Prevention of Thai maize from the infection by *Aspergillus flavus* and aflatoxin contamination in various packages

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P. Siriacha, 川崎浩二*², 斋藤道彦*², P. Tonboon-Ek*¹, D. Buangsuwon*¹:
各種包材によるタイムイズの *Aspergillus flavus* およびアフラトキシン汚染防止

Summary

Each 10 kg of high moisture maize kernels (moisture content (mc) 29.1%) inoculated with *Aspergillus flavus* was packed in various kinds of package. Every bag was sealed with string so as to make the air space to be as little as possible. In traditional jute bag and fabricated polypropylene (PP) bag, *A. flavus* infection occurred completely within 4 to 6 days. Thick polyethylene (PE) bag (125 μm thick) and high density PE bag (40 μm thick) perfectly inhibited the growth of *A. flavus* for over 20 days and no aflatoxin was detected.

Wet maize kernels with various maturity levels and mc (97 to 125 days after planting and mc 21.3% to 33.4%) were also prevented from *A. flavus* infection completely in the thick PE bag.

High moisture maize of which moisture content (mc) is over 20% is widely distributed in Thailand*. It takes several days or even more than a week for the distribution of wet maize. After shelling by local brokers or middlemen, maize kernels in jute bag is sent to upper local merchants (middlemen). They keep maize for enough amounts of truck and then send them to the companies which offer good prices. If weather is rain and road condition is not well, they have to wait some more days. At the busiest maize season, trucks have to spend several days for waiting at the front of company until cargoes are accepted, because there are so many trucks surrounding maize companies (maize exporters or feed meal companies). Drying capacity in these maize companies are not enough, especially if they have to accept high moisture maize. In most cases, local merchants can dry up to several hundreds tons of maize per day. Feed meal companies near Bangkok can dry 50 to 200 tons of maize in one cycle of drying process and reduce its mc by 5%. If mc of maize is around 24%, maize kernels have to be dried twice to get mc lower than 14.5% which is the standard moisture level for the commercial maize kernels.

High moisture maize is infected not only with *Aspergillus flavus* but also by various

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other fungi during such long distribution periods. If high moisture maize could be temporarily stored for a week without fungal infection, wet maize could be safely distributed during that periods from farmer's field to middlemen and further to maize exporters (silo companies) or feed meal companies until the maize being dried.

From the preliminary test, it was found that high moisture maize in plastic bags can be stored without heavy infection of *A. flavus* for over 60 days. Thus, such packaging would enable the distribution of low aflatoxin (AF) contaminated maize and would solve the AF problem in Thai maize.

**Materials and Methods**

1. **Samples**: Maize of 29.1% mc was collected on September 13th, 1988 at Phraphuttat Field Crop Experiment Station, Department of Agriculture (DOA), Thailand. It was 104 days old maize after planting and was shelled at the station immediately after the harvest. Samples of wet maize (2kg each) with various maturity levels and mc were also obtained from the same experimental field. They were 97 days (mc: 33.4%), 111 days (mc: 27.1%), 118 days (mc: 24.9%) and 125 days (mc: 21.3%) maize after planting.

2. **Microorganisms**: *A. flavus* was incubated on sterilized rice for three weeks at ambient temperature. Spores were collected and mixed with sterilized rice powder in aseptic coffee mill. Forty gram of them was mixed with 10 kg of maize. Population of *A. flavus* in the inoculum was 1.2 to 1.5×10⁶/g.

3. **Packaging and inoculation test for high moisture maize (29.1%)**: There were 7 treatments as follows.
   - A. Maize in jute bag (control; traditional package in Thailand).
   - B. Maize in jute bag was covered with plastic film (polyethylene (PE), 125 μm thick) throughout the experiment.
   - C. Maize in jute bag was mixed with 2% (v/w) of methanol (99.5% purity) and then covered with plastic film throughout the experiment.
   - D. Maize in PE film bag (125 μm thick).
   - E. Maize sterilized with γ-rays (25 kGy) in PE bag (125 μm thick).
   - F. Maize in fabricated polypropylene (PP) bag (500 μm thick).
   - G. Maize in double (two layers) plastic bag: fabricated PP bag (500 μm thick, outside) and high density PE film bag (40 μm thick, inside).

   The double bag is widely used for the distribution of agricultural commodities like sugar.

   Two bags (replication) with 10 kg of maize per bag were prepared for each treatment. Jute bags used were pre-sterilized with commercial γ-ray irradiator (25 kGray) to eliminate all the microorganisms exist.

   Following the inoculation of *A. flavus*, each plastic bag was tightly sealed with string to expel the air in the bag as much as possible. All maize kernels were inoculated with *A. flavus* and stored under ambient temperature (25-30°C, RH 70-90%).

4. **Packaging treatment for wet maize**: To know the effect of mc of maize, 2 kg of the wet
maize kernels with various maturity levels and mc were also packed in thick PE bag (125 μm thick) without the inoculation and stored similarly for 15 days.

5. Analyses: During the experiment, maize was sampled regularly after 0, 4, 6, 10, 15 and 20 days and submitted to mycological and chemical analyses. The maize was dried by hot air (80–85°C) to decrease mc less than 12%, and AF was analyzed following the modified CB method. pH value of maize was measured by a pH meter (DKK pH meter PHL-20) for 100 ml of sterile water stirred with 20 g of maize kernels for 5 min.

Results and Discussion

In the inoculation test, A. flavus infection of maize in various packages is shown in Fig. 1.

Jute bag and fabricated PP bag had no effect at all to inhibit the growth of A. flavus. Since most of Thai maize is distributed in jute bag, it could be understood from Fig. 1 how quickly the fungal infection of maize spread in the usual trading. By covering jute bag with PE film, the infection was more stimulated. Fabricated PP bag was failed to inhibit the growth of A. flavus, since PP bag was porous and not air-tight.

In thick PE bag and double plastic bag, A. flavus was drastically inhibited to grow for 20 days during the experimental period. In the same thick PE bag, wet maize kernels with various maturity and mc (97 days after planting, mc: 33.4%; 111 days, 27.1%; 118 days,
24.9%; and 125 days, 21.3%) were also stored. After 15 days, kernels were checked and there was neither A. *flavus* infection nor AF contamination at all in the wet maize. Thus, plastic bag method was applicable in any high moisture maize. In plastic bag it would be relatively more anaerobic, which would be favored by certain microorganisms but unfavored by A. *flavus*. Microflora was expected to be changed and would create the circumstances that inhibit the growth of A. *flavus*.

PE film has rather poor oxygen gas barrier compared to some other plastic films. In our preliminary experiments, single PE bag (40 μm thick) poorly inhibited the growth of A. *flavus*, but double bag fairly well inhibited and triple (3 layers) bag perfectly inhibited. Under modified atmosphere where oxygen concentration was low, AF production was reported to be slight. Peterson et al. showed that A. *flavus* survived under 4.3% oxygen concentration but failed to germinate under 2.3% oxygen. They reported that in the hermetic storage of damp grains, low oxygen and high carbon dioxide atmosphere, as a result of the respiration of the grains and associated microorganisms, will inhibit mold growth and thereby show greatly retard deterioration. The hermetic storage of wet grains was described by Hall and Hyde. Australian scientists are trying to use air-tight plastic bags which are purged with carbon dioxide of phosphine in order to keep grains, but it is for the protection of grain mainly out of insect.

It is interesting that Stoloff et al. reported corn ears could be stored in porous cotton bag but not in nonporous PE bag for several days. If they sealed their PE bag in such a way as air was little as possible, then they could have obtained reverse results. It might be common sense that in air-tight film bag A. *flavus* may infest wet maize in a short time and high moisture maize kernels can not be stored even for a few days. However, only if a PE bag was sealed as to hold least air space, they would have found completely different results. Double plastic bag is physically stable and widely used not only for agricultural products, but for some industrial materials in Thailand.

With the addition of 2% (v/w) of methanol (99.5% purity) in jute bag, A. *flavus* was completely inhibited during first 6 days. After 10 days there was some infection and thereafter infection proceeded quickly and after 15 days of shelling 100% of kernels was infected. Although methanol inhibited the growth of A. *flavus* for more than 6 days, the added methanol gradually evaporated out under the tropical conditions and lost its inhibiting effect. Since the jute bag with methanol was covered with PE film throughout the experimental period, the cover might create favorable conditions for the growth of A. *flavus* and thus the infection might be so rapidly increased after 10–15 days. In our preliminary test, A. *flavus* infection of maize was low for 10 days in jute bag with the addition of 2% (v/w) methanol and covered with PE film for 2 days.

Since the wet maize was prevented from A. *flavus* infection for more than 6 days in jute bag with the addition of methanol, this method could be one of the possible applications to keep maize temporarily until drying. Methanol method is simple and will bring little changes in the present maize trading customs among Thai merchants.

After 5 days pH value of maize dropped from 4.90 to 4.00 which might be caused by
organic acid formation due to the growth of lactic acid bacteria. Later several yeast-like microorganisms become predominant in the plastic bag. Sour smell appeared after several days of storage, indicating occurrence of fermentation in the bag. The smell decreased during heat-drying.

AF production was controlled by several bacteria such as *Bacillus subtilis*, *Brevibacterium linens* and *Streptococcus lactis*. In the case of maize sterilized with γ-rays, however, 9, 4 and 0% of the kernels were found to be infected with *A. flavus* after 4, 15 and 20 days respectively (Fig. 1). Thus, irrespective of microorganism competition, *A. flavus* scarcely invaded into maize kernels in PE bag. It proved that, even without inhibitory activity of other microorganisms, changes of gas composition in the PE bag were strongly effective for controlling the development of *A. flavus* and the AF production.

AF contamination during storage is shown in Table 1. Maize samples in jute bag and fabricated PP bag were heavily contaminated with AF as inferred from the result in Fig. 1. In contrast, maize samples in PE bag and double plastic bag completely free from the AF contamination throughout the all experimental period. In γ-ray sterilized maize, there was little growth of *A. flavus* and so there was no AF detected. Methanol proved to have effectiveness for preserving maize from *A. flavus* infection for several days and AF contamination (Fig. 1) well coincided with the result.

In order to know more idealistic package material, further experiments would be expected in practical scale. Physical and economical suitability, along with some other factors arising from plastic bag (e.g. sour smell), would be estimated. In some cases, the best results could be derived from applying physical and chemical methods together. It would be hoped that any prevention method for AF contamination in maize would bring little changes in the present maize trading systems, otherwise the method would not be widely accepted.

### Table 1. Aflatoxin B1 content of maize stored in various packages

<table>
<thead>
<tr>
<th>Package</th>
<th>Aflatoxin B1 content (ppb)</th>
<th>Days of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Jute bag (control)</td>
<td>ND*4</td>
<td>ND</td>
</tr>
<tr>
<td>Jute bag covered with PE*1 film</td>
<td>ND</td>
<td>51</td>
</tr>
<tr>
<td>Jute bag covered with PE film; maize mixed with 2% (v/w) methanol</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PE bag</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PE bag; maize sterilized with γ-rays</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Fabricated PP*2 bag</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Double plastic bag (outside: fabricated PP; inside: high density PE*3)</td>
<td>ND</td>
<td>ND</td>
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*1 PE: polyethylene (125 μm thick); *2 PP: polypropylene (500 μm thick); *3 high density PE: 40 μm thick; ** ND: not detected.
The study was conducted as part of the collaborative researches on "Quality Prevention of Maize by the Prevention of Aflatoxin Contamination" between the Department of Agriculture of Thailand and the Tropical Agriculture Research Center (TARC) of Japan.

References