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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：
Name　Manita Soontornjanagit

学位論文題目：
Title of Dissertation　Studies on the occurrence of aflatoxin M_1 in powdered milk and ochratoxins in coffee products, which were commercially available in Thailand
(タイ国内で市販されていた粉ミルクのアフラトキシンM_1及びコーヒー製品のオクラトキシン類の汚染に関する研究)

学位論文要約：
Dissertation Summary

1. Introduction

1.1 Major mycotoxins

At least around 400 different mycotoxins are existed. Examples of mycotoxins with great public concerns are aflatoxins (AFs), ochratoxins, trichothecenes; (deoxynivalenol, nivalenol, T-2 toxin, and HT-2 toxin), fumonisins, zearalenone, and patulin (Bosco and Mollea, 2012). Major mycotoxins, their producing fungi, contaminated foods and toxicity were summarized in **Table 1**.

Table 1　Major mycotoxins, their producing fungi, contaminated foods and toxicity

Mycotoxins	Main producing fungi	Contaminated foods	Main toxicity
Aflatoxins (B_1, B_2, G_1, G_2)	*Aspergillus flavus* *A. parasiticus*	Cereals, spices, nuts, oilseeds	Liver cancer for human
Aflatoxin M_1	Metabolite of AFB_1	Milk, dairy products	
Ochratoxin A Ochratoxin B	*A. ochraceous* *A. niger* *Penicillium verrucosum*	Cereals, beans, wine, pork products	Nephrotoxicity Carcinogenicity for animal
Trichothecenes; Deoxynivalenol Nivalenol T-2 toxin HT-2 toxin	*Fusarium graminearum* *F. culmorum* *F. tricinctum* *F. sporotrichioides*	Wheat, barley, corn, adzuki bean	Vomiting Diarrhea Immune suppression
Fumonisins	*F. verticillioides* *F. proliferatum*	Corn	Leukoencephalopathy Pulmonary edema Carcinogenetic promoter
Zearalenone	*F. graminearum*	Wheat, barley, corn	Estrogenic effects
Patulin	*P. expansum*	Apples, its juice	Gastrointestinal hyperemia, bleeding, and ulcer

1.2 Regulated limits of Mycotoxins

These countries at least have regulatory limits for AFB_1 or total AFs (AFB_1+B_2+G_1+G_2). Regulations are also existed for other mycotoxins such as AFM_1, deoxynivalenol, fumonisins, ochratixin A (OTA) and zearalenone. E.U. has the most comprehensive and strict regulations for mycotoxins in foods and feeds. E.U. has harmonized regulations for mycotoxins including AFB_1 and total AFs. In 2002, regulatory limits for AFM_1 are used in 60 countries. Ten-fold difference between the U.S. regulatory limit of 0.5 μg/kg and the E.U. regulatory limit of 0.05 μg/kg have existed for several years (Van Egmond and Jonker, 2004). In 2003, 26 out of 47 countries in Asia have mycotoxins regulations for foods or feeds (Food and Agriculture Organization of the

United Nations, 2004). In Japan, the regulated limit in foods was set up for total AFs at 10 μg/kg in 2011 (Ministry of Health, Labour and Welfare, 2011). The regulated limit for AFM_1 in milk was 0.5 μg/kg (Ikawa, 2015). In Thailand, only the regulated limit of total AFs in foods (20 μg/kg) was set up (Ministry of Public Health, 1986). Some of the regulated limits for mycotoxins in foods were shown in **Table 2**.

Table 2 Some of the regulated limits for mycotoxins in foods.

Countries /organization	Mycotoxins	Limits (μg/kg)	Foods	Ref.
Codex[a]	Total AFs	10	Almonds, hazelnuts and pistachios (ready to eat)	Codex, 2010
		15	Almonds, peanuts, hazelnuts and pistachios (for further processing)	
	OTA	5	Raw wheat, barley, rye	
	Patulin	50	Apple juice	
	AFM_1	0.5	Milk	
E.U.	AFB_1	2	Cereals and products derived from cereals	European Commission, 2010a
	Total AFs	4		
	Deoxynivalenol	500	Bread, biscuits and breakfast cereals	European Commission, 2007
	Fumonisins	4,000	Unprocessed corn	
	Zearalenone	50	Bread, biscuits and breakfast cereals	
	OTA	5	Cereals	European Commission, 2010b
			Roasted coffee	European Commission, 2006a
		10	Instant coffee	
	AFM_1	0.05	Milk	
		0.025	Infant milk	
Thailand	Total AFs	20	All foods	Ministry of Public Health, 1986
Japan	Total AFs	10	All foods	Ministry of Health, Labour and Welfare, 2011
	AFM_1	0.5	Milk	Ikawa, 2015
	Deoxynivalenol	1,100	Wheat	Ministry of Health, Labour and Welfare, 2002
	Patulin	50	Apple juice	Ministry of Health, Labour and Welfare, 2003

[a] ; Codex Alimentarius Commission

1.3 Analytical methods of mycotoxins

Mycotoxins are usually found at μg/kg (ppb) or ng/kg (ppt) levels, therefore difficult to detect and sensitive and reliable methods are required. There are several quantification techniques for mycotoxins detection, and the desire for rapid and economic quantification techniques has increased (Pereira *et al.*, 2014). Sampling is critically important because lead to accepted or rejected of the food. One standard method is not existed to detect mycotoxins because they are varied in structures. The same mycotoxin in a different matrix or similar properties of mycotoxins may not be able to use the same technique to detect. According to the physical and chemical properties, the analytical techniques have been developed (Turner *et al.*, 2009).

In recent years immunologic methods, which are enzyme-linked immunosorbent assay and immuno-affinity column (IAC) linked HPLC methods are used for mycotoxins analysis (Kawamura *et al.*, 2011). The immunologic methods use the specific antigen-antibody reaction which is highly specific. Antigen-antibody reaction is like the relations of a key and the keyhole. Hydrophobic bonds, ionic bonds, hydrogen bonds and van der Waals bonds are powers participated in the antigen-antibody reaction. The reaction is very specific and very delicate which often affected by pH, temperature, lipid concentration, sugar content and others. The methods to use IAC as the clean-up step were frequently used to analysis mycotoxins in foods and feeds recently. The principle was shown in **Figure 1**. The crude extract from foods and feeds was applied to the mini-column, which immobilized an antibody. And then, to remove impurities, the column was washed. Finally, the cleaned-up mycotoxins were eluted form the column and analysed by HPLC, LC-MS or GC-MS. Because the

antigen-antibody reaction is high specificity, the IAC clean-up can remove the impurities which a physicochemical property is close to analysis targets (mycotoxins). It is difficult in physicochemical clean-up method such as liquid-liquid extraction and solid phase extraction. The operation is simple and does not need too high technique in an IAC clean-up method.

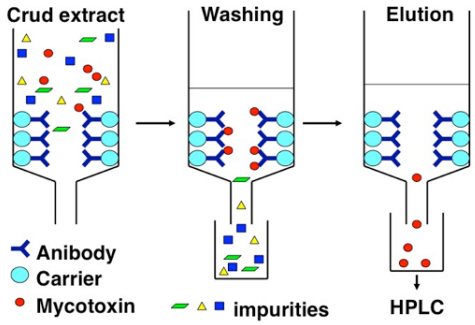

Figure 1 Principle of immunoaffinity column

1.4 Occurrence of mycotoxins in Thailand

The agricultural population accounts for approximately a little less than 40% of the employee in Thailand. Agriculture is important industry in Thailand. Thailand is one of the world's largest foods exporters. Thai government has strengthened Thailand as a kitchen of the world (FAO, 2004a). However, Thailand is located in tropical regions, where is high temperature and humid, an area suitable for growth of fungi. Therefore, possibility contaminated with mycotoxins in the Thai farm products is high. Mycotoxins exposures generally more likely occur in Thailand where poor food handling/storage and few regulations exist. Therefore, mycotoxins exposure as a risk to food safety is an important problem (Songsermsakul, 2015). During 2000 to 2010, among 28 studies, the most analysed mycotoxin in Thailand was AFs (79%). Ochratoxins, fumonisins and deoxynivalenol were included in 5 studies (18%). T-2 toxin and zearalenone were analysed in 4 (14%) and 3 (11%) reports, respectively.

1.5 Objectives

Thailand is located in tropical regions, where is high temperature and humid, an area suitable for growth of fungi. It was considered that heavy contamination of AFM_1 in raw milks was caused by the heavy contamination of AFB_1 in dairy cow feeds. From these results, it was estimated that Thai marketing powdered milks were heavy contaminated with AFM_1. However, there are a few reports on the occurrence of AFM_1 in powdered milks in Thailand. So that, to clarify AFM_1 contamination levels in commercial powdered milks in Thailand, I collected commercial powdered milks marketed in Bangkok, developed an IAC-HPLC method, which was used widely to detect mycotoxins, analyzed, and evaluated the liver cancer risk from the HBV prevalence rate and the intake amount of AFM_1.

The data indicated that Thai green coffees, which were pre-roasted coffee bean, were heavily contaminated with OTA. There are some reports on OTA in coffee cherry and green coffee, and only one report on OTA in roasted coffee, but there are no reports in international journals on ochratoxins in instant coffee in Thailand. So that, to clarify the levels of ochratoxins in commercial roasted and instant coffee in Thailand, I collected 30 roasted Arabica coffees in Chiang Mai City and 38 instant coffees (mainly Robusta) in Chiang Mai and Bangkok in Thailand, analyzed the IAC-HPLC method, and evaluated its risks based on TDI. In additon, Vietnam is the world's second largest coffee producer, mainly of Robusta coffee. There are some reports on contamination by OTA and OTA-producing fungi in Vietnamese green coffee. There is little data on the coffee

that is commercially available in Vietnam. Therfore, I collected 32 Vietnamese Robusta roasted coffees, which was for reference, and analyzed the IAC-HPLC method, and evaluated its risks.

2. Occurrence of aflatoxin M_1 in commercial powdered milk in Bangkok, Thailand

2.1 Introduction

AFs, classified as carcinogenic to humans (Group 1) by IARC, are produced principally by the fungi *Aspergillus flavus* and *A. parasiticus*, which infect food crops such as corn, peanuts, cottonseed and animal feeds (IARC, 2012). Mammals that ingest AFB_1, which is the most potent AF, from contaminated feeds transfer its 4-hydroxylated metabolite known as AFM_1 into milk (Mohammadi, 2011). **Figure 2** shows the structures of AFB_1 and AFM_1.

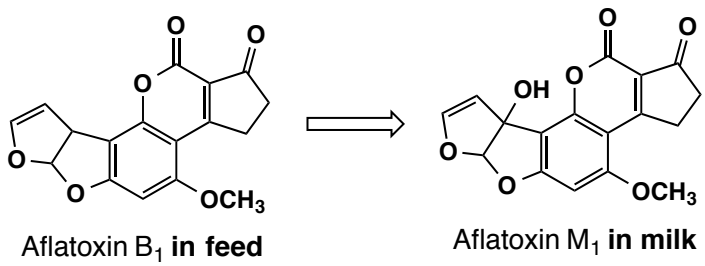

Figure 2 Structures of aflatoxin B_1 in feed and aflatoxin M_1 in milk

Dairy cow feeds in Thailand were heavily contaminated AFs. AFs were found in 21 to 100% of Thai dairy feeds 3 to 26% of Thai dairy feeds were exceeded the regulated limit of U.S. for dairy feed (20 μg/kg). Ruangwises and Ruangwises (2010) were reported that 100% of 240 raw milks were contaminated with AFM_1, which is classified as a possible human carcinogen (Group 2B) by IARC, in the range 0.014 to 0.197 μg/kg. Pimsorn *et al*. (2012) reported that of 115 raw milks and commercial milk samples, 111 (96%) were contaminated with AFM_1 with an overall average of 0.285 μg/kg, 89 samples (77%) exceeded the limit of E.U. (0.05 μg/kg), and 27 (23%) exceeded the limit of Codex (0.5 μg/kg). It was considered that heavy contamination of AFM_1 in raw milks was caused by the heavy contamination of AFB_1 in dairy cow feeds. From these results, it was estimated that Thai marketing powdered milks were heavy contaminated with AFM_1. However, there are a few reports on the occurrence of AFM_1 in powdered milks in Thailand. Therefore, to clarify AFM_1 contamination levels in commercial powdered milks in Thailand, I collected 79 commercial powdered milks marketed in Bangkok, developed an IAC-HPLC method, which was used widely to detect mycotoxins, analyzed, and evaluated the liver cancer risk from the HBV prevalence rate and the intake amount of AFM_1.

Most of these contents were published in Soontornjanagit, M., Kawamura, O. (2015) Occurrence of aflatoxin M_1 in commercial powdered milk in Bangkok, Thailand. *JSM Mycotoxins*, 65, 75-79

2.1 Methods

Powdered milk of 4.20 g was dissolved in hot water (65˚C) to make 30 mL according to the manufacturer's instruction. A mixture of 4.20 g of milk powder, 1.80 g of NaCl, and 18 mL of 0.1 M Tris-HCl buffer pH 7.5 (pre-heated at 65˚C) was heated at 65˚C for 3 minutes, and then vigorously mixed for 30 seconds and sonicated for 10 minutes. The solution was adjusted to 30 mL with 0.1 M Tris-HCl buffer pH 7.5. For recovery tests, AFM_1 solution (300 μL) in acetonitrile was spiked in 30 mL of this reconstituted powdered milk. The final concentrations of AFM_1 in the reconstituted milk were 0.005, 0.025, 0.05 and 0.15 ng/mL.Then, this

reconstituted powdered milk (30 mL) was shaken at 200 rpm for 30 minutes using a shaker. After centrifuging at 3,000 rpm for 20 minutes, the fat layer was removed. The middle layer was collected and filtered using a glass fiber filter (GA-200, Advantec Toyo). The filtrate was filtered again using a GA-55 (Advantec Toyo) glass fiber filter, and 10 mL of the filtrate was applied to the IAC

The in-house IAC was prepared with an anti-AFM_1 monoclonal antibody named AM.3 (Okumura *et al*., 1993). The AM.3 hybridoma was cultured in Hybridoma-Serum Free Media (5 L). The culture supernatant of AM.3 was collected and purified using the protein G column. The purified antibody was dialyzed against PBS. The antibody was coupled to Affi-Gel 10 in the ratio of 5 mg of antibody per 1 mL of the gel, according to the manufacturer's instructions. The gel-coupled AM.3 antibody was stored at 4˚C in PBS containing 0.1% sodium azide until use. Gel (0.3 mL) was packed into a mini column (Muromac column size S).

The IAC was conditioned by passing through 10 mL of PBS. Sample solution (10 mL) was applied to the IAC at a flow rate of less than 1 mL/minute. The column was washed with 4 mL of PBS and then 4 mL of water. For elution of AFM_1 from the IAC, we performed the following operation. The column was added to 3 mL of acetonitrile and closed the cap, and reversed 15 times of top and bottom upside down and leaved for two minutes, and then acetonitrile was eluted from the column. I repeated five times of these operations. The eluate was pooled and evaporated to dryness using a centrifugal evaporator at 40˚C. The residue was dissolved in 1 mL of mobile phase, and analyzed using HPLC.

2.2 Results and discussions

Recovery tests

The average recoveries were in the range 73.6 to 86.3% for spiking levels ranging from 0.005 to 0.15 ng/mL in reconstituted milk. Adequate recoveries were obtained with 60 to 120% at 0.01 to 0.05 ng/mL and 70 to 110% at more than 0.05 ng/mL (European Commission, 2006b). The precision of our method was also high, as estimated by RSD of the recovery for AFM_1, which ranged between 5.2 and 7.4% (**Table 3**).

Table 3 Recoveries of AFM_1 from spiked reconstituted powdered milk

Spiked AFM_1 (ng/mL)	Recovery (%, mean ± SD[a])[b]	RSD[c] (%)
0.15	73.6 ± 4.3	5.8
0.05	78.2 ± 4.7	6.0
0.025	83.9 ± 4.4	5.2
0.005	86.3 ± 6.4	7.4

[a]Standard deviation. [b]Average of 3 determinations. [c]Relative standard deviation.

Occurrence of AFM_1 in commercial powdered milk in Bangkok, Thailand

AFM_1 in 79 powdered milk samples collected in Bangkok during 2010 and 2014 were analysed. 12 samples (15%) were contained AFM_1 with the overall mean of 0.004 ng/mL in reconstituted powdered milk. Two samples (0.066 and 0.135 ng/mL) exceeded the regulation of E.U. None exceeded the regulation of Codex. In a comparison by year, there was no significant difference in the contaminated level of AFM_1 in powdered milks. Unlike the cases of fluid milk, the AFM_1 contamination of powdered milk was considerably low in Thailand.

The incidences of AFM_1 in different formula of powdered milk were below. None of 29 samples in infant formula was contaminated with AFM_1. In 24 samples of the follow-on formula, 3 (13%) were contaminated with AFM_1 at levels below LOQ (0.0015–0.005 ng/mL). The levels of AFM_1 in all samples of infant and follow-on formula were below the limit of E.U. for infants (0.025 μg/kg). In 22 samples of children to adult formula, 5 (23%) was contaminated with AFM_1 with an average of 0.008 ng/mL. All 4 samples of non-specified formula were contaminated with AFM_1 with an average of 0.059 ng/mL. Our results suggested that there is an increasing occurrence of AFM_1 in samples specified for higher-age children. In 34 powdered milks

(43% of all samples) made in Thailand, 26% of which were contaminated with AFM_1 (average 0.031 ng/mL). All 4 powdered milks made by one Thai company were contaminated with AFM_1 (average of 0.059 ng/mL). These powdered milks, which were produced in Thailand, were significantly higher contaminated with AFM_1 than imported. From these results, continuous monitoring of AFM_1 in commercial powdered milk, particularly of the Thai domestic products, and stricter regulation of AFB_1 in Thai dairy cattle feed are necessary to reduce AFM_1 in powdered milks made in Thailand.

Risk assessment of AFM_1 in commercial powdered milk in Thailand

I estimated the liver cancer risk from the HBV prevalence rate and the intake amount of AFM_1. The results were shown that the ratio among liver cancer patients in Thailand, who were caused by an intake of AFM_1 from these powdered milks, was lower than 0.006% in the average consumption and lower than 0.038% in the worst-case scenario, respectively. The liver cancer risk of AFM_1 in Thai commercial powdered milks was very low

3. Occurrence of ochratoxin A and B in commercial coffee in Vietnam and Thailand

3.1 Introduction

Arabica coffee beans are cultivated in the mountains at high altitude in the north as Royal project, whereas Robusta coffee beans are cultivated in the south in Thailand. Coffee has been found to be contaminated with OTA (**Figure 3**), which is a potent nephrotoxin and carcinogen (Group 2B by IARC), at a relatively high frequency. Noonim *et al.* (2008) repoted that 28 (89%) of 32 Arabica green coffees were contaminated with OTA (< 0.6 - 5.5 μg/kg), and all of 32 (100%) Robusta green coffees were contaminated with OTA (1.5 - 8.9 μg/kg). The data indicated that Thai green coffees, which were pre-roasted coffee bean, were heavily contaminated with OTA. There are some reports on OTA in coffee cherry and green coffee, and only one report on OTA in roasted coffee (Noonim *et al.*, 2008; Bucheli *et al.*, 2000; Joosten *et al.*, 2001; Chompurat *et al.*, 2006), but there are no reports in international journals on ochratoxins in instant coffee in Thailand. Red wines in Italy and Spain were contaminated with OTB (**Figure 3**), which was almost the same levels of OTA (Remio *et al.*, 2010; Di Stefano *et al.*, 2015). Because 22 (28.6%) of the 77 red wines in these reports were contaminated with higher concentration of OTB than OTA, we considered that it was preferable to analyze both OTA and OTB in coffee products.

So that, to clarify the levels of ochratoxins in commercial roasted and instant coffee in Thailand, I collected 30 roasted Arabica coffees in Chiang Mai City and 38 instant coffees (mainly Robusta) in Chiang Mai and Bangkok in Thailand during 2012 and 2013, analyzed the IAC-HPLC method, and evaluated its risks based on TDI.

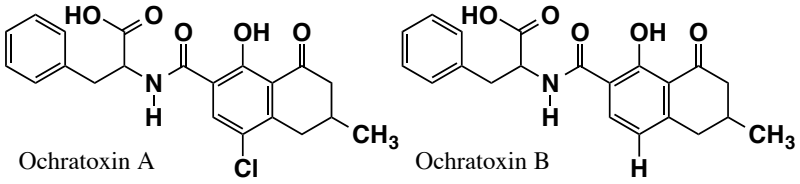

Figure 3 Structures of ochratoxin A and B

In additon, Vietnam is the world's second largest coffee producer, mainly of Robusta coffee. Its Robusta coffee is exported around the world and commonly consumed as a regular coffee in Vietnam. There are some reports on contamination by OTA and OTA-producing fungi in Vietnamese green coffee. However, only one

report on contamination by OTA in Vietnamese roasted coffee has been published (Napolitano *et al.*, 2007). There is little data on the coffee that is commercially available in Vietnam. I collected 32 Vietnamese Robusta roasted coffees, which was for reference, and analyzed the IAC-HPLC method, and evaluated its risks.

Most of these contents were published in Wongworapat, K., Ho, T. H. M., Soontornjanagit, M., Osamu Kawamura, O. (2016) Occurrence of ochratoxin A and ochratoxin B in commercial coffee in Vietnam and Thailand. *JSM Mycotoxins*, 65, (in press).

3.2 Materials and methods

Thirty-two roasted coffees (Robusta) were purchased from conventional supermarkets and stores in Ho Chi Minh City and Can Tho City in Vietnam during 2011 and 2012. Thirty roasted coffees (Arabica) were purchased in Chiang Mai City and 38 instant coffees (mainly Robusta) were purchased in Chiang Mai and Bangkok in Thailand during 2012 and 2013. All samples were kept at 4˚C until analysis. These coffees were analyzed by the method of Kawamura *et al.* (2015).

3.3 Results and discussions

Thai roasted and instant coffees

The OTA and OTB in 68 commercial coffee products in Thailand (30 Arabica roasted and 38 instant coffees) and 32 Vietnamese Robusta roasted coffees, which was for reference, were analyzed. OTA in all coffees were less than the limit of E.U. (5 μg/kg in roasted and 10 μg/kg in instant coffee). Only 4 (13.3%) of 30 samples in Thai Arabica roasted coffee, were contaminated with OTA with an average of 0.66 μg/kg in the range 0.40–1.00 μg/kg. The overall average was 0.17 μg/kg. OTB was detected in only one sample (0.56 μg/kg). The level of OTA in Thai Arabica roasted coffee was relatively lower than previous reports in other countries.

28 (73.7%) of the 38 samples in Thai instant coffee (mainly Robusta) were contaminated with OTA with a positive average of 2.90 μg/kg in the range 1.28–6.20 μg/kg. The overall average was 2.19 μg/kg. It was remarkable that OTB in these samples was not detected. In Vietnamese Robusta roasted coffee samples, 26 (81.3%) of 32 samples were contaminated with OTA with a positive average of 0.90 μg/kg in the range 0.24–4.27 μg/kg. The overall average was 0.75 μg/kg. Additionally, 11 (34.4%) of 32 samples were contaminated with OTB in the range 0.21–0.64 μg/kg. The positive average was 0.40 μg/kg. The positive ratio of OTA was 2.4 times higher than that of OTB. The average concentration of OTA was 2.3 times higher than that of OTB. The finding was that the samples with OTA that larger than 0.6 μg/kg tended to contain OTB. I reported the occurrence of OTA and B in Thai instant coffee and OTB in commercial Vietnamese roasted coffee for the first time.

Vietnamese roasted coffee

Thai roasted coffee (Arabica) was 4.4 times lower than Vietnamese roasted coffee (Robusta) in overall average. These data were indicated that Robusta coffee was easy to be contaminated with OTA than Arabica coffee. Accordingly, it was suggested that the risk of OTA in instant coffee, which was made from Robusta coffee bean, was higher than regular coffee, which was mainly extracted from Arabica coffee bean.

The TDI of OTA was 14 ng/kg b.w./day according to Joint FAO/WHO Expert Committee on Food Additives (2002).

Risk assessment of OTA in commercial coffees in Thailand and Vietnam

I evaluated the risk of the OTA to take in from these coffees. In the average consumption, the intake amount of OTA form Thai roasted and instant coffee was less than 0.12 % of TDI. In the worst scenario, the intake amount of OTA was less than 13.3 % of TDI. The risk of OTA from commercial Thai coffees was an

allowable level. In case of Vietnamese roasted coffee, the intake amount of OTA was estimated 0.21% and 45.1% of TDI in the average consumption and in the worst scenario, respectively. The risk of OTA from Vietnamese Robusta coffee was acceptably low. However, since the data were only for one year, continuing the surveillance of OTA and OTB in commercial coffee in Thailand and Vietnam was suggested

4. Total conclusions

From my studies, the risks of AFM_1 in commercial powdered milk and OTA in coffee product were low in Thailand. Also, OTA in Vietnamese Robusta coffee product was acceptably low. However, powdered milks made in Thailand contained more AFM_1 than made in other countries, which were imported powdered milks. It was thought that this originated in AFM_1 contamination levels of Thai liquid cow milk was high. The limit of AFs in feeds for Thai dairy cows was 200 μg/kg, which was 10 times higher than U.S., 20 times higher than Japan, and 40 times higher than E.U. In order to reduce AFM_1 in liquid cow milk, at first, it is necessary to lower regulation for Thai dairy cows to the same level of at least U.S. (20 μg/kg), and then to enhance the education of the farmers to enforce cultivation and preservation management of the crops for cow feeds at the same time. In addition, the construction of the analysis system of AFs in feeds is important, too.

Arabica coffees are cultivated in the mountains at high altitude in the north as Royal project in Thailand. It was good news that the level of OTA in Thai Arabica roasted coffee was lower than previous reports in other countries. I think that the results were more publicized and the Arabica coffee in northern Thailand should be promoted. However, OTA in Thai instant and Vietnamese roasted coffee were acceptably levels but comparatively high. I think that continuous monitoring of OTA in these coffees is necessary.

My studies indicated that the contamination of mycotoxins in commercial foods in Thailand were acceptably low. But, I analyzed mycotoxins in only two commercial foods, which were powdered milk and coffees. I think that it is necessary the construction of the analysis system of mycotoxins in foods and to analyze various foods continuously. From these results, the risks of mycotoxins in Thai food were made clear, and we should work out effective and necessary measures to reduce the risks. I hope that my studies become part mentioned above.

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(Note)　The Summary should be about 10％ of the entire dissertation and may include illustrations