# Determining appropriate postharvest handling method to minimize fungal infection and aflatoxin contamination in nutmeg (*Myristica fragrans*)

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# Article history

# <u>Abstract</u>

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# <u>Keywords</u>

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Nutmeg (Myristica fragrans) or fragnant nutmeg is an important commodity that has been used in the food and pharmaceutical industries, hence its quality should be monitored. The objectives of this study were (1) to investigate the effect of postharvest handling of nutmeg, i.e. origin, drying and storage on the quality of nutmeg, in terms of fungal infection (including Aspergillus *flavus*) and total aflatoxin content, (2) to recommend proper (appropriate) postharvest handling method (Good Handling Practice) of nutmeg to ensure its good quality during storage. Nutmeg fruits were obtained using two methods, i.e. harvesting nutmeg by picking ripe fruits from the tree and collecting those which had fallen on the ground. Drying of nutmeg was conducted using sun-drying on tarpaulin which was put on the ground or smoke-drying until its moisture content was reduced by 10%. Nutmeg was stored for two and four months under warehouse conditions. The results showed that appropriate postharvest handling method of nutmegs could ensure their good quality in terms of the percentage of damaged kernels, fungal infection and aflatoxin contamination, i.e. nutmeg should be harvested directly from the tree, dried using smoke-drying and stored with its shell. Nutmeg obtained by picking should not be mixed with fallen fruits which have been in contact with the soil. The highest A. flavus infection and aflatoxin contamination were found in nutmeg that had been fallen on the ground.

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# Introduction

Nutmeg (Myristica fragrans) or fragrant nutmeg is an important commodity widely used in the food and pharmaceutical industries, hence its quality should be monitored (Punnathara, 2011). Nutmeg is native to the Moluccas Islands of Indonesia, but nowadays it is also grown in Penang Island in Malaysia, in the Caribbean (particularly Grenada), in the southern state of Karela in India, and in the island of Zanzibar.

Based on the statistical data of Directorate General of Estate Crops in Indonesia, in 2008 the area planted with nutmeg was 75 062 ha. The distribution area of nutmeg covered 19 provinces. The largest plantation area of nutmeg was in North Moluccas (33%), followed by Nanggroe Aceh Darussalam or NAD (23%), North Sulawesi (18%), Moluccas (12%), West Java (5%), and the rest (9%) in other provinces. Indonesia contributes 75% (8 943 tonnes) of nutmeg production in the world (Novarianto, 2010).

According to CBI (2015) Indonesia and Grenada dominate production and export nutmeg to European countries with world market shares of 75 and 20% respectively. India, Malaysia, Papua New Guinea, Sri Lanka, and Caribbean islands such as St.Vincent are also producers and exporters of nutmeg.

During postharvest period (including storage), nutmeg could be infested by insects and microorganisms. Among microorganisms, fungi are the most important cause of deterioration of stored foodstuff. Fungal infection in foodstuff can cause discolouration, decrease in physical quality and nutritional contents, and mycotoxin contamination. Aflatoxins are toxins produced by certain fungi, such as Aspergillus flavus and A. parasiticus. Aflatoxins are considered dangerous due to their association with various diseases in humans and animals such as aflatoxicoses and liver cancer. There are four naturally occurring aflatoxins in many commodities, i.e. aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. The most common and toxic of aflatoxins is aflatoxin B<sub>1</sub> (Basappa, 2009). According to FAO (2004) European Union has determined Maximum Tolerable Limits (MTL) of aflatoxin B<sub>1</sub> and total aflatoxins in nutmeg as 5 and 10 ppb, respectively.

Dharmaputra *et al.* (2015) reported, that postharvest handling method of nutmeg conducted by farmers and collectors in North Sulawesi province was not appropriate. As postharvest handling method of nutmeg can affect the quality of nutmeg, it is important to conduct research on the effect of some methods of postharvest handling on the quality of nutmeg, especially on fungal infection and aflatoxin contamination.

The objectives of the research were : 1) To investigate the effect of postharvest handling (harvesting method of nutmeg fruit, drying and storage) methods on the quality of nutmeg, in terms of fungal infection (including *Aspergillus flavus*), and aflatoxin contamination. Moisture content and the percentage of damaged kernels were also determined, because they affect fungal infection and aflatoxin contamination. 2) To recommend proper (appropriate) postharvest handling method (Good Handling Practice) to ensure good quality of nutmeg during storage.

# **Materials and Methods**

# Time and location of research

Collection of nutmeg fruits and drying of nutmeg with its shell were conducted in the location where nutmeg was planted, i.e. in Kauditan Subdistrict, Minahasa Regency, North Sulawesi Province. Storage of nutmeg was took place in a warehouse located in the Municipality of Bitung, North Sulawesi Province. The determination of moisture content, the percentage of damaged kernels, the population of each fungal species infecting kernels, and aflatoxin content were conducted at SEAMEO BIOTROP, Bogor.

# Collection of nutmeg fruits, drying and shelling of nutmeg

Nutmeg fruits were obtained using two methods, i.e. harvesting nutmeg by picking ripe fruits from the tree and collecting those that had fallen on the ground. The pulp of fruit and the mace were separated from the whole nutmeg seed. Nutmeg was then dried using sun-drying on a tarpaulin or smoke-drying. Sun-drying method was conducted for 16 days until the moisture content of nutmeg was reduced by 10%. The smoke-drying method was preceded by sundrying for one day, followed by smoke-drying for 13 days, until the moisture content of nutmeg reduced by 10%. One portion of nutmegs was still in shell, while another portion was without shell. Shelling of nutmeg was conducted using a wooden stick.

# Packaging and storing of nutmeg

Nutmeg in shell and nutmeg without shell were packed in gunny bags. Each bag contained 1.35 kg of nutmegs in shell and 1.50 kg of nutmegs without shell. In three replicates, each bag was subjected to the following treatments: (a) harvesting of nutmegs by picking ripe fruits from the tree or collected from the ground, (b) drying methods (sun-drying or sundrying followed by smoke-drying), (c) nutmeg in or without shell, and (d) storage duration. Nutmegs were stored for two and four months under warehouse conditions. Thus, the number of experimental units was 72, i.e. 2 origin of nutmeg fruit x 2 drying methods x 2 nutmeg in or without shell x 3 storage durations x 3 replications. The temperature and relative humidity in the storage room were recorded using a thermohygrograph.

# Sampling and obtaining working samples

Sampling of nutmeg was conducted at the beginning of storage, subsequently after two and four months of storage. As much as 72 gunny bags containing nutmegs were packed in SEMAR hermetic plastic bags in order to prevent the change of moisture content during transportation from the locations of sampling to Bogor. Nutmegs in shells were not shelled until they arrived in Bogor.

Insects found in nutmeg were separated from nutmeg using a sieve, they were then preserved in vials containing 70% ethanol. Each sample of nutmeg derived from a bag was mixed thoroughly, then it was divided into four parts, i.e. one part was used to determine the percentage of damaged kernels, while the other three parts for the determination of moisture content, fungal population and aflatoxin content. Nutmegs in shell were shelled using a wooden stick to get its kernels. The three parts were ground using Mill Powder Tech Model RT 04 and mixed thoroughly, they were then divided into eight parts to obtain working samples, i.e. one part for the determination of moisture content, three parts for determination of fungal population, and four parts for the determination of total aflatoxin content.

# Determination of moisture content, percentage of damaged kernels, fungal population, and aflatoxin content

Moisture content of nutmeg kernels (based on wet basis) was determined using distillation method (SNI,1993). Two replicates were used for each sample. Damaged kernels included shrivelled, cracked, and broken kernels, mouldy and insect damaged kernels. The percentage of damaged kernels was determined using the following formula:

The percentage of damaged kernels (% wet basis)

= Weight of damaged kernels (g) x 100%

Weight of working sample used for damaged kernel analyses (g)

Storage duration (month) Treatment 2 0 Δ PADC 8.8 ± 0.7 bcdef 9.8 ± 0.5 ab 6.9 ± 0.3 jj PATC 10.3 ± 0.5 a 9.0 ± 0.3 bcd 8.0 ± 0.3 defghi PJDC 7.5 ± 0.2 ghij 8 8 + 0 4 bcdef 7.8 ± 0.3 efahij 7.0 ± 0.3 hij PJTC 7.0 ± 0.2 hij 8.1 ± 0.3 cdefah TADC 8.9 ± 0.6 bcde 8.0 ± 0.2 cdefgh 8.3 ± 0.4 cdefg TATC 7.4 ± 0.4 ghij 7.7 ± 0.2 fghij 6.8 ± 0.2 j TJDC 9.2 ± 0.6 abc 9.7 ± 0.3 ab 7.8 ± 0.3 efghij TJTC 8.9 ± 0.1 bcde 8.8 ± 0.2 bcdef 7.0 ± 0.2 hij

Table 1. Moisture content of nutmeg caused by various treatments during storage

Numbers followed by the same letter do not differ significantly according to Tukey Test at 95% confidence level.

Notes :

- P = nutmeg fruit picked from the tree
- T = nutmeg fruit collected from the ground
- A = nutmeg dried using smoke- drying method
- J = nutmeg dried using sun-drying method
- DC = nutmeg in shell
- TC = nutmeg without shell

Fungi were isolated using serial dillution method, followed by pour plate method on Dichloran 18% Glycerol Agar (DG18) (Hocking and Pitt, 1980, Pitt and Hocking, 2009). Each fungal species was identified using Pitt and Hocking (2009) as the main reference. Aflatoxin contents were determined using High Performance Liquid Chromatography (HPLC) method (VICAM, 2007). Two replicates were used for each sample.

#### Statistical analysis

The data were analyzed using Completely Randomized Factorial Design with four factors. The first, second, third, and fourth factor were the harvesting method of nutmeg fruit, nutmegs in and without shell, drying methods and storage durations, respectively.

# **Results and Discussion**

# *Moisture content*

Moisture content of foodstuff is one of the important factors that affected the deterioration of foodstuff during storage. High moisture content will give an opportunity for fungal growth. SNI (1993) determines 10% as the maximum moisture content of nutmegs during storage. Based on the analyses of variance, interaction between harvesting method of nutmeg fruit, nutmeg in or without shell, drying method and storage duration gave very significant differences in moisture content of nutmeg.

The pattern of moisture content of nutmeg caused

by various treatments was relatively similar during storage (Table 1). The moisture content of nutmeg decreased after 4 months of storage. In general the moisture content of nutmeg during storage were lower (6.8-9.8%) than maximum limit of moisture content determined by SNI (1993) i.e. 10%, except the moisture content of nutmeg harvested by picking fruit from the tree, drying using smoke-drying and without shell (10.3%). Moisture content is always in equilibrium with the relative humidity in the storage room. It is also affected by the temperature of storage room. In this study, the mean and range of temperature and relative humidity in the storage room decreased after 4 months of storage. Mean and range of temperature and relative humidity in the storage room at 0-2 months of storage were  $29.2 \pm 1.3$  °C  $(28.0 - 32.0^{\circ}C)$  and  $73.6 \pm 3.3\%$  (67.0 - 77.2%), respectively; while at 2 - 4 months of storage they were  $28.4 \pm 1.9^{\circ}$ C (24.2 - 32.8°C) and 72.1 ± 5.8% (54.9 - 83.9%), respectively.

# Percentage of damaged kernels

SNI (1993) determines damaged kernels including damages caused by insect and fungal attacks, cracked, broken and shriveled kernels. In this study, damaged kernels including shrivelled, cracked and broken kernels were only determined at the beginning of storage, because shrivelled kernels were caused by early harvesting of nutmeg fruits and they were not affected by the duration of storage. Cracked and broken kernels were due to shelling using a wooden stick.

Damaged kernels at the beginning of storage (%)		Damaged kernels caused by insect infestation (%)		
Shrivelled kernel	Cracked and broken	Storage duration (month)		
	kernel	0	2	4
30.1	2.6	0	0	5.6 ± 3.8
25.5	5.0	0	0	19.6 ± 17.5
27.5	0.6	0	0	1.7 ± 2.9
33.3	11.6	0	2.3 ± 1.1	10.5 ± 8.6
23.4	2.7	13.5 ± 2.4	15.7 ± 0.6	24.0 ± 0.0
16.8	7.6	15.2 ± 0.0	16.6 ± 0.2	20.2 ± 2.2
17.4	12.9	15.1 ± 1.1	28.5 ± 10.2	10.9 ± 6.4
24.8	12.0	12.8 ± 1.6	18.6 ± 0.8	39.5 ± 16.2
	Damaged kernels at th Shrivelled kernel 30.1 25.5 27.5 33.3 23.4 16.8 17.4 24.8	Damaged kernels at the beginning of storage (%)           Cracked and broken kernel           30.1         2.6           25.5         5.0           27.5         0.6           33.3         11.6           23.4         2.7           16.8         7.6           17.4         12.9           24.8         12.0	Damaged kernels at the beginning of storage (%)         Damaged kernel           Cracked and broken         S           kernel         0           30.1         2.6         0           25.5         5.0         0           27.5         0.6         0           33.3         11.6         0           23.4         2.7         13.5 ± 2.4           16.8         7.6         15.2 ± 0.0           17.4         12.9         15.1 ± 1.1           24.8         12.0         12.8 ± 1.6	Damaged kernels at the beginning of storage (%)         Damaged kernels caused by inserved and broken kernel           Shrivelled kernel         Cracked and broken kernel         Storage duration (m           30.1         2.6         0         0           25.5         5.0         0         0           27.5         0.6         0         0           33.3         11.6         0         2.3 ± 1.1           23.4         2.7         13.5 ± 2.4         15.7 ± 0.6           16.8         7.6         15.2 ± 0.0         16.6 ± 0.2           17.4         12.9         15.1 ± 1.1         28.5 ± 10.2           24.8         12.0         12.8 ± 1.6         18.6 ± 0.8

Table 2. Percentages of damaged kernels of nutmeg at the beginning of storage and percentage of damaged kernels of nutmeg caused by insect infestation in various treatments during storage

Note :Based on analysis of variance, the effect of drying method, nutmeg in shell or without shell, and duration of storage did not show any significant differences on the percentage of damaged kernel caused by insect infestation.

The percentage of shrivelled kernels originating from fruit picked from the tree (25.5-33.3%) was higher than that of nutmegs that fell on the ground (16.8-24.8%). It was assumed that nutmegs picked from the tree were younger than those that fell on the ground. The percentage of cracked and broken kernels originating from fruit picked from the tree was relative similar to those which had fallen on the ground. The percentage of shrivelled, cracked and broken kernels at the beginning of storage is presented in Table 2.

The percentage of damaged kernels caused by fungal and insect attacks was determined at each period of sampling. Based on the analyses of variance (the data were transformed into Acresin), nutmeg with or without shell, drying method and storage duration gave very significant differences in the percentage of damaged kernels caused by fungal infection, while the harvesting method of nutmeg fruit did not give any significant differences. There was no interaction among the four treatments. The pattern of percentage of damaged kernels caused by fungal infection during storage was relatively similar in various treatments.

The percentage of damaged kernels caused by fungal infection in nutmeg dried using sun-drying (28.9 ± 6.4%) was higher and significantly different from nutmegs dried using smoke-drying (26.3 ± 6.3%). The percentage of damaged kernels caused by fungal infection in nutmeg in shell (26.1 ± 5.7%) was lower and significantly different from that nutmeg without shell (29.0 ± 6.8%). The percentage of damaged kernels caused by fungal infection at the beginning of storage, after two and four months of storage were  $23.9 \pm 4.4$ ,  $28.9 \pm 6.0$  and  $29.9 \pm 6.0\%$ , respectively. The percentage of damaged kernels caused by insect infestation in nutmeg in various treatments increased with the increase of storage duration (Table 2). The kernels damaged by insect infestation in nutmegs originating from fruit that fell on the ground were found since at the beginning of storage, while in nutmegs originating from fruit picked from the tree, they were found after 4 months of storage (Table 2).

In this study, the dominant insect species found in nutmegs after 4 months of storage were Araecerus fasciculatus, Carpophilus sp., Oryzaephilus and surinamensis, Tribolium castaneum. Dharmaputra et al. (2013) reported that a significant number of holes caused by insect infestation were found in nutmegs originating from nutmeg fruit that fell on the ground. According to Haines (1991) A. fasciculatus is the most important insect infesting spices including nutmeg. A research by Childers and Woodruff (1980) found that A. fasciculatus is the primary insect pest in stored products such as nutmeg in North and South America.

### Fungal population

As many as 15 fungal species were isolated from nutmegs subjected to various treatments. *Aspergillus niger, Eurotium chevalieri* and *Penicillium citrinum* were often found in nutmeg samples (Table 3). *Aspergillus flavus* was only found in nutmeg that fell on the ground and was dried using sun-drying.

Ichinoe *et al.* (2006) stated that *Eurotium* spp. were the predominant fungi found in 12 of powdered nutmeg samples collected from retailers in Indonesia. *Aspergillus flavus* (1.0 x  $10^2$  CFU/g) was detected in one sample. According to Mandel (2005) peeled seeds of nutmeg imported from India, Sri Lanka, Indonesia and Brazil were infected by

5	1	0
J	+	7

Table 3. Fungal population in nutmegs subjected to various treatments during storage

		Fungal population (CFU/g wet basis)		
Treatment	Fungi	Storage duration (month)		
		0	2	4
PADC	Aspergillus flavus	0	0	0.20 x 10
	A. niger	0.30 x 10	0	0.10 x 10
	- A. tamarii	0	0	0.10 x 10
	A penicillipides	- 0.42 x 10 <sup>2</sup>	0	0
	Cladosporium	0.42 × 10	0	0
	cladosponum	0.30 x 10	0	0.64 x 10 <sup>2</sup>
	ciadosporioides			
	Eurotium chevalieri	0.10 x 10	0.11 x 10°	0
	Fusarium solani	0.20 x 10	0	0
	Penicillium citrinum	0.60 x 10	0.13 x 10 <sup>2</sup>	0.30 x 10
	P. thomii	0	0	0.16 x 10 <sup>2</sup>
PATC	A. niger	0.20 x 10	0.30 x 10	0.20 x 10
	A. penicillioides	0	0	2.51 x 10 <sup>2</sup>
	A. tamarii	0	0	0.10 x 10
	A. versicolor	0	0.68 x 10 <sup>2</sup>	0
	C. cladosporioides	0	0	0.17 x 10 <sup>2</sup>
	E. chevalieri	0	0.58 x 10 <sup>2</sup>	0.20 x 10
	Penicillium citrinum	0.10 x 10	0	0
	Svncephalastrum			
	racemosum	0	0	0.10 x 10
PJDC	A. niger	0.12 x 10 <sup>4</sup>	0.10 x 10	0.10 x 10
	A. wentii	0	0.10 x 10	0
	C. cladosporioides	0	0	0.30 x 10
	Endomyces fibuliger	0.49 x 10 <sup>4</sup>	0	0
	Eurotium chevalieri	0.98 x 10 <sup>4</sup>	0	0.10 × 10
	E solani	$0.10 \times 10^{4}$	0	0
	P. odrigum	0.19 × 104	0.10 × 10	0.10 × 10
	P. cannum	0.18 x 10	0.10 x 10	0.10 x 10
PJTC	A. flavus	0.80 x 10	0	0.20 x 10
	A. niger	0.40 x 10 <sup>2</sup>	0.13 x 10 <sup>2</sup>	1.04 x 10 <sup>2</sup>
	A. tamanii	0	0.30 x 10	0.10 x 10
	E. chevalieri	0	1.35 x 104	0.30 x 10
	E. fibuliger	0.10 x 10	0	0
	F. solani	0.10 x 10 <sup>4</sup>	0	0
	P. citrinum	1.47 x 104	0.44 x 10 <sup>4</sup>	0.30 x 10
TADC	A. flavus	0.16 x 10 <sup>2</sup>	0	0
	A. niger	1.03 x 10 <sup>2</sup>	0	0.10 x 10°
	A. penicillioides	0	0	0.68 x 10 <sup>4</sup>
	Eurotium chevalieri	2.67 x 10 <sup>4</sup>	0.27 x 10 <sup>4</sup>	0.79 x 10 <sup>4</sup>
	P. citrinum	0	0	0.10 x 10'
TATC	4 niger	0.29 × 10*	0.33 × 10*	0.25 x 10 <sup>4</sup>
	A penicillipides	0	0	0.40 × 10
	E obevaliari	~ ^	1 00 104	1.07 - 10-
	E. crievalieri		1.05 X 10"	1.07 X 10°
	r. citinum	U	0.11 x 10*	0.10 x 10'
TJDC	A. flavus	1.26 x 10 <sup>3</sup>	0.11 x 10 <sup>2</sup>	0.33 x 10 <sup>2</sup>
	A. niger	3.33 x 10 <sup>4</sup>	3.23 x 10 <sup>2</sup>	2.64 x 10 <sup>2</sup>
	A. tamarii	1.00 x 10⁴	3.04 x 10 <sup>2</sup>	1.81 x 10 <sup>×</sup>
	E. chevalieri	0	0	2.89 x 10 <sup>2</sup>
	Endomyces fibuliger	1.44 x 10⁴	0	0
	P. citrinum	0	0.12 x 10 <sup>2</sup>	0
	S. racemosum	0	0.18 x 10 <sup>2</sup>	0.36 x 10 <sup>2</sup>
TJTC	A. flavus	4.89 x 10 <sup>2</sup>	0.37 x 10 <sup>2</sup>	0.17 x 10 <sup>2</sup>
. –	A. niger	1.59 x 10 <sup>4</sup>	1.29 x 10 <sup>2</sup>	0.69 x 10 <sup>2</sup>
	A ochraceus	0	0.30 - 101	0.00 x 10
	A. ochraceus	0.00	0.30 X 10	0
	A. tamarii	3.33 x 10 <sup>2</sup>	0.34 x 10 <sup>2</sup>	0.67 x 10 <sup>2</sup>
	Eurotium chevalieri	1.44 x 10 <sup>2</sup>	0.31 x 10 <sup>2</sup>	0.21 x 10°
	Endomyces fibuliger	2.59 x 10 <sup>4</sup>	0	0
	P. citrinum	0.90 x 10 <sup>1</sup>	0.90 x 10 <sup>1</sup>	0
	Trichoderma sp.	0.80 x 10 <sup>1</sup>	0.80 x 10 <sup>1</sup>	0

Treatment	Storage duration (month)			
	0	2	4	
<b>D</b> 4	0.40 x 10* ±	1.26 x 10* ±	1.43 x 10* ±	
PA	0.64 x 10 <sup>2</sup> a	0.92 x 10 <sup>2</sup> abc	1.62 x 10 <sup>2</sup> abc	
	0.50 x 10° ±	0.78 x 10* ±	0.59 x 10° ±	
PJ	1.98 x 10 <sup>2</sup> cd	1.69 x 10 <sup>2</sup> ab	0.10 x 10 <sup>3</sup> ab	
<b>T</b> 4	2.07 x 10 <sup>4</sup> ±	0.13 x 10° ±	0.59 x 10° ±	
IA	3.11 x 10 <sup>2</sup> bcd	1.46 x 10 <sup>2</sup> abc	0.81 x 10 <sup>3</sup> cd	
тJ	0.61 x 10* ±	0.46 x 10° ±	5.83 x 10* ±	
	3.70 x 10 <sup>3</sup> d	5.34 x 10 <sup>2</sup> cd	4.38 x 10 <sup>2</sup> cd	

 

 Table 4. Total fungal population in nutmegs subjected to different harvesting and drying methods during storage

Numbers followed by the same letter do not differ significantly according to Tukey Test at 95% confidence level.

Aspergillus niger, A. flavus and Rhizopus stolonifer. The predominant species was A. flavus. Dharmaputra et al. (2015) reported that dominant fungi infecting nutmeg collected from farmers and collectors in North Minahasa Regency, North Sulawesi Province were A. flavus, A. niger, Endomyces fibuliger, Eurotium repens and P. citrinum.

Toma and Abdulla (2013) reported that twenty fungal species and one yeast species were isolated from sixteen samples of spices and herbal medicines collected in Shekalla market, Erbil City. Five out of the twenty fungal species were *A. flavus* and *A. niger* (1 x 10<sup>3</sup> CFU/g), *A. ochraceus* (2 x 10<sup>3</sup> CFU/g), *A. versicolor* (6 x 10<sup>3</sup> CFU/g), and *E. wentii* (2 x 10<sup>3</sup> CFU/g). Based on analysis of variance (the data were transformed into logarithmic values), interaction between harvesting methods of nutmeg fruits, nutmeg in or without shell, drying method and storage duration showed significant differences in fungal population in nutmegs.

Fungal population in nutmeg subjected to various treatments fluctuated during storage. It decreased after two months of storage, then increased after four months of storage. The highest fungal population was found in nutmeg collected from ground, dried using sun-drying, either in shell (2. 84 x  $10^3 \pm 3.79$  x  $10^3$  CFU/g) or without shell (1.92 x  $10^3 \pm 3.09$  x  $10^3$  CFU/g). Fungal population in nutmeg originating from fruit picked from the tree, either dried using sun-drying or smoke-drying was lower than that of the samples collected from the ground, either dried using sun-drying or smoke-drying during storage (Table 4).

#### Total aflatoxin content

Total aflatoxin content of nutmegs collected from ground, dried using sun-drying, in or without shell (20.7 - 79.5 ppb) was higher than the Maximum Tolerable Limit of nutmeg determined by European



Figure 1. Total aflatoxin content subjected to various treatments during storage.

- Note : P = nutmeg fruit picked from the tree
- T = nutmeg fruit collected from the ground
- A = nutmeg dried using smoke-drying method
- J = nutmeg dried using sun- drying method
- DC = nutmeg in shell
- TC = nutmeg without shell

Union (maximum 10 ppb). Total aflatoxin content of nutmegs in shell was lower than in those without shell. Total aflatoxin content of nutmeg increased with the increase of storage duration, either in nutmeg in shell or without shell (Figure 1). According to Uraih and Ogbadu (1982) smoke-drying method can prevent toxin produced by toxigenic *A. flavus*. The fungistatic efficiency of the wood smoke increased with the decrease of moisture content in fish.

Tabata *et al.* (1993) reported that in Tokyo aflatoxin was found in 3054 of foodstuff and their processed products, among others in nutmeg. The highest aflatoxin contamination was found in nutmeg (80%), while aflatoxin  $B_1$  was found in pistachio (1382 ppb). Takahashi (1993) stated, that in 1986-1991, as much as 29 (43%) of 67 samples of nutmeg collected in Japan were contaminated with aflatoxin. According to Martin *et al.* (2001) three nutmeg samples contained aflatoxin  $B_1$  from 1 to 5 ppb, three

other samples 6-20 ppb, and 2 samples 54 and 58 ppb, respectively.

Romagnoli *et al.* (2007) reported aflatoxins in spices, aromatic herbal and medicinal herbal collected from common market, supermarkets, shop and warehouse in Italy since 2000 - 2005. One of six spices was analyzed, i.e. nutmeg. One of three nutmeg samples was contaminated by aflatoxin. Their aflatoxin  $B_1$  and  $B_2$  contents were 2.27 and 0.47 ppb respectively, while aflatoxin  $G_1$  and  $G_2$  were not detected.

Aspergillus flavus is a saprophytic soil fungus that infects and contaminates preharvest and postharvest seed crops (Amaike and Keller 2011). Consequently, nutmegs which had fallen on the ground are easily infected by aflatoxigenic *A. flavus*. If postharvest handling of nutmeg is not conducted properly nutmeg will be contaminated by aflatoxin.

Comparing with apples, Amiri and Bompeix (2005) reported that apples which have fallen on the ground are not used for apple juice production because the fruits can be infected by *Penicillium expansum*, a fungus which can produce patulin. The inoculum of *P. expansum* can be found in soil.

# Conclusion

Appropriate postharvest handling (Good Handling Practice) of nutmeg to ensure its good quality during storage in terms of the percentage of damaged kernels, fungal infection and aflatoxin contamination, i.e. nutmeg should originate from ripe nutmeg fruit picked from the tree, dried using smoke-drying and stored in shell. Nutmeg obtained by picking its ripe fruit from the tree should not be mixed with that had fallen on the ground.

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