Prevention of Aflatoxin Contamination in Thai Maize

2. Distribution of maize with high moisture content and methods of control of *Aspergillus flavus* infection

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Abstract

The results of a survey conducted among local maize traders, showed that undried wet maize (moisture content over 20%) was widely distributed throughout Thailand (more than 50% of total maize traded). Wet maize became readily infected with *A.flavus*. Aflatoxin problems in wet maize could be resolved by prevening *A.flavus* infection for the initial seven to ten days after shelling. The use of alcohols was effective in inhibiting the growth of *A.flavus*. Hermetic storage of wet maize in plastic bags was a simple but the most practical method to prevent aflatoxin contamination of Thai maize even in local areas.

Additional key words : *Aspergillus flavus*, maize, hermetic storage

Introduction

Aflatoxin contamination of Thai maize[1] is a serious problem for overseas trading and for the health of the Thai people. To solve the problem domestic production and distribution routes have to be examined carefully. Since farmers and maize traders are poorly informed and the infrastructure is under developed in Thailand, the methods of control must be safe and economical as well as locally acceptable and sustainable. Materials produced in the country must be applied for this purpose.

Surveys on the production and distribution of maize with a high moisture content

1) Interviews with local brokers and middlemen

Wet maize with a moisture content(mc) of about 20% to 30% or even higher was readily infected with *A.flavus*. To analyse the distribution process of wet maize and to determine why maize was not sun-dried after shelling, a questionnaire was prepared. A total of 28 local brokers and middlemen in the five main maize-producing areas in Thailand were interviewed, namely: 1.Chiang Mai and Chiang Rai, 2.Phrae, 3.Phetchabun and Loei, 4.Sikhiu and Nakhon Ratchasima, 5.Wang Num Yeng, Chantaburi (Fig.1). The answers were tabulated to outline the situation of the post-harvest distribution processes of undried wet maize.

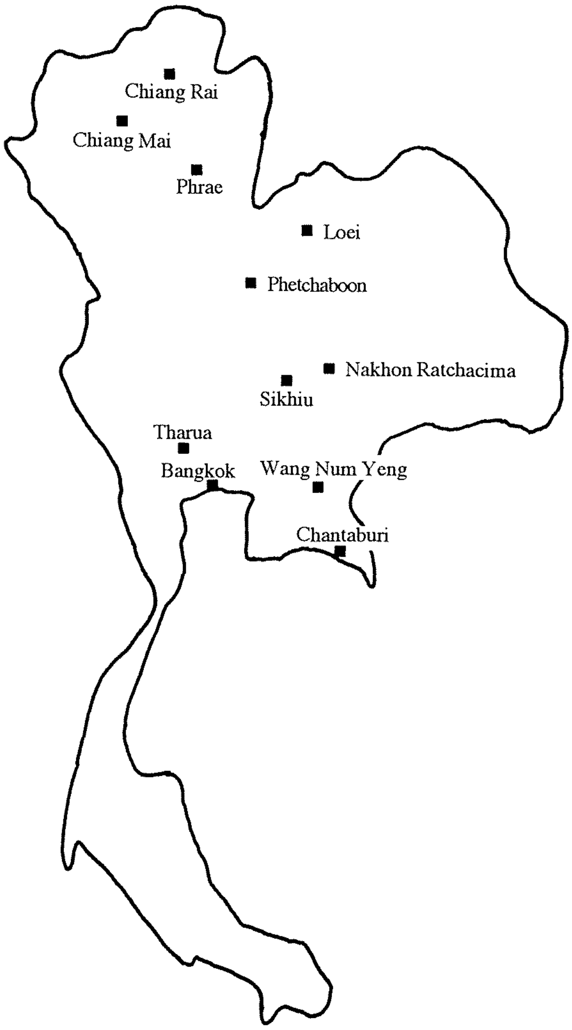

Fig. 1. Areas where interviews were conducted

2) Interviews with feed meal companies and maize exporters

After being handled through the distribution routes, wet maize was finally delivered to big traders. Seven big traders (four feed meal companies and three maize exporters) around Bangkok and Tharua city were interviewed to obtain information on the moisture situation of maize cargoes delivered to them. Seasonal changes of the moisture content in maize kernels were tabulated.

Chemical and physical methods to prevent aflatoxin contamination of maize associated with *Aspergillus flavus* infection

Measures besides drying to prevent aflatoxin contamination in maize have to be developed. It is not advisable to apply sophisticated technologies. Measures should be safe, applied on a small scale, flexible, inexpensive, not associated with chemical residues, and suitable for the social conditions in Thailand.

1) Chemical methods

Pure chemical grade ethanol(95.0%) or methanol(99.5%) was poured(600ml or 900ml) thoroughly into 30 kg of maize(mc 31.3%) packed in traditional jute bags. Bags were then sewn up and covered with thick polyethylene film sheets for two days.

2) Physical methods

(1) Maize in various packages

It was anticipated that the wet maize kernels in hermetic storage[2] like in plastic film bags would consume oxygen and would discharge CO_2, creating anaerobic conditions in the bag, which would protect maize from *A.flavus* infection. Maize kernels (104 days after planting, mc 29.1%, 10kg each) were inoculated with *A.flavus*(10^6/g, 50g of milled rice) and packed in various kinds of bags. A part of the maize kernels was sterilized with gamma rays (25kGy).

Traditional jute bags, polyethylene bags(125 μm) and fabricated polypropylene bags(500 μm) were used for the experiments. Maize kernels at different stages of maturity (97, 111, 118, and 125days after planting, mc 33.4, 27.1, 24.9 and 21.3% respectively) were packed in polyethylene bags(two kg each) after inoculation with *A.flavus* (10^6/g, 20g of milled rice).

(2) Maize kernels subjected to prolonged hermetic storage

In Phraphuttabat FCES, maize with a high moisture content (mc 36.9%, 90 days after planting) was harvested and immediately shelled. One or two bags made of high density polyethylene, 45 μm in thickness were inserted inside the jute bag. Since large plastic bags which fit to jute bags of ordinary size (73×104 cm) were not available, the jute bags were cut to about 2/3 size and packed with 35kg of maize with a high moisture content. There were four groups of bags as follows: C)jute bags as control, SR)jute bags with single plastic bag inside, DR) jute bags with double plastic bags inside, DF)jute bags with double plastic bags, the top end of which was folded in. Each plastic bag was tightly bound with a string except for DF in such a way as to expel the air in the bag as much as possible. The jute bags were finally sewn up and stored at ambient temperature. *A.flavus* was not inoculated.

Results and Discussion

Survey on the distribution of wet maize

1) Interviews with local brokers and middlemen in five districts

It is generally assumed that all the Thai maize is sun-dried on a concrete floor after shelling. However, most of the Thai maize is harvested during the rainy season[3], and since mechanical dryers are not usually available, especially in local areas, a large volume of undried maize circulates in the local markets[4].

In practice, wet maize was widely distributed especially during the main season. It was learned that very few middlemen owned mechanical dryers or even drying floors. The practice of sun-drying is difficult due to technical, socio-economic, climatic, and transportation constraints. Typical monthly moisture contents (mcs) of Thai maize were reported to be 30.0%(July), 24.5%(August), 22.% (September),19.5% (October),16.0% (November)[5]. In another Thai report the average mcs of maize delivered to maize exporters were 22.0%(July), 22.3% (August), 20.4% (September), 17.3% (October)[6]. These data indicate that most of Thai maize was likely to be distributed as wet maize with a mc over 20%. The results of the interviews are summarized as follows:

1. Area with one maize season (July/Nov): Phrae, Phetchabun, Loei, Wan Num Yeng.
2. Area with two maize seasons (June/Sep-Nov, Nov/Feb): Nakhon Ratchasima Sikhiu, Chiang Mai, Chiang Rai.
3. Farmers and middlemen sell maize whenever the price is high, even wet maize. Feed meal companies or maize exporters will buy such maize.
4. At the beginning of the maize season(July to August), there is a strong demand in the market for maize high at prices.
5. Many middlemen do not have a drying floor and trade only wet maize (mc 20-30%).
6. Around 50-90% (depending on the district) of maize is estimated to be distributed as wet maize from July to October(or November).
7. For the transportation and distribution of maize before sun-drying it takes 2-3(maximum 7-10)days.
8. During the second maize season (December to February), maize dries well in the field and wet maize is unlikely to become a problem.
9. Drying floors of middlemen are not large enough to accommodate all the products in the busiest harvest season (late July to the end of September). The capacity of the sun-drying floors is usually 8-15 ton/rai(0.16 ha)/1-3 days with grains laid at a 2-3 inch

thickness.

10. Maize rewet by rain is often sold without further drying.
11. In northern Thailand , maize ears are hanged to dry under roofs. It is a good practice but some alternatives (plastic net bags) should be introduced. Since this practice is laborious and requires a large wall area, farmers prefer to sell their harvested products immediately.
12. Middlemen or creditors often control farmer's maize. They can harvest and sell wet maize at their will when the price is high.
13. Some middlemen collect good quality maize stored and dried as corn ears in mountain areas.
14. A leader has organized a farmers' group to produce good quality maize in Loei. He introduced a field drying system (do not harvest maize until 120 days after planting), producing excellent maize.
15. Maize quality hardly determines the price. An inexpensive, reliable and quick method to detect aflatoxin needs to be developed.
16. Many local traders consider that a mechanical dryer will not be profitable to their business.
17. In general, better quality products are sold to feed companies and inferior ones to maize exporters.
18. Timely shipping is not always possible in remote areas, especially in the rainy season. Muddy roads impede vehicle traffic in the mountain areas. Wet maize has to wait for trucks for several days.
19. Mechanical dryers are usually not available in local areas. However, in the newly developing maize area (Wan Num Yeng, Chanthaburi) many middlemen have introduced mechanical dryers, although electric or mechanical troubles sometimes occur.
20. Farmers are generally poor and they need money as soon as possible after harvest. They sell maize even in the field.
21. Not all of the farmers have storage facilities - for example in the Khon Buri area, the government once prohibited farmers from storing maize in field sheds.
22. Farmers' storehouses have low floors and require better ventilation devices.
23. Field drying is a good method to decrease the maize moisture content but farmers do not like it because : 1) They have to start the next planting and can not wait another one or two weeks for field drying. 2) The product weight decreases. 3) The fresh bio-mass is reduced, and the stalks exhibit lodging, hence the reduction of yields. 4) The products are more susceptible to rodent and insect damages. 5) Farmers are usually indebted. 6) Farmers fear of losing favorable price opportunities.

2) Interviews with feed meal companies and maize exporters

Domestic consumption of Thai maize is rapidly increasing. About 30% of the annual production used to be traded in the internal market. However due to the rapid growth of chicken meat production, 70% to 80% of maize is currently domestically consumed.

1. Feed meal companies offer higher prices and collect better quality maize. Moisture contents of maize kernels are mostly less than 20%(15 to 20%).

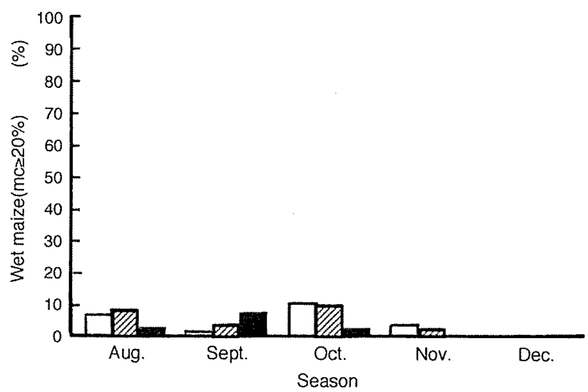

Fig. 2. Trading of maize with high moisture content ($mc \geq 20\%$) in feed meal companies.

Date: □ 1-10th, ▨ 11-20th, ■ 21-30th of every month

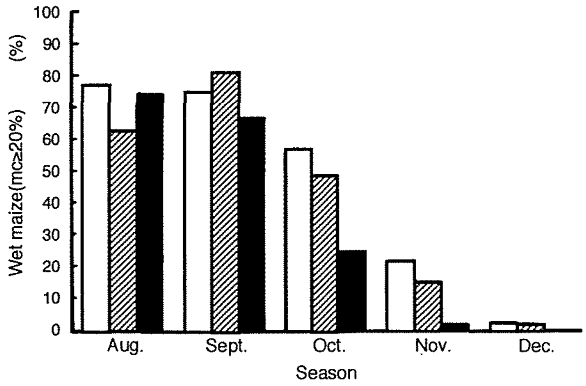

Fig. 3. Trading of maize with high moisture content ($mc \geq 20\%$) by maize exporters (silo).

Date: □ 1-10th, ▨ 11-20th, ■ 21-30th of every month

2.Generally, feed meal companies avoid maize with a high moisture content though sometimes they buy it(Fig.2).

3.Feed meal companies are willing neither to pay for the drying of maize nor to do it on their own.

4.Maize with a high moisture content in hermetic storage produces a sour smell which is erroneously considered to be a sign of aflatoxin contamination.

5.Silos(maize exporters) have a smaller market share and buy second class maize because they usually offer lower prices compared to feed meal companies.

6.Silos buy maize with a high moisture content especially in August, September and October (Fig. 3).

7.Silos know that freshly harvested maize with a high moisture content is free from aflatoxin contamination. They claim that they can process maize with low aflatoxin contamination out of wet maize and prefer to collect such maize from the middlemen nearby.

8.Silos have to pay fuel costs to dry wet maize. However, they can expect higher profits by imposing weight penalties on the high moisture. In the international trade, they demand special premiums for maize, with low aflatoxin contamination.

9.Since feed meal companies are more concerned with quality, they will buy wet maize in future, once they recognize the profit potential of wet maize.

10.It should be realized that the presence of aflatoxin can not be detected from the maize appearance.

11.To inflate the maize volume, a low quality lot is often mixed with a high class one, resulting in a drastic rise of aflatoxin contamination. This is likely to happen more frequently with sun-dried maize.

Chemical and physical methods to prevent aflatoxin contamination of maize associated with *Aspergillus flavus* infection

It took a maximum of seven to ten days for the undried shelled maize to be conveyed in jute bags to feed meal companies or to maize exporters, and to be finally mechanically dried(mc 14 to 15%).

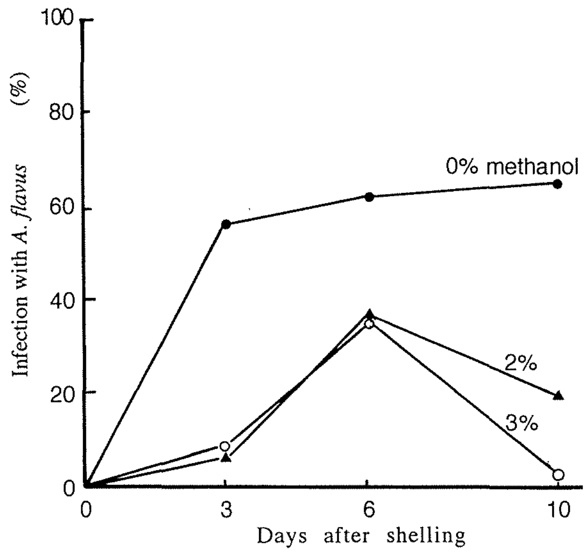

Fig. 4. *A.flavus* infection of wet maize mixed with methanol immediately after shelling
30kg of maize (harvested 100 days after planting, mc 31.3%) in jute bag was mixed with 2% (600ml) or 3% (900ml) of pure methanol and stored at ambient temperature.

During this shipping period, *A.flavus* grew quickly and produced aflatoxins. In practice, if wet maize could be protected from mold infection only for the initial seven to ten days after shelling, aflatoxin problems of Thai maize could be substantially alleviated.

1) Chemical methods

(1) Addition of alcohols immediately after harvest

Moisture content of kernels(100days after planting) decreased gradually from the initial 31.3% to 27.7 and 25.6% after 3, 6 and 10 days of storage in jute bags, respectively.

During the first 3 days, the infection rate of *A.flavus* in the control increased nearly by 60%, but there was no further sharp increase (Fig.4). The surface of the sterilized kernels was quickly covered with *Botryodiplodia* sp. which, caused only limited further infection with *A.flavus*, because in the kernels heavily infected with *Botryodiplodia* sp., *A.flavus* could grow only poorly.

In the control, the accumulation of aflatoxin B_1 during the first three days after the inoculation was nil and after six days it exceeded the legal limit of 20ppb (38ppb) while in the methanol group(2%, 3%), it was less than 20ppb(Fig.5). Ten days after inoculation, in the 3% methanol group, the toxin level was still around 5ppb while in the control it was over 180ppb. In the 2% methanol group, it was around 43ppb which nearly corresponded to the toxin value of the control on the 6th day.

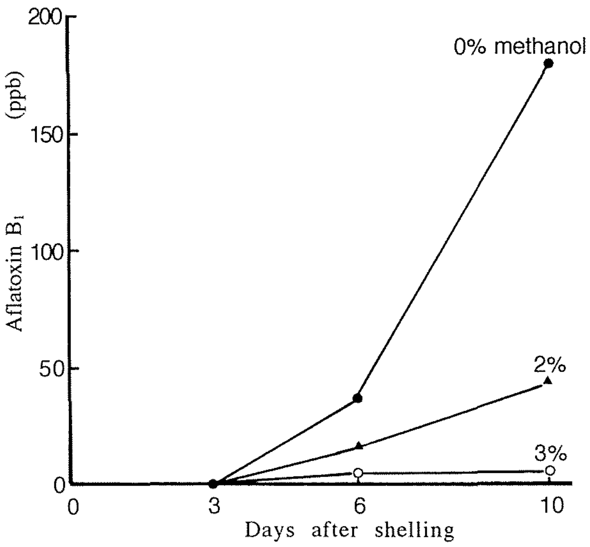

Fig. 5. Aflatoxin B_1 contamination of wet maize mixed with methanol immediately after shelling
30kg of maize (harvested 100 days after planting, mc 31.3%) in jute bag was mixed with 2% (600ml) or 3% (900ml) of pure methanol and stored at ambient temperature.

Thus, by the addition of 3% weight of pure methanol it was possible to control the infection with *A.flavus* and toxin contamination for more than 10 days. In the case of ethanol, aflatoxin contamination did not occur until three days after inoculation. However, after six days, in the control and 2% ethanol group, the toxin levels reached 38 and 22ppb, respectively, and were already over the legal limit level(20ppb). After ten days, the 3% ethanol group still showed a low toxin level(12ppb), and thus ethanol was also found to be effective in controlling aflatoxin contamination. Ethanol and methanol were similarly effective in inhibiting the toxin accumulation in maize kernels.

(2) Addition of alcohols 3days after bagging

During the first three days without treatment *A.flavus* started to grow in the gunny bags. Toxin formation was fairly well inhibited by the addition of 2% methanol. However, it was rather difficult to keep the toxin level under 20ppb. In the 3% methanol group, the toxin level was lower than 20ppb(15ppb) even after 6 days(9 days after harvest). After 10 days the value was 35ppb but was considerably lower than that of the control(250ppb). It was necessary to add chemicals as soon as possible after shelling. However if 3% weight of methanol is mixed within three days after shelling, toxin contamination can possibly be prevented for several more days.

2) Physical methods

The so called UTP system was proposed in Thailand in 1986, with the following steps; shelling of maize within 48hrs of harvest; loading of shelled grains into a dryer within 12 hrs after shelling; and finally completion of drying to mc 14% within 2 days. However, visits to Thai local fields show that practical methods to prevent aflatoxin contamination must be simple, inexpensive and the materials locally available. Considering such constraints, hermetic storage of maize in plastic bags was developed.

(1) Maize in various forms of packages

To confirm the effect of hermetic storage, maize was stored in various forms of packages[7]. The following results were obtained.

1. In traditional jute bags and fabricated plastic bags (polypropylene), *A.flavus* infection occurred in a short period of time (within 4 to 6 days).
2. Thick polyethylene bags (125 μm) and high density polyethylene bags (40 μm) inhibited completely the growth of *A.flavus*. In hermetic storage, maize consumed oxygen and produced CO_2, creating an anaerobic condition inside the bag.
3. Wet maize kernels with various degrees of maturity and various levels of moisture contents (97, 111, 118, 125 days after planting and mc 21.3%, 24.9%, 27.1%, 33.4% respectively) were all fully protected from *A.flavus* infection in thick polyethylene bags for over six weeks.

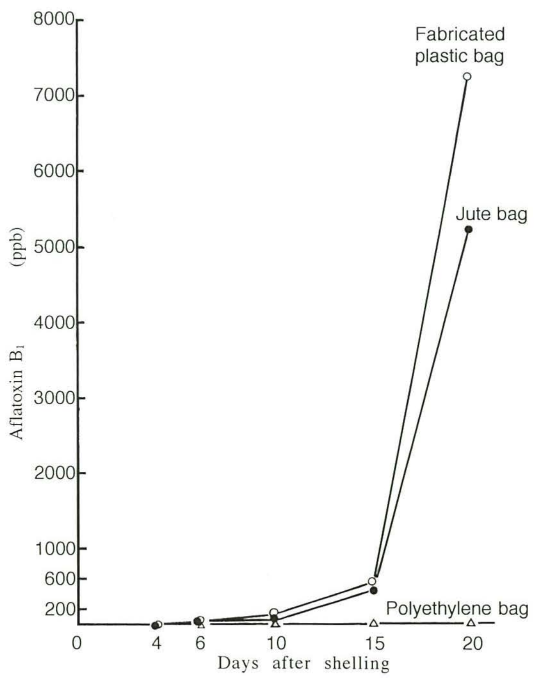

Fig. 6. Aflatoxin contamination of maize in various packages.

4. Since in the gamma ray sterilized maize, the inoculated *A.flavus* scarcely grew, it was assumed that microorganism competition was not the main reason why *A.flavus* failed to grow in the plastic bags.
5. In porous jute bags and fabricated polypropylene bags aflatoxin contamination was severe (2,000 - 7,000ppb), whereas in thick polyethylene bags no aflatoxins were detected (Fig.6).

(2) Maize in prolonged hermetic storage tests

1. Maize with a high moisture content (mc 36.9%, 90days after planting) was packed (35kg) in jute bags or in high density polyethylene bags, one or two of them lining the jute bags, and stored for 60 days at ambient temperature.
2. In the control, after 3 days, 68% of the kernels were infected with *A.flavus*. Then the incidence of infection decreased and within 10 days infection with *A.flavus*, was negligible although every kernel was heavily infected with various kinds of fungi other than *A.flavus*. After 2 weeks, the incidence of infection with *A.flavus* again increased until the end of the experimental period.
3. Maize in jute bags (control) was heavily contaminated with molds and lost its

Plate 1. Inhibition of infection of wet maize with *A.flavus* by hermetic storage
left:in high density polyethylene bag, right:in jute bag maize;initial mc 36.9%, stored at ambient temperature for 20days

commercial value within 5 days, irrespective of *A.flavus* infection.

4. Plastic bags were tightly bound so as to expel the air inside as much as possible. The infection with *A.flavus* was completely inhibited (Plate l).
5. After 10 days not only *A.flavus* but all kinds of fungi completely disappeared in every plastic bag.
6. *A.flavus* was not detected even in the plastic bags which were incompletely closed by folding the top ends.
7. A sour smell was detected after a few days in the plastic bags but after drying the smell became less pronounced. Since such a smell is not appreciated by traders, some promotional tactics are needed to overcome the prejudices.
8. The sour smell in the plastic bags was less pronounced by mixing of methanol during hermetic storage.
9. The growth of *A.flavus* was perfectly controlled in hermetic storage due to the anaerobic conditions (O_2 deficiency, CO_2 accumulation).
10. In ordinary jute bags (control), aflatoxin was not detected before 10 days and then the concentration increased rapidly from 217ppb (14 days) to 1395ppb (30 days) and 977ppb (60 days).
11. Aflatoxin was not detected in any of the grains in hermetic plastic bag storage during the experimental period up to 60 days (Table 1).
12. A new system of maize handling to save the drying cost could be developed, if hermetically stored wet maize were supplied directly to the poultry industry.

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タイメイズのアフラトキシン汚染防止に関する研究

2．高水分メイズの流通と*Aspergillus flavus*汚染の防止法

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摘　要

タイ国産メイズは，収穫後の流通過程中にカビ（*Aspergillus flavus*）が生育し強力な発癌物質のアフラトキシンに汚染される。このため国民の健康上，また貿易取り引き上，大きな障害となっている。我々は現地におけるメイズの生産，流通プロセスをたどりながら，その汚染機構を明らかにすると共に，現地で実現可能な汚染防止法の確立を行うことを目的とした。

*A.flavus*及びアフラトキシン汚染が，生産から流通過程中の主としてどのプロセスで始まるかを明らかにしようとし，メイズを農家，仲買人，輸出業者及びメイズの飼料業者とたどりながら聞き取り調査を実施した。流通過程を追跡調査することにより，タイ国内を流通するメイズには，非乾燥のウエットメイズ（水分含量20%以上）と，仲買人が天日乾燥する乾燥メイズとが，ほぼ半数ずつ存在することをまず明らかにした。ウエットメイズは*A.flavus*で容易に汚染される。脱粒後このウエットメイズが飼料会社や輸出企業に運び込まれて機械乾燥されるまでの最初の７～10日間，*A.flavus*感染を防止すれば，メイズのアフラトキシン汚染は，大幅に低減することができる。

汚染防止法として，化学的，物理的な手法の開発に努めた。化学的防止法としては，アルコールが効果的であった。即ち脱粒直後のウエットメイズをkg当たり，95～99%濃度のメタノールまたはエタノールを30ml程度添加することにより麻袋中で，*A.flavus*の発生を10日間程度遅らせることができた。その時のアフラトキシン（AFB_1）濃度はメタノールで10日間以上20ppm以下に抑制することが可能であった。エタノールも10日後で12ppmであった。次に物理的な防止法として袋内への空気の出入を絶つことにより*A.flavus*の生育が阻止されることを見い出した。タイ国のメイズは麻袋で流通されている。この麻袋の内側に高密度ポリエチレン袋（HD・PE）を挿入し，ウエットメイズを詰め，袋内の空気をできるだけ追い出して口をしっかり固定するだけで，２ヵ月以上室温に貯蔵したが，*A.flavus*による感染は防止された。また，貯蔵開始７～10日で*A.flavus*だけでなく*Penicillium*,*Fusarium*など全てのカビがメイズ粒内部から検出されず，アフラトキシンの汚染も全くなかった。高密度ポリエチレン袋内のメイズを密封することにより数日で乳酸菌等による発酵臭が発生するが，この臭いはメイズを乾燥することにより軽減した。この処理でメイズの商品価値としての色は遜色がなかった。この方法は安全，簡便，安価で小規模の使用にも適用でき，特殊な道具や知識を必要としないので，タイ農村部で実用可能と思われる。

キーワード：アフラトキシン，*Aspergillus flavus*，トウモロコシ，密封貯蔵