OCCURRENCE AND DETOXIFICATION OF MYCOTOXINS IN FOOD

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INTRODUCTION

Agricultural products which are used as food are always exposed to the danger of fungal contamination during their cultivation, harvest, transportation and storage. When the foodstuff, temperature and humidity are suitable for the growth of certain fungi, there is always the danger of mycotoxin production. Indonesia as a tropical country, the temperature and humidity are favorable for the growth of fungi. Among such fungi, some species of the genus *Aspergillus* and *Fusarium*, well-known aflatoxin and *Fusarium* toxins producer are frequently found. Aflatoxin B1 (AFB1) which is the most carcinogenic mycotoxins, produced by *Aspergillus flavus* and *A. parasticus* is the highest mycotoxins contaminant in Indonesian commodities. Aflatoxin B1 is known very stable against cooking condition in daily life and other processing factors. Because of this reason, AFB1 became the most important food hygiene problems.

Removal of aflatoxins by degradation or detoxification is important to reduce a risk to human health from the intake of aflatoxin contaminating agricultural products, because nobody knows which food is already contaminated by aflatoxins. Among degradation methods microbiological degradation are promising methods because the nutritive value and physical properties of foods are not change significantly.

The occurrence of mycotoxins in food.

Indonesia as a tropical country, has a warm and humid climate which are suitable for the growth and toxins production of mycotoxic fungi. From the simplified diagram as shown in Fig 1 , mycotoxins contamination of agricultural products started from the field before harvesting and several factors affect the growth of fungi and toxin production, i. e microbial interaction, bio fertilizer, insect and rodent damage and other local conditions. It was shown that there is always the danger of mycotoxins contamination in food, and it was correlated with the growth of mycoflora on it. The mycoflora of cereal and nuts from Indonesia have been observed, and they were dominated by toxigenic fungi (Sardjono, et al.1992) and similar result were obtained on food commodities (Piit, et al. 1998). About 22,000 isolate were identified, and they were dominated by toxigenic fungi, especially aflatoxins producing fungi. Indonesian peanut and corn reported have the highest aflatoxin contamination if it is compare with peanut and corn from the Phillipines and Thailand (Anonym, 1996, Yamashita et al.,1995). It was also reported by Goto, et al (1999) , the highest AFB1 concentration in corn and peanut from Central Java, East Java and Bali are 299 ppb, 45 ppb , 27 ppb for corn and 43 ppb, 206 ppb, 8 ppb for peanut, while Yamashita et al.(1995) reported the *Fusarium* mycotoxins and aflatoxin of corn from Philippines, Thailand and Indonesia. Over 50% of corn samples from individual countries were contaminated by Fumonisins B1 (FMB1)and Fumonisisns B2 (FMB2). The highest level of Fumonisins and aflatoxin were found in samples from Indonesia, and the average concentration of FMB1, FMB2, AFB1 and AFB2 were 843, 442, 352 and 90 ppb from Indonesia; 580, 251, 63 and 14 ppb from Thailand and 419, 286, 49 , 14 ppb from Philippines. Nurhayati et al (1998) also have found the natural co-occurrence of aflatoxin and *Fusarium* toxins in corn from central Java. It is the first report on the natural co-occurrence of AFB1 and deoxynivalenol (DON) in corn from hot areas of Southeast Asia. The dominant of *Fusarium* species identified as *F. moniliforme* and *F. proliferatum*. It was not clear weather synergist or antagonist between *A. flavus* and *Fusarium moniliforme* on production of AFB1 and fumonisin B1 (FMB1), because the concentration of both mycotoxins are still high (the highest concentration of AFB1 and FMB1 were 428 ng/g and 2440 ng/g respectively), but Picco et al (2000) observed that under optimal condition, the interaction between *A. flavus* and *F. proliferatum* could produce inhibition of AFB1 and stimulation of FMB1.

Aflatoxigenic fungi were known growth on several kind of spices and herbs and almost of them produced aflatoxins (unpublished data). The Indonesian traditional medicines, either pellet or powder type were contaminated by aflatoxin because of un-proper drying process of the raw materials (Soedarini et al, 1993).

The occurrence of mycotoxins in food were not only caused by contaminated raw materials but also by mycotoxins properties, especially aflatoxins and *Fusarium* toxins which were resistant to processing condition either in daily life or food processing industries. Corn, peanut and wheat products were susceptible to aflatoxins and fumonisins contamination, depend on the raw materials and processing conditions. Many types of mycotoxins do not decompose by ordinary cooking methods conducted at home, so that they remain in food (Kamimura, 1999). Using microwave oven, about 72% aflatoxin in ground nut was removed when treated for 7 minutes (Chinaphuti, 1999). In the case of food products, even when mycotoxins are detected in primary materials, it can be removed in manufacturing process, but in general it was remain in final products.

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Table 1. The occurrence of mycotoxins in foods from South-East Asia*)

Commodity	Sampling location	AFB1	AFB2	FMB1	FMB2	DON	Referrence
Peanut	East Java, Indonesia	206	61				Goto, et al (1999)
Peanut	CentralJava, Indonesia	43	trace				Goto, et al (1999)
Peanut	Bali, Indonesia	8	ND				Goto, et al (1999)
Peanut & its product	Bangkok, Thailand	626	160				Supraset and Kamimura (1999)
Roasted peanut	Yogyakarta, Indonesia	50.0					Noviandi, et al (2001)
Flour coated peanut	Yogyakarta, Indonesia	61.7					Noviandi, et al (2001)
Baby food products	Yogyakarta, Indonesia	5.6					Noviandi, et al (2001)
Peanut butter	Yogyakarta, Indonesia	249					Noviandi, et al (2001)
Corn	Surakarta, Indonesia	428	59	2440	376	0	Ali, et al (1995)
Corn	Purworejo, Indonesia	49	9	668	62	32	Ali, et al (1995)
Corn	Yogyakarta, Indonesia	92	9	1390	376	0	Ali, et al (1995)
Corn	Iloilo, Philippines	395					Arim, et al (1999)
Corn chip	Iloilo, Philippines	8					Arim, et al (1999)
Popcorn	Iloilo, Philippines	5					Arim, et al (1999)
Corn and its product	Bangkok, Thailand	1.60	0.12				Supraset and Kamimura (1999)
Peanut oil	Bangkok, Thailand	343					Suttajit, et al. (1999)
Rice noodle	Bangkok Thailand	20.24					Suttajit, et al. (1999)

*) The number shows the highest level of contamination (ppb)

Abbreviations: AFB1 and AFB2, Aflatoxin B1 and B2, FMB1 and FMB2, Fumonisin B1 and B2, DON, Deoxynivalenol

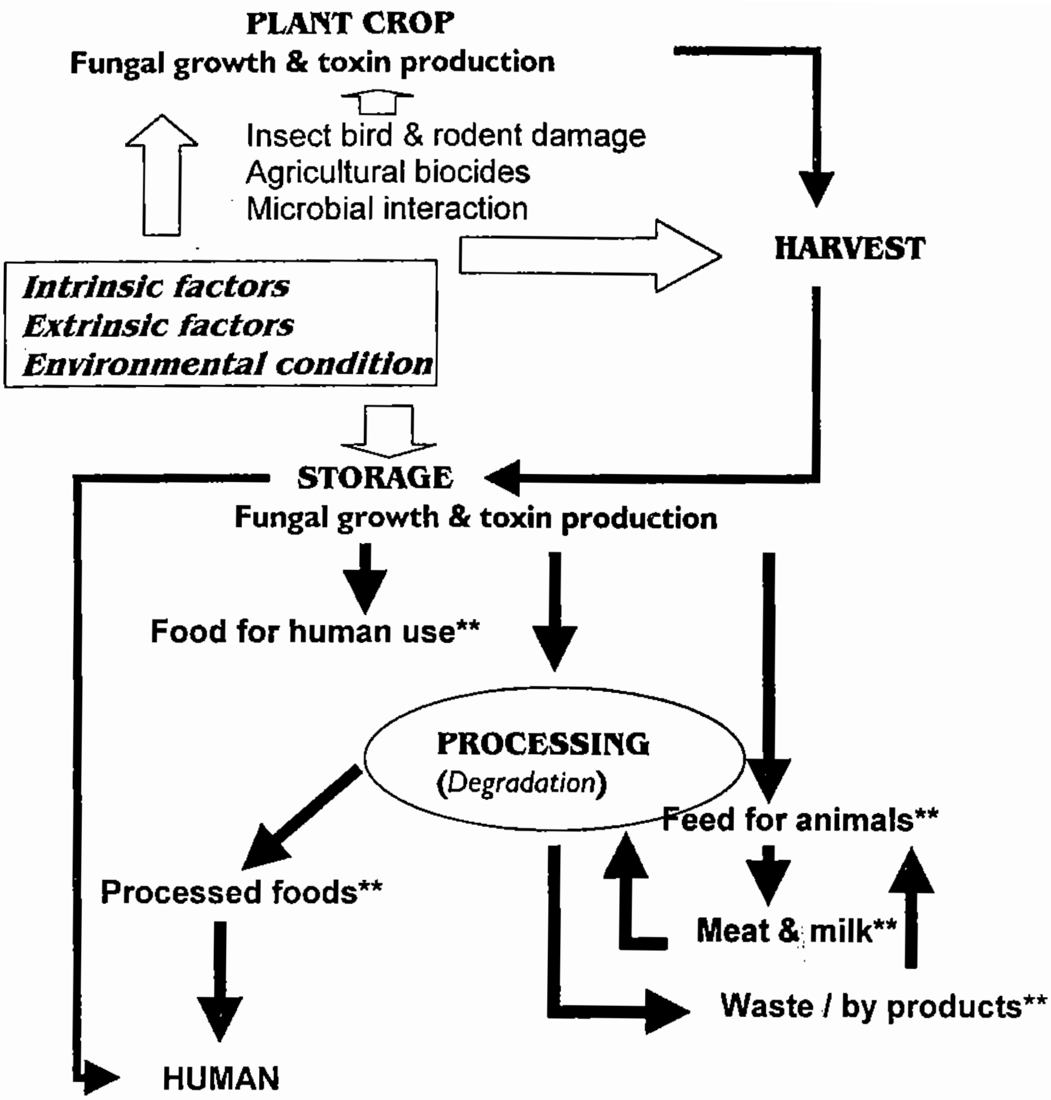

Fig. 1. Simplified diagram representing the route of mycotoxins contamination in foods. (** possible contaminated)

In corn starch processing industry, fumonisins are removed into corn step liquor, germ, fiber and gluten (all are usually used for feed) and was not detected in corn starch (Kamimura, 1999). In peanut oil processing industry, part of aflatoxins are removed during refining process, and have moved to the food layers, but some peanut oil still contain aflatoxins. It means that may be the neutralization process was not carried out and the peanut oil will contaminate process foods as reported by Suttajit., et al. (1999). They found the aflatoxins in rice noodle, because of the contaminated peanut oil is used for coating to reduce stickiness and for flavoring of the noodle.

Uncontrolled mold fermentation in traditional food fermentation were always dangerous for mycotoxins contamination (Sardjono, et al, 1995; Sardjono, 1998). Similarly, small scale of food industries should aware of the mold contamination on their products, especially the intermediate moisture food products that their water activity are favorable for the growth of fungi. From the preliminary research, it was found that "Yogyakarta's foods" such as "geplak" and "yangko" which were sold in market were contaminated by several toxigenic fungi.

Research on prevention and detoxification strategy.

To ensure the safety of foods, prevention strategies have to include all stages of food production with the aim to avoid fungal as well as mycotoxins contamination. It means that at first must determine the critical control point along the food production chains. There are various approaches to control or combat mycotoxins problems:

1. Biological control can be used for the first step to prevent the contamination of mycotoxins in food chain production. Various methods and formulations using bio competitive fungi against *A. flavus* and *A. parasiticus* have been applied to the soil, provides a very effective and economic strategy

to reduce the levels of pre harvest and post harvest aflatoxin contamination level in peanuts . Inoculation of fields with competitive, non-toxigenic strains reduced the aflatoxins ranging from 30-90% (Cole and Dorner, 1999).

2. Post harvest handling management, based on the prevention of the formation of mycotoxins, including storage in low moisture level and prevention of grain damage during processing.

3. Sortation in order to separate the mouldy raw materials (usually peanut and grains) is important step to prevent and reduce aflatoxin contamination level in final products. Sortation machine or apparatus is needed to get the high capacity of sortation process, and apparatus which equipped with Near Infra Red (NIR) photometer has been used by Hirano, et al (1999) to separate the mouldy nut , and it require only few minutes to sort out moldy nuts from several kilograms of peanuts.

4. If the case of prevention strategy are not able to reduce the contamination level, degradation and detoxification strategy must be considered in order to get the safety of products. Degradation or detoxification should be one of the processing steps in food production. Detoxification should meet some basic criteria; mycotoxins must really remove from food materials, the degradation products are not toxic and the nutrition and physical characteristic of food materials are not change significantly. Among degradation and detoxification methods, biological or microbiological degradation is promising than others, because the physical properties of food materials are not change significantly.

Fungal degradation and inhibition of aflatoxins production by fungi were done extensively, therefore there were limited on laboratory scale. A number of fungal cultures were suitable to be used in degradation and inhibition of aflatoxin production. *Rhizopus* sp and *Neurospora* sp inhibit AFB1 production if they growth together with *A. flavus* (Djien, 1974). Similar result were obtained for *A. oryzae* (Sardjono, et al., 1992), *Phoma* sp, *Mucor* sp., *Trichoderme harzianum*, *Sporotricum* groups and *Cladosporium* were observed able to degrade AFB1 (Shanta, 2000). The cell free extract of *Phoma* sp able to degrade 45 ng AFB1/ 100 mL for 120 hr, and possibly a heat stable enzymatic activity in the cell free extract is proposed (Shanta, 2000). Degradation of AFB1 by *Aspergillus oryzae* KKB4 have been studied intently in our laboratory. Four fungal strains, namely *Aspergillus niger, Eurotium herbarum*, *Rhizopus* sp and non-aflatoxin-producing *Aspergillus flavus* could convert AFB1 to aflatoxicol (Nakazato, et al., 1990). Wicklowaf and co workers (1999) reported that the extract of several *Chaetomium* strains strongly inhibit the growth of *A. flavus* at 250 ug/disk.

A number of lactic acid bacteria strains were suitable to bind aflatoxin B1 (El-Nezami, et al, 1998; Pierides, et al , 2000; Haskard, et al., 2000; El-Nezami , et al., 2000; Oatley, et al., 2000), while Kankaanpaa, et al.(2000) observed that AFB1 alters the adhesion capability of *Lactobacillus rhamnosus* strain GG using a Cacco-2 adhesion model, and reduced the adhesion capability from 30% to 5%, so by using these bacteria may reduce the accumulation of aflatoxins in the intestine via increased excretion of an aflatoxin-bacteria complex. The stability of complex formed depend on strain, treatment and environment conditions (Haskard, 2001). Their ability to bind aflatoxins indicate that specific lactic acid bacteria used in dairy products able for decontaminating aflatoxins from milk.

Prevention of mycotoxins contamination of meat and meat products.

Research on mycotoxins contamination of meat and meat products are very rare even the possibility of mycotoxins contamination in meat and meat products is high, as shown in Figure 2. In meat, mycotoxins can occur primarily as a result of indirect transmission from animals exposed to natural contaminated feed. In case of meat products, the possible occurrence of mycotoxins is strongly come from particular recipe, as spices, which are often highly contaminated with mycotoxins.

To ensure the safety of meat and meat products, prevention strategies has to include all stages of food production with the aim to avoid fungal as well as mycotoxin contamination. In recent years, nutritional adjustments have been used to improve the animal's self-defence against mycotoxins or decrease the detrimental consequences of mycotoxin consumption. Since lipid peroxidation plays an important role in mycotoxin toxicity, a protective effect of antioxidants would be expected indeed, in several experiments with various animal species, protective effect of antioxidants against toxic effects of mycotoxins have been observed (Yaroshenko, et al., 2003). In spite of the positive effects of natural antioxidants on animal fed mycotoxin-contaminated diets, the most promising and practical approach has been the addition of adsorbent to contaminated feed. Mycotoxins can be bound to the adsorbent and pass harmlessly through the digestive track. Various clays and zeolites are mainly effective against aflatoxins. In contras, a glucomannan derived from yeast cell wall has been shown to be effective against the wide range of mycotoxins (Yaroshenko, et al., 2003). Concept for risk management and prevention measure is needed, which allow the detection and exclusion of the use of contaminated raw materials and spices.

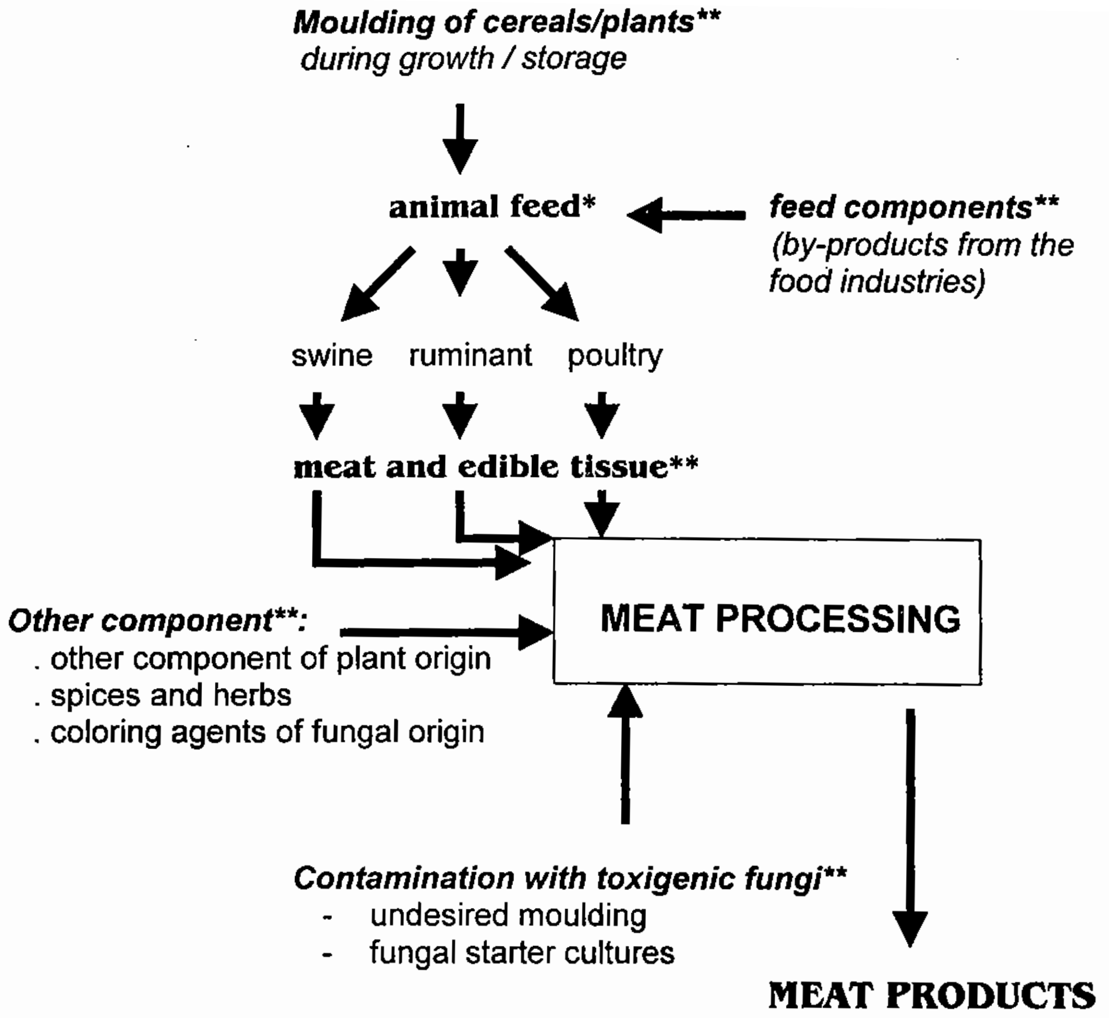

Fig. 2. Source of contamination of meat products with mycotoxins, Possible pretreatment (*) & prevention (**) steps for decontamination

WHAT IS NEEDED TO REDUCE THE MYCOTOXINS RISK IN FOODS.

So far there was no comprehensive study on how to make the food products free from mycotoxins because of the research on these fields were done separately. Comprehensive studies on preventing fungal contamination, mycotoxin production and detoxification of mycotoxins should be encouraged and conducted simultaneously with field management system and post harvest handling, in order to get the efficient methods on preventing mycotoxins contamination in agricultural products. People in agriculture must be made more aware of how important it is to prevent mycotoxins contamination, and the same heightened awareness is also essential for those in food manufacturing.

The term of detoxification should be clarified that it is not only to reduce mycotoxins content, but the most important is absolutely remove the mycotoxins from food and products of degradation or detoxification process should not have toxic effect to human health. It means that toxicity test of degradation products should be done.

The assessment of knowledge, attitude and behavior of a cross section of farmers and food producer toward mycotoxins contamination is necessary in order to enhance the producer's role in food safety. Hazard Analysis Critical Control Point (HACCP) or HACCP-like approach as a frame work to enhance food safety system on farm and in food industries should be proposed, followed by detailed planning and implementation.

REFERRENCE

Ali, N., **Sardjono**, A. Yamashita and T. Yoshizawa, 1998. Natural co-occurrence of aflatoxins and *Fusarium* mycotoxins in corn from Indonesia. J. Food Add. and Contaminant, 5 (4):377-384

Anonim, 1993. ACIAR Project 8806 Annual report 1992-1993.

Arim, H., C.A. Ferolin, R.P.Ramirez, A.R. Aguinaldo and T. Yoshizawa., 1999. Determination of aflatoxins in corn and its processed products in the Philippines by minicolumn method . Proceed. of International Symposium of Mycotoxicology, Japan : 193-196.

Cole, R.G. and J. W. Dorner. 1999. Biological control of aflatoxin and cyclopiazonic acid contamination in peanut. Proceed. of International Symposium of Mycotoxicology, Japan : 70-73.

Chinnaputi, A., 1999. Decontamination of aflatoxin in food using microwafe oven. . Proceed. of International Symposium of Mycotoxicology, Japan : 272-276.

Djien, K.S., 1974. Self protection of fermented foods against aflatoxin. Proceed. IV Int. Congress Food Sci. and Technol., III:24-253

El-Nezami, H., P. Kankaanpaa, S. Salminen and J. Ahokas., 1998. Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B1. J. of Food Chem Toxicol., 36 (4) : 321-6

Gareis, M and R. Scheuer. 1999. Prevention of mycotoxin contamination of meat and meat product. Proceed. of International Symposium of Mycotoxicology, Japan : 101-108.

Goto, T., E. Ginting, S.S. Antarlina, J.S. Utomo, Y. Ito and S. Nikkuni., 1999. Aflatoxin contamination and fungi isolated from Indonesian agricultural commodities. Proceed. of International Symposium of Mycotoxicology, Japan : 211-215.

Haskard, C.A., H. El-Nezami, P.E. Kankaanpaa, S. Salminen and J. T. Ahokas.,2001. Surface binding of afltoxin B1 by lactic acid bacteria. J. Applied and Environment. Mycrobiology., 67(7):3086-91.

Hirano, S.,N. Okawara and S. Narazaki. 1999. Near Infra Red detection of internal moldy nuts . Proceed. of International Symposium of Mycotoxicology, Japan : 74-80

Kankaanpaa, P., E. Tuomola, E., H. El-Nezami, J. Ahokas and S.J. Salminen., 2000. Binding of aflatoxin B1 alters the adhesion properties of *Lactobacillus rhamnosus* strain GG in Caco-2 model. J. Food Prot., 63(3):412-4.

Kamimura, H. 1999. Removal of mycotoxins during food processing. Proceed. Of International Symposium of Mycotoxicology, Japan :88-94

Nakazato, M., S. Morozumi., K. Saito., K. Fujinuma, T. Nishima and N. Kasai., 1990. Appl. Environ Microbiol., 56(5):1465-1470.

Noviandi, C.T., E. Razzazi., A. Agus., J. Bohm, H.W. Hulan, S. Wedastri., Y.B. Maryudhani, Nuryono, **Sardjono** and J. Leibetseder, 2001. Natural occurrence of aflatoxin B1 in some Indonesian food and feed products in Yoygyakarta in year 1998-1999. Proceed. of the 23rd Mycotoxin-Workshop, Vienna, Austria

Pierides, M., H. El-Nezami, K. Peltonen., S. Salminen and J. Ahokas., 2000. Ability of dairy strains of lactic acid bacteria to bind aflatoxin M1 in food model. J. Food Prot. 63(5): 645-50.

Picco, M., A. Nesci, G. Barros, L. Cavaglieri, M. Etcheverry, 1999. Aflatoxin B1 and Fumonisin B1 in mixed cultures of *Aspergillus flavus* and *Fusarium proliferatum* on maize. Natural Toxins, 7(6):331-36.

Pitt, J.I., A.D. Hocking, B.F. Miscamble, O.S. Darmaputra, K.R. Kuswanto., E.S. Rahayu and **Sardjono** . 1998. The mycoflora of food commodities from Indonesia. J. of Food Mycology 1 (1) : 41-60

Sardjono, K. R. Kuswanto and S. Sudarmadji, 1992. Growth and aflatoxin production by *Aspergillus flavus* in mixed culture with *Aspergillus oryzae*.ASEAN Food J., 7 : 30-33.

Sardjono, E. S. Rahayu, A.D. Hocking and J.I. Pitt. 1992. Mycoflora of cereals and nut from Indonesia. Development of Food Sci and Technol in Souteast Asia. Proc. Of the 4th ASEAN Food Conference, Jakarta. Indonesia.

Sardjono, E. S. Rahayu and S. Naruki. 1995. Mycoflora and aflatoxin in soybean and koji for kecap production. J.Biosains (1) : 11-15

Sardjono., 1998. Pencemaran pangan oleh jamur, potensi bahaya dan pencegahannya. AGRITECH, 18 : 23-27

Shanta, T., 1999. Fungal degradation of aflatoxin B1. Natural Toxins 7(5): 175-78.

Soedarini and **Sardjono**., 1993. Cemaran mikroflora dan aflatoksin pada beberapa jamu tradisional. Skripsi. Fakultas Teknologi Pertanian UGM.

Suttajit, M., S. Roytrakul, S. Lipigorngoson and P. Koonanuwatchaided. 1999. Food-process contamination of aflatoxins in rice noodle. Proceed. of International Symposium of Mycotoxicology, Japan : 208-210.

Tanaka, K., N. Hara , T. Goto and M. Manabe. 1999. Reduction of mycotoxins contamination by processing grain. Proceed. of International Symposium of Mycotoxicology, Japan : 95-100.

Yamashita, A., T. Yoshizawa, Y. Aiura, P.C. Sanchez, E.I. Dizon, R. H. Arim and **Sardjono**. 1995 *Fusarium* mycotoxins and aflatoxins in corn from Southeast Asia. Biosci. Biotech. Biochem. 59(9) : 1804-1807

Yaroshenko, F., J. Dvorska and P. Surai. 2003. Mycotoxins in poultry production: problems and solutions. Poultry International, April 2003.