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FUNGAL INFECTION AND AFLATOXIN CONTAMINATION IN STORED NUTMEG (*Myristica fragrans*) KERNELS AT VARIOUS STAGES OF DELIVERY CHAIN IN NORTH SULAWESI PROVINCE

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ABSTRACT

Fragrant nutmeg (*Myristica fragrans*) is an important commodity that has been used in food and pharmaceutical industries, hence its quality should be monitored. The objectives of the research were (a) to obtain information on the postharvest handling of nutmeg kernels; (b) to investigate the occurrence of fungi (including *A. flavus*) and aflatoxin contamination in stored nutmeg kernels and (c) to measure moisture content and percentage of damaged nutmeg kernels. Methods used in this study included survey, interviews and sample collection in the delivery chain. This study was conducted in April and May 2013, in three regions (North Minahasa, Siau Tagulandang Biaro (Sitaro) and Sangihe Talaud) and two cities (Bitung and Manado). The total number of nutmeg kernels samples collected from different points of the delivery chain was 76. It consisted of samples collected from farmers (25 samples), collectors (22) and exporters (29). The results showed that, the moisture content of nutmeg kernels collected from North Sulawesi Province was not higher than the maximum limit of moisture content determined by Indonesian National Standard or SNI (10%). Nutmeg kernels collected from farmers and collectors had a high percentage of damaged kernels. *Aspergillus niger* and *Endomyces fibuliger* were the dominant fungi infecting nutmeg kernels collected from farmers and collectors, while *Eurotium repens* was the dominant fungus found infecting nutmeg kernels stored by the exporters. Aflatoxin B_1 and total aflatoxin contents of nutmeg kernels samples collected from farmers and exporters were relatively high. Based on statistical analysis using non-parametric analysis, the effect of delivery chain did not give any significant differences on moisture content, percentage of damaged nutmeg kernels, total fungal population and total aflatoxin content. Our study suggested that the postharvest handling methods of nutmeg kernels conducted by farmers, collectors and exporters in North Sulawesi Province (North Minahasa, Sitaro and Sangihe Talaud regions), Bitung and Manado cities should be improved to minimize aflatoxin B_1 and total aflatoxin contamination.

Keywords: aflatoxin, delivery chain, fungi, *Myristica fragrans*, North Sulawesi Province, nutmeg kernels

INTRODUCTION

Nutmeg is native to the Moluccas Islands of Indonesia, but nowadays nutmeg is also grown in Penang Island in Malaysia, in the Caribbean (particularly Grenada), in the southern state of Kerala in India and in Zanzibar Islands. Fragrant nutmeg (*Myristica fragrans*) is an important commodity widely used in food and pharmaceutical industries, therefore, it is important to monitor its quality (Direktorat Jenderal Perkebunan 2012).

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Based on statistical data from the Directorate General of Estate Crops in Indonesia, areas planted with nutmeg were 75,062 ha in 2008. The distribution areas 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 (Revitalisasi Perkebunan Pala Siau, Sulawesi Utara 2010). North Sulawesi Province is one of the most important nutmeg producing provinces in Indonesia. Nutmeg from

this province is exported to the Netherlands, Italy, Japan and Vietnam.

During postharvest and storage periods, nutmeg kernels can be infested by insects and colonized by microorganisms. Among microorganisms, fungi are the most important cause of stored foodstuff deterioration. Fungal infection in foodstuff can cause discolouration and mycotoxin contamination, as well as decrease in physical quality and nutritional content (Sauer *et al*. 1992). 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 human and animals, such as aflatoxicoses and liver cancer. There are four naturally occurring aflatoxins in many stored commodities, i.e. aflatoxins B_1, B_2, G_1 and G_2. The most common and toxic aflatoxin is aflatoxin B_1 (AFB_1) (Basappa 2009). The European Union has determined Maximum Tolerable Limits (MTL) of AFB_1 and total aflatoxin in nutmeg kernels to be 5 and 10 ppb, respectively (FAO 2004). Since nutmeg is among important agricultural export commodities for Indonesia, it is important to conform to the nutmeg kernels importation rules from the importing countries. The first step in postharvest handling is to monitor and improve the current procedure of nutmeg kernels storage. In relation to storage monitoring, several objectives in this study were set as follows: (a) to obtain information on nutmeg postharvest handling methods; (b) to investigate fungal population, fungal diversity (including *Aspergillus flavus*) and aflatoxin contamination of stored nutmeg kernels collected from different points of the delivery chain in North Sulawesi Province and (c) to determine moisture content and percentage of damaged nutmeg kernels in the postharvest chain.

MATERIALS AND METHODS

Time and Location of Survey

Survey and samplings of nutmeg kernels were conducted in April and May 2013 at different points of delivery chain, i.e. at farmer, collector and exporter levels in five locations in North Sulawesi Province. The five locations chosen for the survey based on recommendation from the Agricultural Service of North Sulawesi Province were North Minahasa, Siau Tagulandang Biaro (Sitaro) and Sangihe Talaud regions; Bitung and Manado cities (Table 1).

Interview using Questionnaires

Interviews were conducted during the survey to collect information on nutmeg postharvest handling at different points in the delivery chain (farmer, collector and exporter levels). The questionnaires contained questions on postharvest handling procedures carried out by farmers, collectors and exporters, as well as problems encountered by them. The number of respondents from each level of delivery point was different depending on the conditions in the field during the survey.

Sampling Methods

The samples were collected from the places where the respondents obtained the nutmeg kernels. The number of nutmeg kernels samples at each level of delivery point was determined proportionally, based on the number of farmers, collectors and exporters.

As much as 500 g of shelled nutmeg kernels and 1,000 g of in-shell nutmeg kernels were collected randomly from each respondent. Each sample was packed in a clean plastic (polyethylene) bag and then was double packed in hermetic bags to minimize any changes to the nutmeg kernels samples due to long distance transportation between the location of sampling and laboratory in Bogor, where the samples were analyzed.

Method for Obtaining Working Samples

The in-shell nutmegs were shelled manually to obtain the kernels. Each sample was mixed homogeneously, then was divided into four parts. One part was used to determine the percentage of damaged nutmeg kernels and as a reserve sample, while the other three parts were ground using a Mill Powder Tech Model RT 04. The ground nutmeg samples were then divided into eight parts to determine moisture content, fungal population, dominant fungal species infecting kernels and aflatoxin content.

Table 1 Level of delivery chain, sub-district origin of the sample and the number of nutmeg kernels samples at various stages of delivery chain in North Sulawesi Province

Region/ City	Level of delivery chain	Sub-district origin of the sample	Number of samples
North Minahasa	Farmer	Kauditan	16
	Collector	Kauditan	17
	Exporter		-
Siau Tagulandang Biaro (Sitaro)	Farmer	East Siau	1
		West Siau	1
	Collector	East Siau	3
	Exporter		-
Sangihe Talaud	Farmer	Kendahe	2
		Central Tabukan	1
		Tamako	1
		East Tahuna	1
		West Tahuna	1
		Mangawito	1
	Collector	Kendahe	2
	Exporter		-
Bitung	Farmer		-
	Collector		-
	Exporter	Halmahera Island	1
		Lembeh Island	1
		Kauditan	1
		Some locations	1
		Bitung	2
Manado	Farmer		-
	Collector		-
	Exporter	Siau	12
		Tahuna	1
		Manado	10
Total number of samples			76

Determination of Moisture Content, Percentage of Damaged Kernels, Fungal Population and Aflatoxin Content

Moisture content of nutmeg (based on wet basis) was determined using the distillation method (SNI 1993). Two replicates were used for each sample. The percentage of damaged kernels was calculated from the weight of the damaged kernels and divided by the weight of the working sample from which the damaged kernels were taken. Damaged kernels included cracked, broken, shriveled and mouldy kernels.

Fungi were isolated using the serial dilution method followed by the pour plate method with Dichloran 18% Glycerol Agar (DG18) (Hocking & Pitt 1980; Pitt & Hocking 2009). Each fungal species were identified according to Pitt and Hocking (2009) as the main reference. Aflatoxin B_1 (AFB_1) and total aflatoxin contents were determined using HPLC with post-column derivatization (VICAM 2007).

Data Collection and Analysis

Data collection on nutmeg postharvest handling methods at farmer, collector and exporter levels were carried out by conducting interviews with farmers, collectors and exporters.

Data of moisture content (MC), percentage of damaged kernels, fungal total population and total aflatoxin content were collected from farmers (25 samples), collectors (22 samples) and exporters (29 samples) at North Sulawesi Province. Total samples at farmer and collector levels were collected from North Minahasa, Sitaro and Sangihe Talaud regions. Meanwhile, total samples at exporter level were collected from Bitung and Manado cities. Data of MC, percentage of damaged nutmeg kernels, total fungal population and total aflatoxin content were analyzed with non-parametric one way Kruskal-Wallis test.

RESULTS AND DISCUSSION

Results of Interviews with Farmers, Collectors and Exporters

The total number of respondents was 26 (14 farmers, 8 collectors and 4 exporters). Interviews related to nutmeg postharvest handling at farmer and collector levels were conducted only in North Minahasa, Sitaro and Sangihe Talaud regions, while interviews at exporter level were conducted in Bitung and Manado cities.

Method of Collecting Nutmeg

At farmer level, farmers generally picked nutmeg fruits from the trees (±50%). They also collected nutmeg fruits which had fallen on the ground (±50%). Farmers also collected nutmeg fruits that had fallen on the ground, because this method was easier to be conducted compared to harvesting nutmeg fruits directly from the trees. Price of nutmegs picked directly from the trees was similar to price of nutmegs that had fallen on the ground. Therefore, farmers did not pay attention to the proper method of nutmeg postharvest handling.

At collector level, a total of 57% of collectors bought nutmegs from farmers in three conditions, i.e. wet, semi-dry and dry conditions; for both shelled and in-shell nutmegs. If a collector bought in-shell nutmegs, then a wooden stick was used for conducting the shelling process. As much as 12.5 and 87.5% of farmers sold nutmegs in semi-dry and dry conditions, respectively. In general, farmers sold nutmegs in dry condition, because the price was higher than those sold in semi-dry or wet conditions.

At exporter level, up to 75% of exporters bought shelled nutmegs from collectors, while 25% of exporters bought a mixture of shelled and in-shell nutmegs. Most of exporters bought shelled nutmegs, because the weight of shelled nutmegs was lighter than that of in-shell nutmegs, which could decrease the transportation cost.

Method of Drying and Storing Nutmegs

At the farmer level, 87.5% of farmers dried shelled and in-shell nutmegs using sun-drying method by spreading the nutmegs on tarpaulin placed on the ground. The dried nutmegs were subsequently stored in plastic bags. After 1 kg of nutmegs were collected and shelled, they were sold to collectors. In general, farmers did not do any attempts to sort dried nutmegs based on physical quality, because they wanted to sell dry nutmegs as soon as possible.

At the collector level, drying of nutmegs was conducted using two methods, i.e. sun-drying and smoke drying methods. In general, collectors used smoke drying method, especially during rainy season. The smoke drying method used coconut shells as fuel. Up to 57% of collectors dried both shelled and in-shell nutmegs using sun-drying method on tarpaulin placed on the ground. A total of 43% of collectors smoke-dried nutmegs on wire and wooden racks, followed by sun-drying method. For better result, collectors dried the nutmeg kernels using wire and wooden racks at elevated position ±1 m from the floor, to avoid contamination by animal faeces or dirt. In the night or when it rained, the semi-dried and wet nutmeg kernels were packed in the same plastic bags. As much as 71% of collectors sold nutmeg kernels to exporters, while 29% of nutmeg kernels were sold to large traders, without being sorted. Before being sold, the nutmeg kernels were stored for about a month. During storage, 71% of collectors did not monitor nutmeg kernels for aflatoxin contamination, due to lack of aflatoxin detection equipment, although they knew the impact of aflatoxin on human and animal health.

At the exporter level, 75% of exporters grouped the nutmeg kernels manually based on qualities. The exporters usually stored the sorted nutmeg kernels in gunny and plastic (polypropylene) bags. Those exporters were aware of potential aflatoxin contamination and the impact on human and animal health. They monitored the nutmeg kernels for aflatoxin contamination using a long wave ultraviolet lamp, in which aflatoxin contaminated nutmeg kernels would produce blue green yellow fluorescence and be removed prior to export.

Source and Number of Samples

The total number of nutmeg kernels samples collected from different points in the delivery chain was 76. This consisted of nutmeg kernels samples from farmers (25 samples), collectors (22 samples) and exporters (29 samples) (Table 1).

Moisture Content

The moisture content (MC) of foodstuff at the beginning of storage is one of the important factors influencing the quality of foodstuff during storage. A high initial MC value at the beginning of storage provides an opportunity for growth and development of spoilage and mycotoxigenic fungi. According to Indonesian National Standard or SNI, maximum moisture content value for nutmeg kernels and their processed products should be 10% (SNI 01-0006-1993).

The range and mean MC values of nutmeg kernels collected from farmers, collectors and exporters in North Minahasa, Siau Tagulandang Biaro (Sitaro) and Sangihe Talaud regions; Bitung and Manado cities are presented in Table 2.

The highest and the lowest MC values of nutmeg kernels collected from farmers in North Minahasa region were 18.00 and 8.00%, respectively. For collectors, those MC values were 15.50 and 7.50%, respectively. The mean MC value of nutmeg kernels collected from farmers was similar to that collected from collectors, i.e. 10.88 and 11.07%, respectively. The mean MC value of nutmeg kernels collected from farmers and collectors were higher than the maximum limit determined by SNI. The high MC value of nutmeg kernels collected from farmers was probably due to the short period of drying after the harvesting process, low intensity of sun light during the sun-drying process, limited storage space and short duration of storage.

The highest and lowest MC values of nutmeg kernels from farmers in Sitaro region were 10.50 and 10.00%, respectively, while the highest and lowest MC values of nutmeg kernels from collectors were 11.50 and 6.50%, respectively. The MC values of nutmeg kernels from farmers in Sitaro region exceeded the maximum limit determined by SNI.

In Sangihe Talaud region the range and mean of MC values obtained from the farmers' and collectors' samples were lower than the maximum limit determined by SNI. The highest and lowest MC values of nutmeg kernels from farmers were 9.00 and 6.98%, respectively; while the highest and lowest MC values of nutmeg kernels from collectors were 8.00 and 7.99%, respectively.

The range of MC values of nutmeg kernels collected from exporters in Manado (7.00 - 11.50%) was wider than that collected from Bitung (7.50 - 10.00). The mean MC value of nutmeg kernels from exporters in Bitung (8.75%) and Manado (9.48%) cities were lower than the maximum limit determined by SNI.

Table 2 Range and mean of moisture content, undamaged and damaged kernels of nutmeg collected from farmers, collectors and exporters in North Sulawesi Province

Region / City	Level of delivery chain	Range (mean) of moisture content (% wet basis)	Range (mean) of undamaged kernels (%)	Range (mean) of damaged kernels (%)
North Minahasa	Farmer	8.00 – 18.00 (10.88)	0 – 88 (22.78)	12.00 –100 (77.22)
	Collector	7.50 – 15.50 (11.07)	0 – 81.42 (21.20)	18.58 – 100 (76.12)
	Exporter	-	-	-
Siau Tagulandang Biaro (Sitaro)	Farmer	10.00 – 10.50 (10.25)	52.91 – 61.88 (57.40)	38.13 – 47.09 (42.61)
	Collector	6.50 – 11.50 (8.83)	0 – 47.85 (15.95)	52.15 – 100 (84.05)
	Exporter	-		
Sangihe Talaud	Farmer	6.98 – 9.00 (7.85)	0 – 61.54 (36.71)	38.46 – 100 (63.29)
	Collector	7.99 – 8.00 (8.00)	0 – 58.78 (29.39)	41.22 – 100 (70.61)
	Exporter	-	-	-
Bitung	Farmer	-	-	-
	Collector	-	-	-
	Exporter	7.50 – 10.00 (8.75)	0 – 75.31 (35.25)	24.69 – 100 (64.75)
Manado	Farmer	-	-	-
	Collector	-	-	-
	Exporter	7.00 – 11.50 (9.48)	0 – 71.33 (26.42)	28.67 – 100 (73.58)

The mean MC value of nutmeg kernels collected from farmers and collectors in North Minahasa region were higher than that collected from other delivery chains in North Sulawesi Province.

Overall, the highest mean MC value was recorded from nutmeg kernels collected from collectors in North Minahasa region, followed by those collected from farmers in Sitaro region, from exporters in Manado city, from collectors in Sitaro region, from exporters in Bitung city and from farmers and collectors in Sangihe Talaud region.

Nutmeg kernels are very hygroscopic, therefore, storage at semi-dried condition caused the kernels to absorb moisture which led to initiation of mould growth, spoilage and mycotoxin contamination.

Based on non-parametric statistical analysis, delivery chain did not significantly influence moisture content (MC) values of nutmeg kernels (Table 3).

Percentage of Undamaged and Damaged Kernels

Damage to nutmeg kernels may contribute to infection by mycotoxigenic fungi such as *A. flavus* and increase the chances for aflatoxin contamination during the postharvest storage. The range of damaged nutmeg kernels percentage in North Minahasa region from farmers and collectors were 12.00 – 100 and 18.6 – 100%, respectively. In Sitaro region, the range of damaged nutmeg kernels from farmers and collectors were 38.1 – 47.1% and 52.2 – 100%, respectively. Means of damaged nutmeg kernels percentage from farmers and collectors were 42.6 and 84.1%, respectively.

In Sangihe Talaud region, the mean of damaged nutmeg kernels percentage collected from farmers (63.29%) was lower than that collected from collectors (70.6%).

In Bitung city the percentage of damaged nutmeg kernels collected from exporters was 64.8%. In Manado city the percentage was 73.6% (Table 2). Means of damaged nutmeg kernels percentage collected from farmers and collectors in North Sulawesi Province were higher than those collected from exporters. This might be due to improper sorting regimes used by farmers and collectors. In addition, many farmers and collectors still manually shelled the nutmegs using wooden stick, which can increase the percentage of damaged nutmeg kernels.

Based on statistical analysis using non-parametric analysis, delivery chain did not significantly influence the percentage of damaged nutmeg kernels (Table 3).

Fungal Population Diversity and Dominance

The highest diversity of fungal species was found in nutmegs collected from farmers and collectors in North Minahasa region, i.e. 13 and 12 species, respectively, while the lowest number of fungal species were observed in samples obtained from farmers in Sitaro region (7 species) and those obtained from collectors in Sangihe Talaud region (2 species; Tables 4, 5, 6).

Aspergillus flavus was found in 56% samples from farmers and in 53% samples from collectors in North Minahasa region. Fungal population isolated from samples from farmers was more contaminated than those from collectors (Table 5). The dominant fungi found in nutmeg samples from farmers was *Penicillium citrinum* (81%) followed by *A. niger* (69%) and *Eurotium repens* (63%). Three dominant fungi found in nutmegs collected from collectors were *Endomyces fibuliger* (76%), *A. niger* (76%) and *P. citrinum* (76%) which may have been caused by high moisture content.

Table 3 The effect of nutmeg delivery chain on moisture content (MC), percentage of damaged nutmeg kernels, fungal total population and total aflatoxin

Level of delivery chain	MC (%)	Percentage of damaged kernels (%)	Fungal total population (cfu/g wet basis)	Total aflatoxin content (ppb)
Farmer	9.98 ± 2.62 a	70.55±29.66 a	$3.9x10^5$±$1.7x10^6$ a	141.10±392.11 a
Collector	10.49 ± 2.52 a	76.70±32.39 a	$1.3x10^6$±$3.2x10^6$ a	2.15±4.54 a
Exporter	9.33 ± 1.13 a	71.75±30.27 a	$9.9x10^3$±$1.1x10^4$ a	50.63±213.43 a

Note: Means in the same group followed by the same letter in a column are not significantly different at 5% level

No *A. flavus* was isolated from nutmeg samples obtained from collector in the Sitaro region. At farmer level, *A. niger, A. penicillioides, Eurotium repens* and *P. citrinum* were isolated from all samples, dominated by *A. penicillioides.* Only a small number of nutmeg samples were colonized by *A. flavus* in the Sangihe Talaud region. No nutmeg samples from collectors appeared to contain *A. flavus*. All samples of nutmegs obtained from farmers and collectors in all sampling sites contained *A. niger.*

The total fungal population and diversity of samples from exporters were lower in Bitung city than in Manado city (Table 7). In Bitung city, *A. flavus* was only isolated from 2% of samples. The dominant fungi were xerophilic spoilage fungi such as *E. repens* (all samples) followed by *E. chevalieri* (83%). In Manado city, much higher number of samples contained *A. flavus* (39%). Again, the dominant fungal population isolated were *E. repens* (87%), followed by *A. niger* (70%) and *A. penicillioides* (65%).

Aspergillus flavus was found in 56% samples from farmers and in 53% samples from collectors in North Minahasa region. Fungal population isolated from samples from farmers was more contaminated than those from collectors (Table 5). The dominant fungi found in nutmeg samples from farmers was *Penicillium citrinum* (81%) followed by *A. niger* (69%) and *Eurotium repens* (63%). Three dominant fungi found in nutmegs collected from collectors were *Endomyces fibuliger* (76%), *A. niger* (76%) and *P. citrinum* (76%) which may have been caused by high moisture content.

No *A. flavus* was isolated from nutmeg samples obtained from collector in the Sitaro region. At farmer level, *A. niger, A. penicillioides, Eurotium repens* and *P. citrinum* were isolated from all

Table 4 Fungal populations and diversity in nutmeg samples obtained from farmers and collectors in North Minahasa region

No	Fungi	Number (%) samples infected by fungi		Range (mean) of fungal population in nutmeg (cfu/g wet basis)	
		Farmer	Collector	Farmer	Collector
1.	*Aspergillus flavus*	9 (56)	9 (53)	0.5 x 10 – 8.8 x 10^3 (1.5×10^3)	0.7 x 10 – 5.2 x 10^3 (7.8×10^2)
2.	*A. niger*	11 (69)	13 (76)	0.2 x 10 – 2.2 x 10^4 (4.3×10^3)	0.1 x 10^2 – 8.8 x 10^3 (2×10^3)
3.	*A. ochraceus*	-	1 (6)	-	0.7 x 10 (0.7 x 10)
4.	*A. penicillioides*	2 (13)	3 (18)	2.2 x 10^2 – 1.3 x 10^3 (7.8×10^2)	4.5 x 10 – 2.7 x 10^2 (1.7×10^2)
5.	*A. tamarii*	8 (50)	4 (24)	0.8 x 10 – 1.2 x 10^3 (3.3×10^2)	1.5 x 10^2 – 7.3 x 10^2 (3.7×10^2)
6.	*A. sydowii*	2 (13)	1 (6)	1.2 x 10 – 1.8 x 10^3 (9.2×10^2)	8.2 x 10^2 (8.2×10^2)
7.	*A. wentii*	1 (6)	-	1.2 x 10^2 (1.2×10^2)	-
8.	*Endomyces fibuliger*	7 (44)	13 (76)	5.2 x 10 – 8.4 x 10^6 (1.3×10^6)	1.5 x 10 – 1.1 x 10^7 (2.2×10^6)
9.	*Eurotium chevalieri*	5 (31)	3 (18)	1.5 x 10 – 0.4 x 10^4 (1.2×10^3)	3.3 x 10 – 7.3 x 10^3 (2.6×10^3)
10.	*E. repens*	10 (63)	9 (53)	0.5 x 10 – 6.8 x 10^3 (1.7×10^3)	1.3 x 10 – 3.2 x 10^3 (9.0×10^2)
11.	*E. rubrum*	-	7 (41)	-	0.2 x 10 – 3.5 x 10^3 (9.4×10^2)
12.	*Penicillium citrinum*	13 (81)	13 (76)	0.3 x 10 – 0.6 x 10^5 (6.3×10^3)	1.3 x 10 – 0.4 x 10^4 (7.8×10^2)
13.	*Rhizopus* sp.	2 (13)	-	2.5 x 10^2 – 7.2 x 10^2 (4.8×10^2)	-
14.	*Syncephalastrum racemosum*	-	1 (6)	-	0.2 x 10^3 (0.2×10^3)
15.	*Trichoderma* sp.	2 (13)	-	0.1 x 10^2 – 1 x 10^3 (5.1×10^2)	-

Notes: Number of samples collected from farmers: 16
Number of samples collected from collectors: 17

Table 5 Fungal population and diversity in nutmeg samples obtained from farmers and collectors in Siau Tagulandang Biaro (Sitaro) region

No	Fungi	Number (%) samples infected by fungi		Range (mean) of fungal population in nutmeg (cfu/g wet basis)	
		Farmer	Collector	Farmer	Collector
1.	*Aspergillus niger*	2 (100)	2 (67)	0.2 x 10 – 2.5 x 10 (1.3 x 10)	5.5×10^2 – 4.2×10^4 (2.1×10^4)
2.	*A. penicillioides*	2 (100)	1 (33)	1.8 x 10 – 2.0×10^3 (0.1×10^4)	3.7×10^2 (3.7×10^2)
3.	*Endomyces fibuliger*	1 (50)	-	0.5 x 10 (0.5 x 10)	-
4.	*Eurotium chevalieri*	1 (50)	1 (33)	6.7 x 10 (6.7 x 10)	2.8 x 10 (2.8 x 10)
5.	*E. repens*	2 (100)	1 (33)	0.2 x 10 – 3.3×10^2 (1.7×10^2)	1.8 x 10 (1.8 x 10)
6.	*E. rubrum*	1 (50)	1 (33)	1.7 x 10 (1.7 x 10)	0.3 x 10 (0.3 x 10)
7.	*Penicillium citrinum*	2 (100)	-	0.2 x 10 – 0.8 x 10 (0.5 x 10)	-

Notes: Number of samples collected from farmers: 2
Number of samples collected from collectors: 3

Table 6 Fungal population and diversity in nutmeg samples obtained from farmers and collectors in Sangihe Talaud region

No	Fungi	Number (%) samples Infected by fungi		Range (mean) of fungal population in nutmeg (cfu/g wet basis)	
		Farmer	Collector	Farmer	Collector
1.	*Aspergillus flavus*	1 (14)	-	0.2 x 10 (0.2 x 10)	-
2.	*A. niger*	7 (100)	2 (100)	0.7 x 10 – 2.4×10^5 (5.1×10^4)	4.8 x 10 – 0.1×10^5 (5.4×10^3)
3.	*A. penicillioides*	3 (43)	-	0.1×10^2 – 3.7×10^2 (1.3×10^2)	-
4.	*Endomyces fibuliger*	1 (14)	-	1.5×10^5 (1.5×10^5)	-
5.	*Eurotium repens*	4 (57)	-	0.5 x 10 – 8.3×10^2 (2.2×10^2)	-
6.	*E. rubrum*	3 (43)	-	0.3 x 10 – 0.5×10^3 (1.7×10^2)	-
7.	*Fusarium solani*	1 (14)	-	5.2×10^4 (5.2×10^4)	-
8.	*Penicillium citrinum*	3 (43)	1 (50)	0.3×10^2 – 1.8×10^4 (6.8×10^3)	4.3×10^2 (4.3×10^2)

Notes: Number of samples collected from farmers: 7
Number of samples collected from collectors: 2

samples, dominated by *A. penicillioides.* Only a small number of nutmeg samples were colonized by *A. flavus* in the Sangihe Talaud region. No nutmeg samples from collectors appeared to contain *A. flavus*. All samples of nutmegs obtained from farmers and collectors in all sampling sites contained *A. niger.*

The total fungal population and diversity of samples from exporters were lower in Bitung city than in Manado city (Table 7). In Bitung city, *A. flavus* was only isolated from 2% of samples. The dominant fungi were xerophilic spoilage fungi such as *E. repens* (all samples) followed by *E. chevalieri* (83%). In Manado city, much higher number of samples contained *A. flavus* (39%). Again, the dominant fungal population isolated were *E. repens* (87%), followed by *A. niger* (70%) and *A. penicillioides* (65%).

Nutmegs imported from India, Sri Lanka, Indonesia and Brazil were infected by *A. niger*, *A. flavus* and *Rhizopus stolonifer*. The dominant fungi in these samples were *A. flavus* (Mandel 2005). The water availability of semi-dried and damaged nutmeg kernels provided environmental conditions which are conducive to xerophilic and xerotolerant fungi, including mycotoxigenic species. The boundary conditions for growth and mycotoxin production suggested that aflatoxigenic and ochratoxigenic fungi may be able to thrive under storage conditions (Lacey & Magan 1991; Sanchis & Magan 2004; Magan & Aldred 2007).

Table 7 Fungal population and diversity in nutmeg samples collected from exporters in Bitung and Manado cities

No.	Fungi	Bitung city		Manado city	
		Number (%) samples infected by fungi	Range (mean) of fungal population in nutmeg (cfu/g wet basis)	Number (%) samples infected by fungi	Range (mean) of fungal population in nutmeg (cfu/g wet basis)
1.	*Aspergillus flavus*	2 (33)	0.5 x 10 – 7.7 x 10^2 (3.9 x 10^2)	9 (39)	0.1 x 10^2 – 5.3 x 10^2 (1.3 x 10^2)
2.	*A. niger*	3 (50)	0.3 x 10 – 1.7 x 10^2 (7.1 x 10)	16 (70)	0.7 x 10 – 0.4 x 10^5 (3.1 x 10^3)
3.	*A. penicillioides*	2 (33)	0.5 x 10^2 – 3.5 x 10^3 (1.8 x 10^3)	15 (65)	1.3 x 10^2 – 2.9 x 10^4 (6.6 x 10^3)
4.	*A. sydowii*	1 (17)	0.5 x 10 (0.5 x 10)	-	-
5.	*A. tamarii*	1 (17)	0.2 x 10^3 (0.2 x 10^3)	1 (4)	2.2 x 10^2 (2.2 x 10^2)
6.	*A. versicolor*	-	-	2 (9)	4.5 x 10^2 – 1.2 x 10^3 (8.1 x 10^2)
7.	*Endomyces fibuliger*	2 (33)	1.7 x 10^2 – 6.7 x 10^2 (4.2 x 10^2)	4 (17)	2.8 x 10^2 – 5.3 x 10^3 (1.7 x 10^3)
8.	*Eurotium chevalieri*	5 (83)	0.2 x 10 – 2.5 x 10^3 (5.4 x 10^2)	5 (22)	0.5 x 10^2 – 3.7 x 10^3 (1.1 x 10^3)
9.	*Eurotium repens*	6 (100)	0.1 x 10^2 – 8.8 x 10^2 (3.6 x 10^2)	20 (87)	2.5 x 10^2 – 1.8 x 10^4 (0.5 x 10^4)
10.	*E. rubrum*	1 (17)	6.8 x 10^2 (6.8 x 10^2)	15 (65)	2.8 x 10 – 0.2 x 10^4 (5.2 x 10^2)
11.	*Paecilomyces variotii*	-	-	1 (4)	4.5 x 10^2 (4.5 x 10^2)
12.	*Penicillium citrinum*	4 (67)	0.5 x 10 – 9.3 x 10^2 (2.7 x 10^2)	11 (48)	1.8 x 10 – 1.3 x 10^3 (2.4 x 10^2)

Notes : Number of samples collected from exporters in Bitung city : 6
Number of samples collected from exporters in Manado city : 23

Based on statistical analysis using non-parametric analysis, delivery chain did not significantly influence the total fungal population (Table 3).

Aflatoxin B_1 and Total Aflatoxin Contents

The range of AFB_1 and total aflatoxin content in nutmeg samples from farmers in North Sulawesi Province were 0.40 – 1,632.19 ppb and 0.58 – 1,831.48 ppb, respectively. Survey conducted at a farmer level provided information that postharvest handling was not conducted properly, i.e. the farmers mixed nutmegs picked from the trees with those fell on the ground; consequently the AFB_1 and total aflatoxin content in these samples were high (Table 8).

According to Horn (2003) soil serves as a reservoir for *A. flavus* and *A. parasiticus* that produce aflatoxins in agricultural commodities. Aflatoxigenic fungi reside in soil as conidia, sclerotia and hyphae, while act as primary inocula for directly infecting peanuts (and possibly nutmeg fruit which fell on the ground). Range of AFB_1 and total aflatoxin content in nutmeg samples obtained from collectors in North Sulawesi Province were 0.11 – 14.59 ppb and 0.11 – 16.65 ppb, respectively. Sun-drying method was faster and more effective than smoke-drying method. However, if the weather is extreme, sun-drying method is not recommended, because it could reduce the quality of atsiri oil in nutmeg kernels. Therefore, many collectors used smoke-drying method, because the temperature can be controlled. The weakness using smoke-drying method includes the need of long drying duration, which may allow colonization of spoilage and mycotoxigenic fungi.

Based on statistical analysis using non-parametric analysis, delivery chain did not

Table 8 Aflatoxin B_1 and total aflatoxin contents in nutmeg collected from farmers, collectors and exporters in North Sulawesi Province

Region/City	Level of delivery chain	Number of samples	Number (%) samples contaminated by aflatoxin	Range (mean) of AFB_1 content in contaminated samples (ppb)	Range (mean) of total aflatoxin content in contaminated samples (ppb)
North Minahasa	Farmer	16	6 (37.50)	0.40 – 762.24 (128.52)	0.58 – 910.48 (153.28)
	Collector	17	5 (29.41)	0.19 – 14.59 (4.70)	0.19 – 16.65 (5.67)
	Exporter	-	-	-	-
Siau Tagulandang Biaro (Sitaro)	Farmer	2	2 (100)	1.03 – 1.44 (1.23)	1.44 – 1.55 (1.49)
	Collector	3	3 (100)	0.11 – 0.69 (0.40)	0.11 – 1.34 (0.58)
	Exporter	-	-	-	-
Sangihe Talaud	Farmer	7	7 (100)	1.65 – 1 632.19 (335.92)	1.65 – 1831.48 (371.99)
	Collector	2	2 (100)	3.28 – 13.94 (8.61)	3.28 – 13.94 (8.61)
	Exporter	-	-	-	-
Bitung	Farmer	-	-	-	-
	Collector	-	-	-	-
	Exporter	6	3 (50)	1.40 – 799.25 (267.74)	1.40 – 1112.58 (372.18)
Manado	Farmer	-	-	-	-
	Collector	-	-	-	-
	Exporter	23	15 (65.22)	0.10 – 266.72 (18.37)	0.18 – 334.49 (20.69)

significantly influence total aflatoxin content of nutmeg (Table 3).

Several exporters in North Sulawesi Province possess facilities for drying, shelling, sorting and storage facilities. Thus, aflatoxin contamination could be minimized. In this research, we also collected a sample of sorted nutmeg kernels visually assessed using the long wave ultraviolet lamp. This potentially contaminated sample from an exporter contained a total aflatoxin content of 0.18 – 1,112.58 ppb. Tabata *et al.* (1993) reported that aflatoxin was found in 3,054 foodstuff and their product samples, especially nutmeg samples. The highest aflatoxin contamination was found in nutmeg (80%), while AFB_1 was also found in pistachio nuts (1,382 ppb). Takahashi (1993) reported that in 1986 until 1991, as much as 29 (43%) of 67 nutmeg samples collected from Japan, were contaminated by aflatoxin. According to Okano *et al.* (2012) the distribution of aflatoxigenic fungi in 25 imported Indonesian nutmeg samples were contaminated with aflatoxins B or B and G. The incidence of aflatoxigenic fungi in the samples contaminated with high levels of aflatoxin was significantly higher than that in the samples with low levels of the toxins (r = 0.752). The toxin production of isolates from the samples in cultures of Yeast Extract Sucrose broth was examined by means of TLC and HPLC analyses. The ability of isolates to produce aflatoxins did not correlate with the contamination levels of aflatoxin in the samples. Overall, the postharvest handling procedures need to be standardized to minimize aflatoxin contamination.

CONCLUSIONS

Generally, moisture content (MC) of nutmeg samples collected from North Sulawesi Province was below the maximum recommended limit of Indonesian National Standard or SNI. Nutmeg samples collected from farmers and collectors generally had a higher percentage of damaged kernels. *Aspergillus niger* and *E. fibuliger* were the

dominant fungi in nutmeg kernels from farmers and collectors, while *E. repens* was the dominant species in nutmeg samples obtained from exporters in North Sulawesi province. Aflatoxin B_1 and total aflatoxin contents in nutmeg samples collected from farmers and exporters were relatively high. Although based on statistical analysis using non-parametric analysis, delivery chain did not significantly influence moisture content (MC), percentage of damaged nutmeg kernels, total fungal population and total aflatoxin content of nutmeg, the method of nutmeg postharvest handling, especially at farmer and collector levels should be conducted appropriately to minimize aflatoxin contamination.

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