韓 國 菌 學 會 誌 第 10 卷 第 4 號
Kor. J. Mycol. Vol. 10, No. 4, 225~231, 1982

日本에 輸入된 食品에서 發生分離된 Mycotoxin 生産菌類

宇 田 川 俊 一
日本國 國立衛生硏究所 微生物部

Detection and Occurrence of Mycotoxin-Producing Fungi in Foods Imported to Japan

Shun-ichi Udagawa
Division of Microbiology, National Institute of Hygienic Sciences, Tokyo, 158, Japan

Except for a few speciality crops like rice, the Japanese have become the world's largest importers of agricultural products, even of traditional Japanese farm-products, as well as of marine products. During the 1970s the Japanese greatly increased their consumption of animal proteins from livestock and, thus, increased their consumption of vegetable protein feed ingredients, which involved international trade. With the present pressure on Japan to increase its imports, and with its heavy dependence upon other countries for foodstuffs, especially the increasing imports of agricultural products (Table I), we need to recognize that mycotoxicological problems with imported foods strongly influence the marketing and distribution of food between countries and often have impact on world food sanitation.

Table I. Japan production and consumption of agricultural products in 1978.

	Domestic consumption (A)**	Domestic production (B)**	Self-sufficiency %(A/B)
Rice	11.36	12.59	111
Wheat	5.86	0.37	6
Soybeans	4.19	0.19	5
Vegetales	16.86	16.41	97
Fruits	7.90	6.16	78
Milk, dairy products	7.01	6.26	89
Meats	3.47	2.76	80
Eggs	2.04	1.98	97
For total agricultural products*	—	—	73
For staple food grains*	—	—	68
For feeds*	—	—	29
For food & feed grains*	—	—	34

*Self-sufficiency, **Millions of metric tons.

A general mycotoxin-monitoring system for imported foods has been established as follows: (1) mycological detection of common fungi found as contaminants in foods, (2) taxonomical identification of potential mycotoxin-producing strains among the fungal isolates, particularly *Aspergillus* and *Penicillium*, (3) chemical surveys of the mycotoxin-producing potentials of identified fungi by inoculation tests on solid substrates, and (4) detection of the natural occurrence of mycotoxins by chemical analysis. In Japan, a chemical monitoring system for most imported cereals and feed grains including corn, peanuts and beans has been established for aflatoxins, sterigmatocystins, ochratoxins, and some *Fusarium* toxins.

A recent survey by Hitokoto *et al.*[1] showed that high levels of fungal contamination existed in most of 604 samples of dried beans which included considerable samples of imported beans. The *Aspergillus*, *Penicillium*, and *Wallemia* species predominated. Toxigenic species found included *A. flavus*, *A. ochraceus*, *A. versicolor*, *P. islandicum*, and *Fusarium* spp. Thus, the most recent survey on mycotoxins and their occurrence in imported foods in Japan has dealt with the *Aspergillus* and *Penicillium* species

Table II. List of spices samples examined in 1976-1977*.

Spice	No. sample	Imported from	Spice	No. sample	Imported from
Allspice	3	Guatemala, Honduras	Hatomugi(*Coix lachryma-jobi*)	1	China
Anise seed	1	France			
Basil leaves	1	France	Laurel	1	Turkey
Black pepper	3	Malaysia, India, Korea	Liquorice roots	1	China
Cassia	2	China	Mace	5	Indonesia
Cardamon	2	India, Guatemala	Marjoram	1	France
Celery	1	India	Mustard	2	Canada
Chilli pepper	4	Korea, Pakistan, Indonesia	Nutmeg	3	Indonesia
			Oregano	2	Greece, Mexico
Chinese pepper	1	China	Paprika	1	Hungary
Cinnamon	1	Sri Lanka	Sage	1	Greece
Cloves	1	Madagascar	Sansho (Japanese pepper)	1	China
Dill	1	USA			
Dry Hibiscus flower	1	Sudan	Star anise	2	China
Fennel	3	China	Thyme	2	France
Fenugreek	2	India	Turmeric	3	India, China
Ginger	1	China	White pepper	2	Malaysia, India

*In addition, 28 retailed samples (domestic or imported spices) were also examined.

Table III. List of spices samples examined in 1980*.

Spice	No. sample	Imported from	Spice	No. sample	Imported from
Allspice	3	USA	Ginger	2	uncertain
Basil	3	USA	Horseradish	2	USA, Korea
Black pepper	14	USA, India, Malaysia	Laurel	2	USA
			Lemon peels	1	USA
Caraway	2	USA	Mace	1	uncertain
Cardamon	5	USA, India	Marjoram	2	USA
Celery	2	uncertain	Mustard	1	uncertain
Chilli pepper	23	USA, Korea, China, Papua New Guinea, Pakistan, Sri Lanka	Nutmeg	5	USA, Indonesia
			Onion powder	3	USA
			Oregano	3	USA
			Paprika	4	Spain, USA
			Parsley	1	USA
Cinnamon	7	USA, Sri Lanka, Singapore, China	Rosemary	1	USA
			Sage	3	USA, Greece
			Sansho (Japanese pepper)	1	China
Cloves	3	uncertain			
Coriander	5	China, Roumania	Star anise	1	uncertain
Cumin	5	USA, India	Tarragon	1	USA
Dill	1	USA	Thyme	2	USA
Fennel	2	USA	Turmeric	7	USA, Sri Lanka, China, India
Feungreek	2	India			
Garlic powder	2	uncertain	White pepper	14	USA, Malaysia

*In addition, the following 63 Nepalese samples were also examined: black cardamon, black pepper, chilli pepper, cinnamon, cloves, coriander, cumin, fenugreek, ginger, green cardamon, mace, nutmeg, and parsley.

that grow on cereals or feed grains; only limited work on other products has been done.

As spices are usually minor ingredients in native Japanese foods, little information, especially on imported spices, has been available on the incidence and numbers of potential mycotoxin-producing fungi such as *A. flavus*, *A. versicolor*, *A. ochraceus*, *Emericella nidulans*, and *P. citrinum*. A high level of mycotoxin contamination in some spices, however, is suspected for two reasons: (1) Many spices are cultivated and processed in warm tropical areas where onditions favor fungal growth. (2) These spices often come from areas where sanitary practices are primitive. Moreover, the increasing consumption of processed meat products has led to a remarkable expansion in the use of spices in Japanese food industries. These circumstances have been sufficient to focus our attention on a mycological survey of imported spices, with special emphasis on mycotoxins. For the past 4 years, we have been engaged in surveying the incidence and extent of contamination by mycotoxin-producing fungi in more than 280 samples of imported (Tables II, III) and retailed (domestic or imported) spices. Particular emphasis has been placed on aflatoxin production.

Fungi in spices usually vary to some extent with the spice, but as shown in Tables IV and V, about 30% of the samples of the imported whole spices tested had total ufngal counts of more than 10^4/g. In the Nepalese whole spices examined, 30.2% of the samples also carried heavy loads of fungi, ranging from 1.0×10^4/g to 2.1×10^5/g.

The mycoflora of 20 different ground spices samples and three mixtures in U.K., with the isolation of aflatoxin-producing strains of *A, flavus* from these spices, has been previously studied by Flannigan and Hui[2]. The mycological analysis for 23 imported spices samples in France is also informative because, despite the diverse origin of tested peppers, there was a remarkable similarity in the fungal contamination found (Moreau and Moreau[3]). As a conclusion drawn from these European reports and our survey, black pepper, cardamon, chilli pepper, cinnamon, coriander, and white pepper were contaminated significantly with various fungi, including potential mycotoxin-producing fungi such as *A. flavus*, *A.*

Table IV. Grouping of imported whole spices in 1976-1977, based on fungal contamination.

	No contam. or very low (-10^2)*	Contamination (*propagules per g) Low (10^2-10^3)*	Heavy (10^4)*	Extremely heavy (10^5-)*
Sample	13/57	27/57	8/57	9/57
%	23	47	14	16
Spice	Celery seed	Various	Allspice	Allspice
	Cloves		Cardamon	Basil leaves
	Fennel		Cassia	Cassia
	Ginger		Chilli pepper	Chilli pepper
	Kosho (Chinese pepper)		Oregano	Cinnamon
	Mace		Thyme	Laurel
	Mustard		White pepper	Oregano
	Sansho (Japanese pepper)			Sage
	Star Anise			White pepper

Table V. Grouping of imported whole spices in 1980, based on fungal contamination.

	No contam. or very low (-10^2)*	Contamination (*propagules per g) Low (10^2-10^3)*	Heavy (10^4)*	Extremely heavy (10^5-)*
Sample	54/136	42/136	39/136	1/136
%	40	31	28	1
Spice	Basil	Various	Black pepper	White pepper
	Caraway		Chilli pepper	
	Cloves		Cinnamon	
	Fennel		Coriander	
	Garlic powder		Marjoram	
	Ginger		Oregano	
	Horseradish		Rosemary	
	Nutmeg		Sage	
	Onion powder		Thyme	
	Parsley		White pepper	
	Star Anise			

Table VI. Comparison of each the heavily moldy spices between the two European reports and our survey.

Flannigan & Hui (1976)		Moreau & Moreau (1978)		Udagawa (1976-1977)		Udagawa (1980)		Udagawa (Nepalese)	
Bl pepper	10^5	Wh pepper	10^6	Allspice	10^5	Wh pepper	10^5	Bl cardamon	10^5
Coriander	10^5	Bl pepper	10^5	Basil leaves	10^5	Bl pepper	10^4	Bl pepper	10^5
Allspice	10^4	Caraway	10^5	Cassia	10^5	Ch pepper	10^4	Ch pepper	10^5
Ch pepper	10^4	Coriander	10^5	Ch pepper	10^5	Cinnamon	10^4	Coriander	10^5
Cinnamon	10^4	Cinnamon	10^4	Cinnamon	10^5	Coriander	10^4	Cumin	10^5
Nutmeg	10^4	Cloves	10^4	Laurel	10^5	Marjoram	10^4	Ginger	10^5
Wh pepper	10^4	Nutmeg	10^4	Oregano	10^5	Oregano	10^4	Fenugreek	10^4
Aniseed	10^3	Paprika	10^4	Sage	10^5	Rosemary	10^4	Gr cardamon	10^4
Fennel	10^3	Turmeric	10^4	Wh pepper	10^5	Sage	10^4	Parsley	10^4
Fenugreek	10^3	Onion powder	10^3	Cardamon	10^4	Thyme	10^4	Cinnamon	10^3

Propagules per g.; Bl: black, Ch: chilli, Gr: green, and Wh: white.

Table VII. Incidence of potential toxin-producing fungi in spices (1).

Fungus	Flannigan & Hui (1976)		Moreau & Moreau (1978)		Udagawa (1976~1977)		Udagawa (1980)		Udagawa (Nepalese)	
A. flavus	Allspice	10^2	Bl pepper	10^{3-4}	Ch pepper	10^3	Bl pepper	10^{2-4}	Bl pepper	10^2
	Bl pepper	10^3	Caraway	10^{3-4}	Marjoram	10^3	Ch pepper	10^{3-4}	Ch pepper	10^2
	Ch pepper	10^3	Coriander	10^4			Coriander	10^2	Cinnamon	10^2
	Coriander	10^3	Turmeric	10^{3-4}			Sansho	10^2	Coriander	10^2
	Fennel	10^2	Wh pepper	10^4			(Jap. pepper)		Cumin	10^3
	Nutmeg	10^3					Turmeric	10^2	Fenugreek	10^2
	Wh pepper	10^3					Wh pepper	10^{3-4}	Mace	10^2
A. ochraceus			Bl pepper	10^4			Blpepper	10^3	Bl pepper	10^3
			Wh pepper	10^4			Coriander	10^2	Coriander	10^3
A. fumigatus	Coriander	10^3	Nutmeg	10^{3-4}	Thyme	10^3	Bl pepper	10^{2-4}		
	Fennel	10^2	Turmeric	10^{3-4}			Marjoram	10^2		
	Wh pepper	10^3	Wh pepper	10^4						

Propagules per g,; bl: black, ch: chilli, and wh: white.

fumigatus, *A. ochraceus*, *A. versicolor* and *Emericella* spp. (Tables VI-VIII). *Aspergillus versicolor* was also a prominent component (counts of to 10^4/g) of the mycoflora of allspice, cassia, cumin, ginger, laurel, oregano and sage.

In view of the fairly considerable number of *A. flavus* isolates capable of producing aflatoxins on various agricultural commodities, selected strains of *A. flavus* from the imported spices were tested for the ability to produce aflatoxins during growth on solid medium. As shown in Table IX, under experimental conditions in which a rice medium was used, 28% of 72 strains of *A. flavus* isolated from the spices imported in the 1980s were aflatoxigenic. The aflatoxigenic *A. flavus* strains have been encountered frequently on spices of Malaysia, Sri Lanka and Pakistan origins. The heavy producers of aflatoxins, however, are AF-34 strain found on chilli pepper of

Table VIII. Incidence of potential toxin-producing fungi in spices (2).

Fungus	Flannigan & Hui (1976)	Moreau & Moreau (1978)	Udagawa (1976-1977)	Udagawa (1980)	Udagawa (Nepalese)
A. versicolor	Allspice 10^2 Bl pepper 10^3 Ch pepper 10^2 Coriander 10^4 Fennel 10^2 Wh pepper 10^3	Caraway 10^{3-4}	Allspice 10^4 Cardamon 10^3 Cassia 10^4 Fennel 10^2 Laurel 10^4 Marjoram 10^3 Oregano 10^4 Sage 10^4 Thyme 10^3 Wh pepper 10^4	Bl pepper 10^{3-4} Celery 10^3 Coriander 10^3 Turmeric 10^2 Wh pepper 10^{2-4}	Bl pepper 10^4 Cardamon 10^3 Cinnamon 10^3 Coriander 10^4 Cumin 10^4 Fenugreek 10^2 Ginger 10^4 Parsley 10^3
Emericella spp.	Cardamon 10^2 Ch pepper 10^2 Coriander 10^2 Wh pepper 10^3	Caraway 10^{3-4} Coriander 10^{3-4} Wh pepper 10^4	Anise seed 10^3 Wh pepper 10^3	Coriander 10^3 Cumin 10^2	Coriander 10^2 Cumin 10^2 Fenugreek 10^2

Propagules per g,; Bl: black, Ch: chilli, and Wh: white.

Table IX. Aflatoxin production on rice medium by selected isolates of *Aspergillus flavus* from imported spices.

Source	*A. flavus* strain* (imported from)	Aflatoxin production (μg/g of rice)**			
		B_1	B_2	G_1	G_2
Black pepper	AF-29 (Malaysia)	2.34	0.15		
	AF-57	30.8	0.88		
Celery	AF-1	12.7	0.25		
Chilli pepper	AF-12 (Pakistan)	4.69	0.05		
	AF-15 (Pakistan)	2.5	0.05		
	AF-24	0.36	0.03		
	AF-32 (Pakistan)	2.84	0.05		
	AF-33 (Pakistan)	35.2	1.64		
	AF-34 (China)	53.4	2.26	62.2	2.08
	AE-61 (Sri Lanka)	5.26	0.10		
	AF-73 (China)	7.76	0.14		
Curry powder	AF-4	89.6	2.14		
	AF-60	32.8	1.06		
Mixed spice	AF-19	8.0	0.15		
Sage	AF-47	11.3	0.27		
Turmeric	AF-50 (India)	11.9	0.40		
	AF-56 (Sri Lanka)	16.0	0.51		
	AF-58 (Sri Lanka)	11.8	0.22		
White pepper	AF-26 (Malaysia)	3.01	0.13		
	AF-46 (Malaysia)	2.59	0.12		
Control (*A. parasisicus*)	ATCC-15517	79.2	2.48	67.6	2.56

*The remaining 52 strains tested were negative for aflatoxin production and omitted in this table.
**Cultural condition: Each isolate was grown on 30 g of sterilized polished rice at 25°C, 7 days. Aflatoxin estimation was made by TLC-spectrophotometric determination.

Table X. Inoculation tests of aflatoxin-producing strains of *Aspergillus flavus* group onto spices.

Spice	Experimental condition	Aflatoxin production	Authors
Red pepper (*Capsicum frutescens*) "crushed"	*A. flavus* M-93 strain, 14-20 days	B_1+G_1 18-84 μg/g B_1+G_1 46-300 μg/ half fresh pepper	Schindler & Elsenberg (1968)
Whole ginger (broken)	*A. flavus* isolated from each spice, 25°C, 17 days	B_1+B_2 35 μg/g	Flaannigan & Hui (1976)
Jamaica pepper (whole)		B_1+B_2 254 μg/g	
Red pepper (whole)		B_1 4 μg/g	
White pepper (whole)		B_1 24 μg/g	
Black pepper (corns)	*A. parasiticus* NRRL 3145, 28°C, 30 days, RH 85 %	B_1 60 μg/kg B_2, G_1, G_2 trace	Seenappa & Kempton (1980)
Indian red pepper (whole)	Storage at 85% RH, 28°C, 10 days, without inoculatioo	B_1 90 μg/kg	Seenappa *et al.*(1980)
	Storage at 95% RH, 28°C, 10 days, without inoculation	B_1 180 μg/kg	

Table XI. Natural occurrence of aflatoxins in spices.

Spice	Sample No. (detected/examined)	Aflatoxin concentration (μg/kg substrate)	Author
Chilli pepper	11/106	Total 125 (av.), 966 (max.)	Shank et al.(1972)[7]
Black pepper "ground"	3/7	G_1 1.8-3.7, G_2 1.1	Suzuki et al(1937)[8]
Celery seed "ground"	1/9	G_1 3.7	
Nutmeg "ground"	3/5	B_1 2.5-5.5, B_2 0.75-1.1	
Cayenne pepper	8/33	B_1 3.0-8.0	Scott & Kennedy (1973)[9]
Indian chilli pepper	4/6	B_1 3.6-6.0, G_1 3.0	
Coriander	1/9	B_1+B_2 61.5	Soott & Kennedy 1975)[10]
Ginger	8/15	B_1 trace-12.5, B_2 trace-15, G_1 trace-5, G_2 trace-4	
Nutmeg	4/13	B_1 2.5-87.5, B_2 trace-15	
Turmeric	6/7	B_1 trace-3.8, B_2 trace-1.3	
Nutmeg "ground" & "powder"	30/32	Total 2.3-36.5, B_1 1.0-23.2	Beljaars *et al.* (1975)[11]
Bay leaf	1/1	B_1 5.1	
Fenugreek (fresh)	1/3	B_1 2.0	Girgis *et al.* (1977)[12]
Fenugreek (12 months stored)	2/3	Total 6.8-7.0	
Nutmeg "powder"		B^I 0.5-2.9, B_2 0.3	Naoi et al.(1981)
Chilli pepper		B_1 0.2	
Chilli pepper	2/8	B_1 54.2, B_1+B_2 133.0	Our survey for 1980s imported spices

Chinese origin and AF-34 strain of mixed curry powder.

Aflatoxins have been also synthesized by the *A. flavus* isolates growing on some spices under experimental conditions (Flannigan and Hui[2), Schindler and Elsenberg[4), Seenappa and Kempton[5), Seenappa *et al.*[6); see Table 10).

Finally we have drawn up a mycotoxicological profile from data of our survey and previous reports on the natural occurrence of aflatoxins in spices for each of the 10 common spices (Table XI). Except for the relatively high concentiation of aflatoxins found in chilli pepper and nutmeg, aflatoxin contamination of the spices usually appears to be less serious. However, reports of considerably high incidence of *A. flavus* and other potential mycotoxin-producing Aspergilli in some imported spices would support the conclusion of Moreau's investigation[3) that "The use of polluted spices in various food industries may produce a contamination by molds." Moreover Moreau and Moreau emphasized that "If some of them only give rise to alterations or a bad aspect, others which are toxinogen, may be dangerous." Because of primitive aspects of harvest and postharvest treatment in spces prodiuction, mycotoxin contamination seems to be an unavoibable problem. Therefore, routine monitoring system of spices (e.g. black pepper, cardamon, chilli pepper, coriander, ginger, nutmeg, turmeric, and white pepper particular) is actually needed as a part of the mycotoxin examination for commodities susceptible to aflatoxin contamination.

Our results also indicate that further analytical surveys of highly moldy spices, in particular spices imported from tropical or subtropical areas, are necessary if we are make an adequate evaluation with respect to the natural occurrence of aflatoxins, sterigmatocystins and other *Aspergillus* toxins.

References

本 論文은 1982, 10, 18 水原農村振興廳 農業技術研究所에서 있었던 特別講演內容임.

Hitokoto, H., Morozumi, S., Wauke, T., Sakai, S., and Kurata, H. (1981). Fungal contamination and mycotoxin-producing potential of dried beans. *Mycopathologia 73*, 33-38.

Flannigan, B., and Hui, S.C. (1976). The occurrence of aflatoxinproducing strains of *Aspergillus flavus* in the mould floras of ground spices. *J. Appl. Bacteriol. 41*, 411-418.

Moreau, C., and Moreau, M. (1978): La contamination des epices, ses consequences dans les industries alimentaires. *Ind. Aliment. Agric. 95*, 497-502.

Schindler, A.F., and Elsenberg, W.V. (1968): Growth and production of aflatoxins by *Aspergillus flaves* on red pepper (*Capsicum frutescens* L). *J. A. O. A.C. 51*, 911-912.

Seenappa, M., and Kempton, A.G. (1980): *Aspergillus* growth and aflatoxin-production on black pepper. *Mycopathologia 70*, 135-137.

Seenappa, M., Stobbs, L.W., and Kempton, A.G. (1980): *Aspergillus* Colonization of Indian red pepper during storage. *Phytopathology 70*, 218-222.

Shank, R.C., Wogan, G.N., Gibson, J.B., and Nondasuta, A. (1972): Dietary aflatoxins and human liver cancer. II. Aflatoxins in market foods and foodstuffs of Thailand Hong Kong. *Fd. Cosmet. Toxicol.*