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# THE EFFECTS OF DRYING AND SHELLING ON *ASPERGILLUS FLAWS* INFECTION AND AFLATOXIN PRODUCTION OF MAIZE*

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**ABSTRACT**

The effects of drying and shelling on *Aspergillus fla\-us* infection and aflatoxin production of maize stored under laboratory conditions were investigated together with the intactness of grain and change of moisture content during the storage period.

Fully matured maize var. Arjuna and CPI-2 were harvested at 90 and 97 days after planting, respectively, after which they were unhusked and divided into 4 pans. The 1st and the 2nd parts were sun dried up to 20^ moisture content (m.c.) and then shelled and re-dried up to 17 and *14%* m.c.. respectively. The 3rd part was sun dried up to 17% m.c. and then shelled but not re-dned. The 4th pan was sun dried up to 17% m.c. and then shelled and re-dried up to 14% m.c. The maize was sun dried by spreading either the cobs or the kernels on the paved floor.

The nail-down wood and mechanical sheller were used for shelling the maize. After drying and shelling, maize samples were stored in the jars which were covered with muslin cloth for 3 months under laboratory conditions.

*A. flavus* was isolated using dilution method on Aspergillus Flavus and Parasiticus Agar (AFPA). The damaged kernel analysis was carried out at the beginning of storage to obtain the percentage of damaged kernel caused by shelling. The m.c. and aflatoxin were determined using oven and High Performance Liquid Chromatography (HPLC) methods, respectively.

The m.c. decreased at 1 month of storage and then it was almost constant at 2 and 3 months of storage.

The percentage of damaged kernels of maize var. CPI-2 was higher than those of var. Arjuna. The percentage of damaged kernels of maize shelled at 20% m.c. was higher than that shelled at 17% m.c. The percentage of damaged kernels of maize shelled by mechanical sheller was higher than that shelled by nail-down wood.

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Population of *A. flavus* on maize var. Arjuna was higher than that of var. CPI-2. The population on maize stored at the initial m.c. of 17% was higher than that of 14%, The population on maize shelled by mechanical sheller was higher than that shelled by nail-down wood, but there was no significant difference. The population increased at 1 and 2 months of storage and then decreased at 3 months of storage.

Total aflatoxin Bi content of maize var. CPI-2 was higher than that of var. Arjuna. The content of maize dried up to 17% m.c. and then shelled but not re-dried was the lowest compared with the other methods of drying. The content of maize shelled by nail-down wood was not significantly different than shelled by mechanical sheller. The content increased with the increase of storage duration.

**Key words:** Drying/Shelling/X5perg///MJ/Zav«j/Aflatoxin

## INTRODUCTION

Before and after harvest, maize *(Zea mays)* could be infected by *Aspergillus flavus,* a fungus that can produce aflatoxin (Butler 1974; Lillehoj and Hesseltine 1977). The most notorious toxin is aflatoxin, as it is an extremely potent carcinogen affecting several animal species. Cole *et al.* (1982) reported that among mycotoxins, aflatoxin was often found in maize.

Survey conducted *by* BIOTROP team in 1992 revealed that 35 maize samples collected from farmers and traders in Lampung Province contained 23-367 ppb of aflatoxin. Thirty samples contained aflatoxin of more than 30 ppb (Dharmaputra *et al* 1993).

Another survey conducted during 1993/94 showed that 108 maize samples collected from **fanners** in Central Lampung and Kediri regencies contained 5-291 and 9-283 ppb of aflatoxin BI during dry and wet seasons, respectively. Of these, 87 samples contained more than 30 ppb of aflatoxin B1. While 32 maize samples collected from **traders** in the two regencies contained 10-104 and 21-115 ppb of aflatoxin Bl during dry and wet seasons, respectively, 26 of which contained more than 30 ppb (Dharmaputra *et al. 1994).*

The high level of aflatoxin content in most maize samples could be caused by the methods of handling that were not carried out properly.

Limits on the amount of aflatoxins which are permitted in foods vary between countries and products. Most European countries have moved towards a limit of below 30 ppb for total aflatoxin concentration in all foods and less than 0.05 ppb of M aflatoxin hi milk (Gilbert 1991). In Asia, Taiwan tolerates up to 50 ppb of aflatoxin Bi in cereals and peanuts, while Thailand has a tolerance of 20 ppb for B and G aflatoxins hi all foods and Japan 10 ppb tolerance for B aflatoxin in all foods. The Philippines has a 20 ppb tolerance for aflatoxin Bi in coconut and peanut products for export (Van Egmond 1991).

Postharvest handling (among others, drying and shelling) could affect fungal infection. In general, the moisture content of freshly harvested maize is still high, making it a good substrate for fungal growth. Shelling could cause mechanical damage, and fungal spores can infect the kernel through the damage. Consequently, the kernels should be shelled using a proper tool at certain moisture content to reduce the damage of kernels.

According to Covanich (1991) drying is particularly a vital operation in gram handling chain, since moisture is the most important factor determining the extent grains are liable to deterioration during storage. Dried grains are less susceptible to insect and mold attacks. Overdrying of grains in the sun, for example, can cause breakage, loss of viability, etc.

In general, the moisture content of freshly harvested maize was between 23-33%. Drying of maize was conducted up to 17-20% moisture content (SFCDP 1990). Most farmers in Lampung and East Java shelled cob of maize using mechanical sheller, while in South Sulawesi fanners use a nail-down wood (SFCDP 1990). According to Purwadaria (1987) the mechanized maize sheller could reduce the quantity of losses as much as 5% and lowered the shelling cost to 67-40% of the manual shelling cost.

The objectives of this study were : 1) to determine the effects of some methods of drying and shelling on *Aspergillus flavus* infection and aflatoxin production of maize stored under laboratory conditions; and 2) to determine the effect of drying and shelling on the intacmess of kernel, as well as the effect of storage period on the change of moisture content.

## MATERIALS AND METHODS

### Maize variety

Two maize varieties (Arjuna and CPI-2) were used hi this study. They were grown at the experimental plot of the Research Institute for Food Crop Biotechnology, Bogor, harvested at 90 and 97 days after planting and unhusked immediately after harvest.

### Drying and shelling

Cobs of maize were divided into 4 parts. The 1st and the 2nd parts were sun dried up to 20% moisture content (m.c.) and then shelled and re-dried up to 17 and 14% m.c., respectively. The 3rd part was sun dried up to 17% m.c. and then shelled but not re-dried. The 4th part was sun dried up to 17% m.c. then shelled and re-dried

up to 14% m.c. All of the maize samples were sun dried by spreading either the cobs or the kernels on the paved floor.

Nail-down wood and mechanical sheller type Yanmar TF 55-di with cylinder rotation of 500-700 rpm were used for shelling the maize.

### Storing of maize and method of sampling

After drying and shelling, 500 g of maize of each treatment were placed in 3.3 L jar covered with muslin cloth, and stored for 1, 2, and 3 months under laboratory conditions. Two replicates were used for each treatment. The ambient temperature and relative humidity of the storage were recorded using a Wilh. Lambrecht thermohygrograph type 252.

Initial sample was derived from each replicate (jar) at the beginning of storage, and then at 1, 2, and 3 months of storage. This sample was divided twice using a sample divider to obtain working samples for moisture content. damaged kernels, population of *A. flavus,* and total aflatoxin Bi content analyses.

### Moisture content, damaged kernels, population of A. *flavus* and alfatoxin Bi content analyses

Moisture content (based on wet basis) was determined using oven method at 130° C for 2 hours (BSI 1980). The analyses for damaged kernels were carried out at the beginning of storage to obtain the percentage of damaged kernel caused by shelling.

*A. flavus* was isolated using dilution method on Aspergillus Flavus and Parasi-ticus Agar (AFPA) (Pin and Hocking 1985). Aflatoxin content was determined using High Performance Liquid Chromatography (HPLC) method (Rodriquez and Mahoney 1994).

### Statistical Analysis

The data were analyzed using Completely Randomized Factorial Design with 4 factors. The 1st, 2nd, 3rd and 4th factors were maize variety, method of drying and shelling, and duration of storage, respectively.

## RESULTS AND DISCUSSION

### The effect of maize variety, methods of drying and shelling, and storage period on moisture content and damaged kernel

Based on statistical analysis, the effects of drying, duration of storage and their interaction gave very significant differences in moisture content, while variety and shelling did not give significant differences.

At the beginning of storage, moisture content on each drying method (I, II, III and IV) was 16.84, 14.10, 17.11 and 14.35%, respectively. At 1 month after storage they decreased (13.98, 13.51, 14.05 and 13.75%, respectively), and then they were almost constant at 2 and 3 months after storage (Table 1). Moisture content of maize at 2 and 3 months after storage would approach the equilibrium moisture content (EMC) with relative humidity of the storage. During storage m.c. of grains would move towards the EMC. According to Hall (1957), Henderson and Perry (1976), EMC was

Table 1. Moisture content of maize treated with different methods of drying during storage

| Drying method | Moisture content (%) | | | |
|---|---|---|---|---|
| | Duration of storage (month) | | | |
| | 0 | 1 | 2 | 3 |
| I | 16.84 a | 13.98 cd | 13.18 g | 13.08 g |
| II | 14.10 bc | 13.51 ef | 13.04 g | 13.02 g |
| III | 17.11 a | 14.05 bcd | 12.95 g | 13.21 g |
| IV | 14.35 b | 13.75 de | 12.99 g | 13.01 g |

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level

I = Cobs of maize were sun dried up to 20% moisture content, and then shelled and re-dried up to 17% moisture content.
II = Cobs of maize were sun dried up to 20% moisture content, and then shelled and re-dried up to 14% moisture content.
III = Cobs of maize were sun dried up to 17% moisture content, and then shelled but not re-dried.
IV = Cobs of maize were sun dried up to 17% moisture content, and then shelled and re-dried up to 14% moisture content.

reached when the grains did not absorb or release vapor any more. Brooker *et al.* (1974) reported that EMC of grains were affected by temperature, humidity, variety and maturity of grains. Range of temperature and relative humidity of the storage were 21.75-29.25°C and 47.88-88.25%, respectively. (Table 2).

The effects of maize variety, drying and shelling gave very significantly differences to **damaged kernels,** while their interaction was not significantly different.

Table 2. Range of temperature and relative humidity in the storage room

| Duration of storage (month) | Temperature (C) | Relative humidity (%) |
|---|---|---|
| 1 | 21.75 - 28.38 | 61.00 - 88.25 |
| 2 | 21.75 - 28.38 | 61.00 - 88.25 |
| 3 | 21.75 - 29.25 | 47.88 - 88.25 |

The percentage of damaged kernels of maize var. CPI-2 (5.0%) was higher than that of var. Arjuna (3.6%) (Table 3). Based on visual observation, the kernels of var. CPI-2 were bigger and less solid than var. Arjuna, therefore it could be more easily broken during shelling.

Table 3. the effect of maize variety, methods of drying and shelling on damaged kernels at the beginning of storages *

| Effect | Damaged kernels (%) |
|---|---|
| **Maize variety** | |
| Arjuna | 3.6 a |
| CPI-2 | 5.0 b |
| **Method of drying** | |
| I | 4.7 cd |
| II | 5.4 c |
| III | 3.7 de |
| IV | 3.2 e |
| **Method of shelling** | |
| Nail-down wood | 2.9 f |
| Mechanical sheller | 5.7 g |

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level

*Damaged kernels analysis was carried out only at the beginning of storage

I = Cobs of maize were sun dried up to 20% moisture content, and then shelled and re-dried up to 17% moisture content.
II = Cobs of maize were sun dried up to 20% moisture content, and then shelled and re-dried up to 14% moisture content.
III = Cobs of maize were sun dried up to 17% moisture content, and then shelled but not re-dried.
IV = Cobs of maize were sun dried up to 17% moisture content, and then shelled and re-dried up to 14% moisture content.

The percentage of damaged kernels of maize shelled at 20% m.c. (drying methods I and II) (4.7 and 5.4%, respectively) was higher than maize shelled at 17% m.c. (drying methods HI and IV) (3.7 and 3.2%, respectively). According to SFCDP (1990), in general, the percentage of damaged kernels increased if the m.c. was more than 18%. IDRC (1988) reported that the maize quality deteriorated more when the shelling process took place directly after harvest at a high moisture level of grain (around 37% wb) compared to the process where maize shelling was done after the initial sun drying which brought the grain moisture content down to around 25% wb.

The percentage of damaged kernels of maize shelled by mechanical shelter (5.7%) was higher than that shelled by nail-down wood (2.9%). Shelling of each cob of maize using a nail-down wood did not cause friction among the cobs, while shelling using mechanical shelter created a friction. Moreover, shelling done by manpower

using a nail-down wood can be controlled to reduce the friction between sheller and maize. According to Suprayitno (1980), the percentage of damaged kernels of maize shelled using mechanical sheller was high, because there was a friction among intact kernels, between intact kernels and the cylinder of mechanical sheller.

**The effect of maize variety, methods of drying and shelling, and storage duration on population of *Aspergillus flavus***

Based on statistical analysis, the effects of maize variety gave significant differences to population of *A. flavus;* drying and duration of storage gave very significant differences; while shelling and interaction among maize variety, drying, shelling and duration of storage did not give significant differences.

Population of *A. flavus* on maize var. Arjuna (697.2 colonies/g) was higher than that of var. CPI-2 (306.4 colonies/g) (Table 4). Wilson *el al.* (1983) reported that the growth of *A. flavus* and aflatoxin production were affected by some metabolites (groups of alcohol and aldehyde) produced by maize.

Population of *A. flavus* on maize stored at the initial m.c. of 17% (drying methods I and III) (570.9 and 1224.4 colonies/g) was higher than that of 14% (drying methods II and IV) (84.2 and 127.7 colonies/g) (Table 4). Christensen and Kaufmann (1974) reported that the minimum m.c. for the growth of *A. flavus* on maize was 18-18.5%. According to Kawashima and Kawasugi (1988). grain which was sun dried immediately after shelling on concrete drying floor with m.c. less than or around 15% could be stored with low contamination of *A. flavus* (1.5-8%) for 56 days in middle-men's storage. If the grains (with m.c. more than 20%) were not dried after shelling, *A. flavus* contamination prevailed quickly. However, if m.c. was less than 17% the development of *A. flavus* could be inhibited.

Population of *A. flavus* on maize shelled by mechanical sheller (573.4 colonies/g) was higher than shelled by nail-down wood (430.2 colinies/g), but it did not give significant difference (Table 4). Also the percentage of damaged kernels shelled by mechanical sheller was higher than those shelled by a nail-down wood. The damaged kernels were more easily infected by *A. flavus*. Dharmaputra *et al.* (1994) reported that there was a positive correlation between the damaged kernels and the percentage of kernels infected by *A. flavus* of maize obtained by farmers and village traders in Central Lampung and Kediri regencies.

Population of *A. flavus* increased at 1 and 2 months of storage, from 48.5 colonies/g to 586.8 and 890.7 colonies/g, respectively, because the m.c. of grain, temperature, relative humidity of storage and nutrition content of grams still supported the fungal growth. Garraway and Evans (1984) revealed that the growth and development of fungi was very much affected by nutrition contents and substrate. According to

Chatterjee *et al.* (1990) *A. flavus* growth was highly and linearly correlated with the decrease in the percentage of healthy germ and germinability, root-shoot growth, total carbohydrate and protein of grain. Pomeranz (1992) reported that during storage deterioration of grain occurred, consequently nutrients were lost because of changes among others in carbohydrates. However, at 3 months of storage, population of *A. flavus* (481.1 colonies/g) decreased (Table 4). It was assumed that m.c., relative humidity and nutrition contents decreased and there were competitive fungi to *A. flavus.* According to Christensen (1955), Mills and Abramson (1982), one kernel can be infected by more than one fungal species. In this study, the predominant fungi in addition to *A. flavus* were *Acremonium strictum, Fusarium moniliforme* and *Penicillium citrinum.* Zummo and Scott (1992) reported that *F. moniliforme* inhibited *A. flavus* infection and aflatoxin production on maize.

Table 4. The effect of maize variety, methods of drying and shelling, and duration of storage on population of *Aspergillus flavus*

| Effect | Population of *A. flavus* (colonies/g) | |
|---|---|---|
| | Not transformed | Transformed into log *A. flavus* + 1 |
| **Maize variety** | | |
| Arjuna | 697.2 | 4.662 a |
| CPI-2 | 306.4 | 4.096 b |
| **Method of drying** | | |
| I | 570.9 | 5.068 c |
| II | 84.2 | 3.589 d |
| III | 1224.4 | 5.386 c |
| IV | 127.7 | 3.474 d |
| **Method of shelling** | | |
| Nail-down wood | 430.2 | 4.301 e |
| Mechanical sheller | 573.4 | 4.457 e |
| **Duration of storage (month)** | | |
| 0 | 48.5 | 3.040 f |
| 1 | 586.8 | 4.758 g |
| 2 | 890.7 | 4.984 g |
| 3 | 481.1 | 4.735 g |

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level

I = Cobs of maize were sun dried up to 20% moisture content, and then shelled and re-dried up to 17% moisture content.
II = Cobs of maize were sun dried up to 20% moisture content, and then shelled and re-dried up to 14% moisture content.
III = Cobs of maize were sun dried up to 17% moisture content, and then shelled but not re-dried.
IV = Cobs of maize were sun dried up to 17% moisture content, and then shelled and re-dried up to 14% moisture content.

## The effect of maize variety, methods of drying and shelling, and storage duration on total aflatoxin $B_1$ content

According to Heathcote and Hibbert (1978) production of aflatoxin was affected by strain of fungi, relative humidity, temperature, oxygen, carbohydrate and kind of commodities.

In this study, four kinds of aflatoxins were determined, i.e. aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$

Based on statistical analysis, the effects of maize variety, drying, interaction between drying and shelling and interaction among maize variety, shelling and duration of storage gave significant differences to total aflatoxin $B_1$ content; duration of storage gave very significant difference, while interaction among maize variety, drying, shelling and duration of storage did not give significant difference.

Total aflatoxin Bi content of maize var. CPI-2 (35.97 ppb) was higher than that of var. Arjuna (32.36 ppb) (Table 5). This could be attributed to the effect of metabolites produced by maize.

Total aflatoxin Bi content of maize dried up to 17% m.c. then shelled but not re-dried (30.34 ppb) was the lowest compared with the other methods of drying (drying methods I. II and IV) (36.73, 34.79 and 34.60 ppb, respectively) (Table 5), although the population of *A. flavus* of maize was the highest. According to Diener and Davis (1969) aflatoxin production depended on the strain of *A. flavus.*

Total aflatoxin BI content of maize shelled by nail-down wood (32.78 ppb) was lower and not significantly different with that shelled by mechanical sheller (35.45 ppb) (Table 5). It was assumed that the high damaged kernels caused by shelling using mechanical sheller were more easily infected by *A. flavus,* consequently, aflatoxin producing strains had more chance to infect the kernels.

Total aflatoxin $B_1$ content of maize increased with longer storage. At the beginning of storage, it was 0.49 ppb, while at 1, 2 and 3 months of storage they were 8.19, 50.83 and 76.95 ppb, respectively, because aflatoxin could not be easily decomposed, thus, it accumulated. Melting points of aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ were more than 230 C, therefore, they cannot be decomposed by ordinary heating (Betina 1989).

As a secondary metabolite, aflatoxin production was dependent on stadia of fungal growth, aflatoxin biosynthesis pathways and on factors affecting enzymatic reaction. According to Davis and Diener (1983) aflatoxin production is as follows: as fungal growth and primary metabolism proceed, little or no aflatoxin was formed initially. Eventually, phosphate, nitrogen, or some trace elements become limited and primary growth is retarded. As primary metabolism becomes disorganized, various primary metabolites accumulate. Metabolites such as pyruvate, malonate, acetate, various amino acids, etc. trigger the development and stimulate the activity of en-

Table 5. The effect of maize variety, methods of drying and shelling, and duration of storage on total aflatoxin $B_1$ content

| Effect | Total aflatoxin $B_1$ content (ppb)* |
|---|---|
| **Maize variety** | |
| Arjuna | 32.36 a |
| CPI-2 | 35.97 b |
| **Method of drying** | |
| I | 36.73 c |
| II | 34.79 c |
| III | 30.34 d |
| IV | 34.60 c |
| **Method of shelling** | |
| Nail-down wood | 32.78 e |
| Mechanical sheller | 35.45 e |
| **Duration of storage (month)** | |
| 0 | 0.49 f |
| 1 | 8.19 g |
| 2 | 50.83 h |
| 3 | 76.95 i |

Numbers followed by the same lener do not differ significantly according to Duncan's Multiple Range Tea at 95 % confidence level Aflatoxin $B_1$. $B_2$. $G_1$ and $G_2$ contents were convened into total aflatoxin $B_1$

I = Cobs of maize were sun dried up to 20% moisture content, and then shelled and re-dried up to 17% moisture content.
II = Cobs of maize were sun dried up to 20% moisture content, and then shelled and re-dried up to 14% moisture content.
III = Cobs of maize were sun dried up to 17% moisture content, and then shelled but not re-dried.
IV = Cobs of maize were sun dried up to 17% moisture content, and then shelled and re-dried up to 14% moisture content.

zymes of secondary metabolism, such as those of the polyketide biosynthetic pathway that in turn promote aflatoxin biosynthesis over fatty acid biosynthesis in toxigenic isolates *of A. flavus*. According to Betina (1984) there were acetyl Co-A and malonyl Co-A in aflatoxin biosynthesis. There are active ingredients of acetate and malonate which compose lipid.

Aflatoxin arises naturally when a toxin-producing strain of *A. flavus* grows on a substrate where environmental conditions are suitable for the development of the fungus. In view of the fact that *A. flavus* was ubiquitous and capable of development over a wide range of temperature on substrates of high carbohydrate content (Heathcote and Hibbert 1978).

## CONCLUSIONS

1. Moisture contents of maize decreased at 1 month of storage, and then they were almost constant at 2 and 3 months of storage.
2. Maize var. CPI-2 was more resistant than var. Arjuna to *Aspergillus flavus* infection, although the percentage of damaged kernels of maize var. CPI-2 was higher than those of var. Arjuna.
3. In general, the best drying method was as follows: maize was sun dried up to 17% m.c., and then shelled and re-dried up to 14% m.c.
4. *A. flavus* population of maize shelled by mechanical sheller was insignificantly different with nail-down wood, although the percentage of damaged kernels of maize shelled by mechanical sheller was higher than that shelled by nail-down wood.
5. Population of *A. flavus* increased at 1 and 2 months of storage, and then decreased at 3 months of storage.
6. Total aflatoxin Bi content increased with the increase of storage period.

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