists also began to screen the presence of mycotoxins including aflatoxin in food commodities consumed by Korean population. published papers are summarized in Table 7.

Lee & Lee (1969) screened 15 samples of fermented soy foods including meju, soy paste and soy sauce and found that 6 samples contained aflatoxin B_1, having the same $R_f$values on TLC plate with the authentic compound. They, however, concluded that it was an aflatoxin-like substance since it showed different UV spectrum and color reactions from authentic aflatoxin B_1. Chung & Kwon (1969) also screened 35 samples of soybean, meju, soy paste and soy sauce by AOAC method and confirmed the presence of aflatoxins G_1 and G_2 by means of TLC and UV spectrum. It is, however, regretted that their confirmation step was not sufficient.

Lee et al. (1970) surveyed 228 samples of cereals and fermented foods collected throughout whole country and suspected for the presence of aflatoxin-related substance since it showed the same R_f value on TLC plate but different UV spectrum. Kim (1975) and Han et al. (1978) screened 55 samples of cereals, soy paste, red pepper paste and 40 samples of *Aspergillus* starter and found a fluorescent substance of the same R_f values on TLC plate

with authentic aflatoxin. But the substance showed different R_f value in two-dimensional TLC and UV spectrum and they concluded that the samples contained no aflatoxin. Joo & Kwon (1980) studied the formation of aflatoxin during the storage of dried persimmon and reported the presence of aflatoxin-like substance upon TLC and UV spectrum.

Table 7. Detection of mycotoxins from Korean foods

Year	Researcher	Results
1969	Lee & Lee	Detection of aflatoxin-related from fermented soy foods
1969	Chung & Kwon	Unconfirmed detection of aflatoxin G_1, G_2 from fermented soy foods
1975	Kim	Absence of aflatoxin in cereals and fermented foods
1977	Kim et al.	Detection & quantitation of aflatoxin from fermented soy foods
1979	Chun & Shin	Detection of mycotoxins from dried fruits & nuts
1980	Joo & Kwon	Detection of aflatoxin-related from dried persimmon
1984	Kim & Roh	Detection of zealarenone from imported corn

In reviewing the preceding papers, all of them showed the presence of fluorescent substances with similar R_f values on TLC plate but they failed to prove the identity of their UV spectra with authentic aflatoxins. The presence of aflatoxin in Korean foods was merely to be suspected. It appears that the purification of aflatoxin for UV spectrum was done only by TLC plate and this was not sufficient to remove interfering contaminants. The author (Kim et al., 1977), therefore, undertook a series of experiments to isolate, identify and quantify aflatoxins. From households in the major cities of the country, 54 samples of meju and 125 samples of soy paste were collected after checking for home-made products and screened for the presence of aflatoxins. As shown in Table 8, aflatoxin was detected at the frequency of 7.4% for meju and 8.8% for soy paste. Aflatoxin was detected only in the samples form Yeongnam area (southeast provinces of Korea) and the highest was found to be B_1 66 ppb, B_2 13 ppb, G_1 non-detectable, G_2 5 ppb and total 84 ppb in one soy paste sample from Busan. This level was 2.8 times higher than the tolerance (30 ppb) established by FAO/WHO Joint Expert Committee on Nutrition (1968) for the sake of world food supplies. It must be noted that the most potent carcinogen aflatoxin B_1 was of the highest content.

Table 8. Detection of aflatoxins in Korean fermented soy foods

Location (City)	Meju (Molded Soybean)	Doenjang (Soybean Paste)
Seoul	0/20	0/40
Chuncheon	0/1	0/9
Daejeon	—	0/8
Jeonju	—	0/8
Kwangju	—	0/10
Jeju	—	0/10
Daegu	0/16	6/20
Busan	4/17	5/20
Total	4/54	11/125
(% Frequency)	(7.4%)	(8.8%)

Only one doenjang sample from busan was detected for B_1B_2 & G_1 and all others for G_2 only. Lowest detection limit: 5 ppb for B_1G_1 & 2.5 ppb for B_2G_2 From Kim et al. (1977)

Identification of aflatoxin was undertaken by means of physical, chemical and biological methods as described below for the sample isolated from soy paste of the highest content:
(1) Comparison of R_f values and fluorescence in thin-layer chromatography
(2) R_f values of derivatives (acetate & water adducts) on TLC plate
(3) Melting point
(4) UV and IR spectra
(5) Growth inhibition of *Bacillus megaterium* NRRL B-1368 (the most sensitive microbial species for aflatoxin)
(6) Chicken embryo bioassay
This constitutes the first report for confirmative testing of aflatoxin in Korean foods. On the basis of this observation, it was requested to establish the tolerance of aflatoxin for Korean foods, but any action was not taken as yet. The regulatory authority of health shall establish the official method of assay and tolerance of aflatoxins for the sake of public health as well as domestic and international trades of food and farm products.

Chun & Shin (1979) screened the mycotoxin contamination of dried food products in trade. As shown in Table 9, aflatoxin, sterigmatocystin, ochratoxin A and citrinin were detected at the level of 1 ppb and the levels were relatively high in dried persimmon and jujube. Recently Kim & Roh (1984) surveyed the contamination of imported corn samples with HPLC method and detected 0.2-3 ppm of zearalenone in 8 out of 20 tested samples.

Table 9. Detection of mycotoxins in dried Korean foods

Mycotoxin	Persimmon	Jujube	Peanut	Pinenut	Walnut	Chestnut
Aflatoxin B_1	0.5-5.0	0.5-1.5	0.3-0.5	—	—	0.3
Aflatoxin G_1	0.3-0.5	—	—	—	—	0.3
Sterigmatoxystin	—	0.3-0.5	—	0.5	0.3	—
Ochratoxin A	0.3-1.0	—	—	—	—	—
Citrinin	—	0.3	—	—	—	—

From Chun & Shin (1979)

3. Toxicity and elimination studies

Studies on the toxicity testing, elimination trials of chemical aspects of mycotoxins are relatively limited in Korea. The reasons may be due to the lack of laboratories or researchers of traditional toxicology and this area must be fulfilled by efforts of toxicologists in future. Studies on this subject are summarized in Table 10.

As mentioned earlier, the comment by Crane et al. (1970) on the close link between the high incidence of stomach cancer and ingestion of fermented foods by Koreans is considered very important. Lee et al. (1970) undertook an animal experiment to give aflatoxin orally to albino rats for 100 days and observe their growth and histopathological changes of major organs. Abnormality was observed at a high dosage of aflatoxin but tumor formation was not observed at all. A similar result was also reported by Sung et al.(1970) in an experiment to feed meju to albino rats for one year.

Chung et al.(1971) reported that gamma-ray irradiation toward *A. flavus* had no effect on its aflatoxin formation. Lee et al.(1973) studied the chemical stability of aflatoxin and reported that aflatoxin was decomposed easier in alkaline than in acidic solution and mostly decomposed by artificial gastric juice or bile acid in 60 minutes.

There are also reports on the effect of aflatoxin as a photosensitizer in the photochemical changes of malonaldehyde using a model system (Kwon, 1969) and on the charge-transfer complex formation between aflatoxin B_1 and benzene to explain the binding mechanism of aflatoxin B_1 with proteins or DNA (Noh & Lee, 1974).

Table 10. Toxicity and elimination studies on mycotoxins in Korea

Year	Researcher	Results
1969	Kwon	Effect of aflatoxin as a photosensitizer
1970	Crane et al.	Suspected high incidence of stomach cancer by dietary aflatoxin
1970	Lee et al.	Pathology of aflatoxin in rat
1970	Sung et al.	Pathology of meju in rat
1971	Chung et al.	Effect of γ-ray on aflatoxin formation
1973	Lee et al.	Chemical stability of aflatoxin
1973-5	Cho et al.	Bioassay of fungal toxicity by HeLa
	Koh et al.	Cell and mouse effect of fungal extracts to HeLa cell
1974	Noh & Lee	Charge-transfer complex formation of aflatoxin
1975-6	Choi et al.	Radiosensitivity of mycotoxin-producers effect of aflatoxin on Bac. megaterium.
1976-7	Oh; Oh & Lee	Effect of sunlight and amino acids on aflatoxin stability in meju
1983	Lee & Kim	Persistence of aflatoxin in the preparation & cooking of fermented soy foods

Toxicity testing for culture filtrates of some isolated fungi were undertaken by use of Hela cell and mouse (Cho et al., 1973; Koh et al., 1974a; Koh et al., 1974b; Koh et al., 1974c; Koh et al., 1975). As shown in Table 11, many of *Aspergillus* and *Fusarium* species exhibited a high toxicity whereas only a few of *Penicillium* and *Alternaria* species exhibited a toxicity. It was also observed by an electron microscope for the effect of culture filtrates of *Aspergillus* and *Penicillium* species on the fine structure of HeLa cells.

Table 11. Animal toxicity of culture filtrates from some fungi

Genus	Cytotoxicity*	Animal Toxicity**
Aspergillus	5/9***	3/9
Penicillium	1/9	1/9
Alternaria	2/31	2/31
Fusarium	5/5	3/5

* Toxicity testing with HeLa cell by toplin (1959)
** Histopathological examination of internal organs after IP injection to mouse
*** Frequency of tested strains showing toxicity higher than medium
From Cho et al. (1973); Koh et al. (1974b; 1974c)

The author (Choi et al., 1975; Choi & Lee, 1975) investigated the radio sensitivity of 7 mycotoxin-producing fungal species isolated from deteriorated rice. As shown in Table 12, decimal reduction dose (D_{10} value) was 14-33 krad and induction dose was 12-56 krad for conidia of tested species and a remarkable synergistic effect of γ-ray and heat treatment was observed for conidia of *A. flavus* and *P. islandicum*. These results to indicate that a low radiation dose is sufficient to eliminate mycotoxin-producting fungi whereas a high radiation dose is required to decompose mycotoxin itself suggest the possiblity of utilizing

Table 12. Radiosensitivity of mycotoxin-producing fungi

Fungus	D_{10}Value (Krad)	Induction Dose(Krad)
Aspergillus clavatus	29	24
Aspergillus flavus	24	48
Aspergillus fumigatus	33	48
Aspergillus ochraceus	17	56
Penicillium citrinum	14	21
Penicillium implicatum	23	12
Penicillium islandicum	26	42

From Choi et al. (1975)

a radiation source to control the formation of mycotoxins in food and feed samples.

Choi et al.(1976) studied the effect of aflatoxin on the growth of *Bacillus megaterium* which was known to be the most sensitive species to aflatoxin among microorganisms. The result showed that aflatoxin inhibited the growth of the tested bacteria in liquid culture and caused the cell to abnormal elongation by lack of septum formation. The abnormal cell returned to normal cell upon returning to culture media containing no aflatoxin.

Oh (1976) inoculated *A. flavus*ATCC 15517 and NRRL 3000 to meju, confirmed the formation of aflatoxin and exposed the meju molded in 3 different sizes to sunlight. As shown in Table 13, the inactivation ratio of aflatoxin after 20 days exposure was 16% for $(5\ cm)^3$ size, 60% for $(1cm)^3$ and 95% for $(3\ mm)^3$ size of the meju. Inactivated substances showed UV and IR spectra different from aflatoxin B_1. Oh & Lee (1977) also studied the effect of free amino acids on the stability of aflatoxin B_1 in a model system. They found that aflatoxin remained 29% after 10 minutes and 17% after 15 minutes of heating.

Table 13. Inactivation of aflatoxin B_1 in meju by sunlight irradiation

Meju Sample*	% Inactivation by Sunlight after days				
	5	10	20	40	60
Powder, 2.8 MM	53	80	95	96	96
Lump, $(1\ cm)^3$30	50	60	70	74	
Lump, $(5\ cm)^3$10	15	16	15	17	

* Level of Aflatoxin B_1in original Meju was 1.27 ppm.
From Oh (1976)

Lee & Kim (1983) prepared soy sauce and paste by an indigenous method after artificial contamination of *A. flavus* and observed the variation in aflatoxin content during the preparation and cooking processes. As shown in Tables 14 & 15, total aflatoxin expressed as B_1 toxicity equivalent was formed to the level of 8.5 ppm in ripened meju, of which 45% disappeared by sun-dring. Partition ratio of the aflatoxin in meju soaking was 8% in soy sauce and 69% in soy paste. Aflatoxin in the soy paste remained 77% in cooking as a stew or soup. It was, therefore, concluded that aflatoxin is more troublesome in soy paste than in soy sauce and once contaminated foods cannot be detoxified even in cooking.

In order to see the exact picture for the occurrence of aflatoxin in fermented soy foods consumed in Korea and the effect of their consumption upon the incidence of cancer in Korean population, a systematic epidemiological survey should be undertaken. The recent report by Kimura (1984) that ethyl linolenate isolated from soy paste showed an anticarcinogenic activity according to Ames test may give a clue to answer the above question.

Table 14. Behavior of aflatoxins during the preparation of fermented soy foods

Process	Aflatoxin Content (mg/kg meju solids)				
	B_1	B_2	G_1	G_2	B_1tox. eq.*
Meju after shaping	Nd	Nd	Nd	Nd	Nd
Meju after 40 days	2.90	0.59	11.70	0.69	8.51
Meju after sun-drying	1.50	0.30	6.85	0.54	4.80
Soy sauce after separation	0.08	0.04	0.26	0.09	0.22
Soy sauce after 90 days	0.04	0.03	0.17	0.17	0.15
Soy sauce after separation	2.30	0.05	5.48	0.62	4.92
Soy sauce after 90 days	0.05	3.68	0.60	3.27	

* Aflatoxin B_1 toxicity equivalent=B_1+ 0.215 B_2+ 0.464 G_1+0.106 G_2
From Lee & Kim (1983)

Table 15. Persistence of aflatoxins in cooking of fermented soy foods

Food	Boiling Time (min)	Aflatoxin Content (mg/kg Soy Paste Solids)				
		B_1	B_2	G_1	G_2	B_1 tox. eq.
Paste Soup	0	2.17	0.20	3.13	0.56	3.72
(7% Solids)	15	1.18	0.20	3.42	0.56	2.87
	45	1.21	0.20	3.13	0.47	2.76
Paste Stew	0	2.82	0.29	5.23	0.16	5.33
(15% Solids)	15	1.17	0.24	2.96	0.33	2.63
	45	1.17	0.24	2.96	0.33	2.63

From Lee & Kim (1983)

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