BIOTROPIA Vol. 29 No. 3, 2022: 185 - 192 DOI: 10.11598/btb.2022.29.3.1393

POSTHARVEST QUALITY IMPROVEMENT OF NUTMEG (*Myristica fragrans*)

OKKY SETYAWATI DHARMAPUTRA*, SANTI AMBARWATI, INA RETNOWATI AND NIJMA NURFADILA

Phytopathology Laboratory, Science Innovation and Technology Department, SEAMEO BIOTROP, Bogor 16134, Indonesia

Received 11 August 2020/Accepted 19 September 2021

ABSTRACT

Nutmeg (*Myristica fragrans*) or fragrant 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 to: 1) identify Critical Control Points (CCP) in nutmeg's postharvest handling process and prepare nutmeg HACCP (Hazard Analysis and Critical Control Point) System and 2) provide a recommendation on GHP (Good Handling Practices) of nutmeg in order to maintain its quality in relation to food safety issue which is very important for international trade. Ripe fruits of nutmeg were collected after the fruits had reached maturity and fallen from their trees. A paranet was placed under each nutmeg tree to prevent the ripe nutmeg fruits from falling on the ground. The subsequent processes were taking out the nutmeg seeds from the fruits and separating the nutmeg seeds from the pulps and maces. After that, the nutmeg seeds underwent the drying process by using the smoke- and oven-dried methods until the moisture content of the nutmeg seeds was reduced by 10%. Subsequently, the nutmeg seeds were divided into two parts, prior to the storing process. The first part was fumigated by using phosphine (2 g/m^3) for eight days and the second part was not fumigated. The sampling of nutmeg seeds was conducted at the beginning of storage and after four months of storage. The parameters observed were moisture content, percentage of damaged kernels, the population of each fungal species, and aflatoxin content. The results showed that moisture content, fungal population, aflatoxin B_1, and total aflatoxin contents of nutmeg kernels having been dried by using the smoke- and oven-dried methods with and without fumigation still complied with the requirements related to food safety, although the nutmegs were stored for four months. The results of this research could also determine the Critical Control Point (CCP) in the postharvest handling process of nutmegs, i.e., 1) choosing only ripe nutmeg fruits to be harvested; 2) harvesting method by preventing the ripe nutmeg fruits from falling on the ground; 3) drying process of nutmeg seeds should be conducted immediately after separating the nutmegs from the maces by using the smoke- or oven-dried methods; and 4) nutmeg seeds were stored with the shells.

Keywords: nutmeg, postharvest, quality, shells, storage

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 nutmeg is also grown on Penang Island in Malaysia, in the Caribbean (particularly Grenada), in the southern state of Kerala in India, and on the island of Zanzibar.

*Corresponding author, email: okky@biotrop.org

Djaelani (2018) reported that North Moluccas is the largest nutmeg producer in Indonesia. According to CBI (2015), Indonesia and Grenada dominate nutmeg production and export 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 the postharvest period (including storage), nutmeg could be infested by insects and microorganisms. Among microorganisms, fungi are the most important cause of stored

foodstuffs deterioration. Fungal infection in foodstuffs can cause discoloration, a decrease in physical quality and nutritional contents, and mycotoxin contamination. Aflatoxins are toxins produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxins are considered dangerous due to their association with various diseases in humans and animals, such as aflatoxicosis and liver cancer. There are four naturally occurring aflatoxins in many commodities, i.e., aflatoxins B_1, B_2, G_1, and G_2. The most toxic aflatoxin is aflatoxin B_1 (Basappa 2009). According to FAO (2004), European Union has determined the Maximum Tolerable Limits (MTL) of aflatoxin B_1 and total aflatoxins in nutmeg as 5 and 10 ppb, respectively.

Dharmaputra *et al.* (2015) reported that the postharvest handling method of nutmeg conducted by farmers and collectors in North Sulawesi Province was not appropriate. As the 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 to: 1) identify Critical Control Point (CCP) in the nutmeg postharvest handling process and prepare the nutmeg HACCP (Hazard Analysis and Critical Control Points) System and 2) provide a recommendation on GHP (Good Handling Practices) of nutmeg in order to maintain its quality in relation to food safety issue which is very important for international trade.

MATERIALS AND METHODS

Time and Location of Research

Collection of nutmeg fruits and drying of nutmegs with their shells were conducted in the location where nutmeg trees were cultivated, i.e., in Kauditan Subdistrict, North Minahasa Regency, North Sulawesi Province. Storage of nutmeg took place in a warehouse located in Bitung Municipality, North Sulawesi Province. The determination of moisture content, percentage of damaged kernels, the population of each fungal species infecting kernels, and aflatoxin content were conducted at the Food and Feed as well as the Phytopathology Laboratories, SEAMEO BIOTROP, Bogor.

Collecting Nutmeg Fruits, Nutmegs Drying and Fumigation

Ripe fruits of nutmeg were collected one week after they had fallen from nutmeg trees. A paranet was placed under each nutmeg tree to prevent the ripe nutmeg fruits from falling on the ground. The paranet was placed at 1 m above ground (Fig. 1).

Figure 1 A paranet installed under each nutmeg tree to catch the ripe nutmeg fruits that naturally fell from the nutmeg trees

The pulps and maces of nutmeg fruits were then separated from the whole nutmeg seeds. Nutmeg seeds were dried until the moisture content was reduced by 10%. The drying process was conducted by using: 1) smoke-dried and 2) oven-dried methods (Fig 2). Subsequently, the nutmegs were divided into two parts prior to storage, i.e., 1) fumigated by using phosphine (2 g/m^3) for 8 days to prevent the occurrence of insect infestation during storage and 2) not fumigated. The drying methods and fumigation were replicated three times.

(a) (b)

Figure 2 Drying of nutmeg using: (a) smoke-dried and (b) oven-dried methods

Packaging and Storing of Nutmeg

The fumigated and non-fumigated nutmegs in the shell were packed in gunny bags. Each bag contained 5 kg of nutmegs-in-shells and was stored for four months under warehouse conditions (Fig. 3). In three replicates, each bag containing nutmegs was treated as follows: (a) drying methods; (b) fumigated and not fumigated; and (c) storage durations, i.e., at the beginning of storage and four months of storage. The sampling of nutmegs was conducted at the beginning of storage and after four months of storage. The number of experimental units was 24, i.e., 2 drying methods x 2 fumigated and not fumigated x 2 storage durations x 3 replications. The temperature and relative humidity of the storage were recorded using a thermohygrograph.

Sampling and Obtaining Working Samples

The sampling of nutmegs was conducted at the beginning of storage and after four months of storage. Insects found in nutmeg were separated from nutmeg using a sieve. The insects were then preserved in vials containing 70% ethanol. Each sample of nutmeg seeds was mixed homogeneously. Nutmegs-in-shell were then shelled using a hammer to get nutmeg kernels. After that the nutmeg samples were separated into eight parts i.e., two parts were used for determining the percentage of damaged kernels, while the other six parts were used for determining the moisture content, fungal population, and aflatoxin content. Subsequently, the six parts were ground using Mill Powder Tech Model RT 04 and mixed homogenously on a plastic tray (40 x 30 x 5 cm). The ground nutmeg was then divided into eight parts to be used as working samples, i.e., one part for determining moisture content, three parts for determining fungal population, and four parts for determining total aflatoxin content.

(a) (b)

Figure 3 Condition at the outside (a) and the inside (b) of the warehouse used for storing nutmeg seeds for four months

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

The moisture content of nutmeg kernels (based on a wet basis) was determined based on ISO 939, i.e., the distillation method (SNI 2015). Two replicates were used for each sample. Damaged kernels included shriveled, cracked, broken kernels, moldy and insect-damaged kernels. The percentage of damaged kernels was determined using the following formula:

$$\frac{\text{Weight of damaged kernels (g)}}{\text{Weight of working sample used for damaged kernel analysis (g)}} \times 100\%$$

Fungi were isolated using the serial dilution method, followed by the pour plate method on Dichloran 18% Glycerol Agar (DG18) (Pitt & Hocking 2009). Each fungal species was identified following Pitt and Hocking (2009) and Samson *et al.* (2010). 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 a Completely Randomized Block Factorial Design with three factors, i.e., the drying methods, fumigated and not fumigated nutmeg seeds, and storage durations, respectively.

RESULTS AND DISCUSSION

Moisture Content

One of the important factors causing the deterioration of foodstuff during storage is moisture content. SNI (2015) determined 10% as the maximum moisture content of nutmeg seeds during storage.

Based on the analysis of variance, there were no significant differences in moisture content among the applied treatments and their interaction (drying methods, fumigation, and storage duration). Ranges of moisture content of nutmegs with various treatments at the beginning of storage and after four months of storage were 7.4 - 7.6% and 7.1 - 7.7%, respectively. Those percentages were lower than the maximum limit of moisture content determined by SNI (2015) (Table 1).

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

Treatment	Moisture content (%)	
	Storage duration (months)	
	0	4
Smoke-dried and fumigated	7.4 ± 0.3[a]	7.4 ± 0.3[a]
Oven-dried and fumigated	7.5 ± 0.3[a]	7.1 ± 0.3[a]
Smoke-dried and unfumigated	7.6 ± 0.1[a]	7.7 ± 0.3[a]
Oven-dried and unfumigated	7.5 ± 0.3[a]	7.3 ± 0.4[a]

Moisture content is always in equilibrium with the relative humidity of a storage room. The humidity of the storage environment will be absorbed by foodstuff stored in the storage room having high relative humidity. On the other hand, foodstuff will lose its humidity if it is stored in a storage room having low relative humidity. Moisture content is also affected by the temperature of a storage room. In this study, the mean and range of temperature and relative humidity of the storage room decreased after four months of storage. The mean and range of temperature and relative humidity of the storage room at 0 - 4 months of storage were 27.9 ± 1.6 °C (23.2 - 32.8 °C) and 73.7 ± 4.2% (59.0 - 84.6%).

Percentage of Damaged Kernels

SNI (2015) determined damaged kernels including damages caused by insects and fungal attacks, cracked, broken, and shriveled kernels. Based on the analysis of variance, storage duration contributed to the significant differences in the percentage of damaged kernels, while drying methods, fumigation and their interactions did not contribute any significant differences. The percentage of damaged kernels of nutmeg increased after four months of storage (Table 2).

Table 2 Percentage of damaged kernels of nutmeg during storage

Storage duration (months)	Damaged kernels (%)
0	40.5 ± 5.3[a]
4	45.7 ± 3.8[b]

In this study, the damaged kernels were arguably caused by the occurrence of insects in nutmeg during storage. There were three insect larvae in the fumigated nutmegs. As many as 20 adult insects and 7 insect larvae were found in nutmeg that were not fumigated. These findings indicated that some insects were resistant to phosphine. According to Gautam *et al.* (2016), phosphine resistance in stored product insects occurs worldwide and is a major challenge to the continued effective use of this fumigant. Phosphine resistance is present in *Tribolium castaneum* and *Plodia interpunctella* populations in California almond storage and processing facilities.

Dharmaputra *et al.* (2018) reported that the dominant insect in nutmeg after being stored for four months was *Araecerus fasciculatus*. Haines (1991) and Rees (2004) also reported that *A. fasciculatus* is the most important insect infesting spices, including nutmeg. According to Childers and Woodruff (1980), *A. fasciculatus* is a primary insect pest in stored products, such as nutmegs in North and South America, Africa, Asia, Australia, and Europe.

Total Fungal Population

As many as eight fungal species were isolated in nutmeg in this research. Yeast was the dominant fungal species and was often isolated in nutmeg samples at the beginning of storage (Table 3). In all treatments, the population of each fungal species (except yeast) was relatively low (< 10 cfu/g wet basis). *Aspergillus flavus* was not found. These findings indicated that the postharvest handling method conducted in this research, from harvesting up to storing was appropriate to ensure good quality of nutmegs.

Based on analysis of variance (data transformed in log (x+1)), the interaction between drying methods and fumigation contributed to significant differences in total fungal population in nutmeg, while storage duration contributed to very significant differences.

Fumigated nutmeg, dried using smoke- and oven-dried methods did not show any significant differences in total fungal population. On the other hand, the total fungal population in unfumigated nutmeg, dried using the smoke-dried method was lower than that of using the oven-dried method (Table 4). The total fungal population in nutmeg after four months of storage was lower than that of the population at the beginning of storage (Table 5). It was assumed that the yeast did not grow in nutmeg having moisture content suitable for storing (< 10%) during four months of storage.

Table 3 Population of each fungal species

Treatment	Fungi	Fungal population (cfu/g)	
		Storage duration (month)	
		0	4
Smoke-dried and fumigated	*Aspergillus flavus*	1	0
	A. chevalieri	0	3
	A. ochraceus	1	0
	Cladosporium cladosporioides	4	1
	Penicillium citrinum	0	1
	P. islandicum	0	1
	Yeast	12	0
Smoke-dried and not fumigated	*C. cladosporioides*	2	1
	P. citrinum	1	0
	Yeast	2	0
Oven-dried and fumigated	*A. niger*	1	0
	C. cladosporioides	1	1
	P. citrinum	0	1
	Yeast	23	0
Oven-dried and not fumigated	*C. cladosporioides*	0	2
	P. citrinum	0	2
	Yeast	4,660	0

Table 4 Total fungal population in nutmeg

Drying method	Total fungal population (cfu/g wet basis)	
	Fumigated	Unfumigated
Smoke-dried	13 ± 9[b]	3 ± 3[a]
Oven-dried	14 ± 16[b]	2,332 ± 5,395[c]

Table 5 Total fungal population in nutmeg during storage

Storage duration (months)	Total fungal population (cfu/g wet basis)
0	1,178 ± 3,832[a]
4	4 ± 4[b]

According to Dharmaputra *et al.* (2015), the dominant fungal species in nutmeg collected from farmers and collectors in North Minahasa Regency were *Penicillium citrinum, A. niger, Eurotium repens, A. flavus,* and *Endomyces fibuliger*.

Ichinose *et al.* (2006) reported that *Eurotium* spp. was the predominant fungi found in 12 powdered nutmeg samples collected from retailers in Indonesia. *Aspergillus flavus* (1.0×10^2 cfu/g) was detected in one sample. According to Mandeel (2005), peeled seeds of nutmeg imported from India, Sri Lanka, Indonesia, and Brazil were found to be infected by *Aspergillus niger*, *A. flavus,* and *Rhizopus stolonifer*. The predominant species was *A. flavus*.

Toma and Abdulla (2013) reported 20 fungal and one yeast species isolated from 16 samples of spices and herbal medicines in Shekalla market, Erbil City, Iraq. Five of the 20 fungal species that contaminated nutmeg were *A. flavus* and *A. niger* (1×10^3 cfu/g, respectively), *A. ochraceus* (2×10^3 cfu/g), *A. versicolor* (6×10^3cfu/g), and *A. wentii* (2×20^3 cfu/g).

Aflatoxin Content

Aflatoxin B_1 and total aflatoxin of nutmeg in various treatments were lower than the limit detection determined by HPLC (< 0.92 μg/kg). These findings indicated that the postharvest handling implemented in this research was appropriate to ensure the good quality of nutmeg seeds on the occurrence of fungi and aflatoxin contamination during four months of storage.

Studies by Dharmaputra *et al.* (2018) found that: a) the total aflatoxin content of nutmegs originating from hand-picked ripe fruits was lower than that from ripe fruits fell on the ground; b) total aflatoxin content in nutmegs with shells was lower than that in nutmegs without shells; and c) total aflatoxin content in nutmegs was lower for those dried using the smoke- and oven-dried methods compared to those dried using the sun-dried method.

The smoke-dried method could prevent toxin production produced by toxigenic *A. flavus* (Uraih & Ogbadu 1982). Tabata *et al.* (1993) reported that aflatoxin was found in 3,054 of foodstuffs 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 (1,382 ppb). Takahashi (1993) also reported 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 with 54 and 58 ppb, respectively.

Aflatoxin is also detected in spices, aromatic herbs, and medicinal herbs collected from common markets, supermarkets, shops, and warehouses in Italy in the period of 2000 - 2005. Nutmeg was one of the six spices which were analyzed for aflatoxin content. One of the three nutmeg samples was contaminated with aflatoxin. The contents of aflatoxin B_1 and B_2 in nutmeg were 2.27 and 0.47 ppb, respectively, while aflatoxin G_1 and G_2 were not detected (Romagnoli *et al.* 2007).

As many as 52 samples of nutmeg were imported from India and Indonesia. Twenty-two samples were heat treated, while the other 30 samples were not heat-treated. The heat-treated samples were less contaminated by aflatoxin than those in the untreated samples. Nutmeg in powder form had more contamination than that of the whole nutmeg. Of the powdered nutmeg subjected to steam treatment, 72.5% of samples were positive for total aflatoxin contamination, with a range of 0 - 17.2 ppb (Pesavento *et al.* 2016).

CONCLUSION

The moisture content, fungal population, aflatoxin B_1, and total aflatoxin contents in nutmeg dried using smoke- and oven-dried methods, fumigated and not fumigated, still complied with requirements related to food

safety, although they were stored until four months. The results of this research could also determine the Critical Control Point (CCP) in the postharvest handling process of nutmegs, i.e., 1) choosing only ripe nutmeg fruits to be harvested; 2) harvesting method by preventing the ripe nutmeg fruits from falling on the ground; 3) drying process of nutmeg seeds should be conducted immediately after separating the nutmegs from the pulps and maces by using the smoke- or oven-dried methods; and 4) nutmegs were stored with the shells. The CCP can be used as a recommendation for farmers, collectors, and exporters concerning appropriate postharvest handling methods (Good Handling Practice) to ensure the good quality of nutmeg during storage.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the Government of Indonesia. Thanks are due to the CV Multi Rempah Sulawesi for the cooperation in conducting the preparation of the research, storing, and sampling of nutmeg; to Mr. Sunjaya, Mr. Edi Suryadi, Mrs. Ratnaningsih and Mrs. Syifa Fauzia for their assistance.

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