Fungal Population of Nutmeg (*Myristica fragrans*) Kernels Affected by Water Activity during Storage

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ABSTRACT

The aim of this investigation was to determine the effect of various water activities (a_w) on fungal population in nutmeg kernels during storage. The seed nutmegs were obtained from ripe fruits one week after they fell on the ground in North Minahasa Regency, North Sulawesi Province, Indonesia. The kernels (moisture content ± 10%) were stored 0, 15, and 30 days in various a_w (0.75, 0.80, 0.83, 0.90, 0.97) using saturated salt solutions at 29 °C in sorption containers. Serial dilution method followed by a pour-plate method in Dichloran 18% Glycerol Agar (DG18) was used to isolate and quantify the fungal population. Results revealed that kernels stored at a_w = 0.75 was not significantly ($p < 0.05$) different from at a_w = 0.80-0.83. Fungal population of kernels determined a_w and significantly ($p < 0.05$) influenced by duration of storage. Range of a_w 0.80-0.83 has a smaller total fungal population than $a_w \geq 0.90$. Thirteen different genera/species were isolated and identified including *Aspergillus* and *Eurotium* (6 species), *Penicillium* (3 species), *Fusarium* (2 species), 1 species each of *Cladosporium* or *Syncephalastrum,* and isolate A. The largest total fungal population (5.0×10^5 CFU g^{-1}) was present at the beginning of storage (a_w = 0.97) and it was dominated by *Penicillium citrinum* (2.6×10^5 CFU g^{-1}) followed by *Cladosporium cladosporioides* (1.7×10^5 CFU g^{-1}). After 30 days of storage (a_w = 0.97) the population of *P. citrinum* was still dominant with a population of 2.4×10^4 CFU g^{-1}. *Eurotium chevalieri* followed with a population of 1.2×10^4 CFU g^{-1}.

Keywords: Fungal population; moisture content; nutmeg; water activity

INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt.) is one of the most important export commodities. Approximately 75% (8,943 tones) of the world's nutmeg originates from Indonesia (Codex Alimentarius Commission, 2014). A large number of aromatic compounds render Indonesian nutmeg strong in taste with a distinctive aroma. In addition, the production practices of nutmeg are organic and do not rely on the use of chemical fertilizer or pesticides (Codex Alimentarius Commission, 2014). Based on the statistical data of the Directorate General of Estate Crops (2013), 133,732 hectares were in planted with nutmeg in 2012 in Indonesia to yield a production of 25,233 tons. The distribution area covered 19 provinces. The largest total area cultivated with nutmeg was located in North Moluccas (33%), followed by Nanggroe Aceh Darussalam (23%), North Sulawesi (18%), Moluccas (12%), West Java (5%), and the remaining provinces (9%).

Nutmeg mainly produced by small-scale farmers where harvest and warehouse storage condition often damage quality of the kernels. Nutmeg is harvested by collecting the seeds from the ground, drying them in open air on the ground and storing them in warm, humid conditions. During the drying process, the temperature and relative humidity (RH) are beyond control of the majority of farmers. As a result, stored nutmegs are susceptible to infection by fungi (Martins *et al*., 2001; Dharmaputra *et al*., 2015).

Soil is the source for contamination of diverse strains of fungi on spices in the fields (Kneifel *et al*., 1994). Postharvest practices such as drying, transportation and storage increase additional contamination (Hussain *et al*., 2012). Dried-stored nutmeg like other stored products are hygroscopic and tend to absorb water vapor from the environment. This process leads to an increase in kernel moisture content that results in accelerated deterioration. The deterioration of most spices is caused by filamentous fungus that survive during drying and storage (Stankovic *et al*., 2006; Toma and Abdulla, 2013). The hard shell of the nuts is a good barrier against fungal infection (Campbell *et al*., 2013), however, the degree of fungal infection depends on environment, moisture content, temperature, matrix composition, and physical damage (Chiou *et al*., 1984; Varga *et al*., 2009; Škrinjar *et al*., 2012,). Only a few studies concerning fungal infection related to water activity on nutmeg. To inhibit fungal development during storage, the moisture content of the surrounding air should be lowered (Jayas and White, 2003). The objective of this study was to investigate the effect water activities on fungal population on nutmeg kernels during storage.

MATERIALS AND METHODS

Collecting Nutmeg Seeds, Shelling, and Drying

We obtained nutmeg kernels from ripe fruits within one week falling on the ground (*i.e.,* the fruits naturally matured). We collected the fruits from plants cultivated in Kauditan sub-district, North Minahasa Regency, North Sulawesi Province, Indonesia in June 2015. The seeds were dried for 7 days by sun-drying and smoke-drying until their moisture content reach ± 10%. The seeds were shelled using a wooden hammer. Only intact kernels were used in this experiment.

Preparation of Saturated Salt Solutions

Five water activities (a_w) were controlled, *i.e.*, 0.75, 0.80, 0.83, 0.90 and 0.97 by saturated salt solutions *i.e.* NaCl, $(NH_4)_2SO_4$, KCl, $BaCl_2$ and K_2SO_4 respectively. The preparation of each salt solution followed the procedure of Kitic *et al*. (1986). As much as 200 g of salt was placed in a 5 l sorption container containing 200 l of distilled water. The solution was mixed homogenously until saturated and selected water activity (a_w) was obtained. We used distilled water ($a_{w =}$ 0.99) as a control. During storage the salt solutions were saturated at 29 °C. Based on thermo-hygrograph data obtained at every sampling, the a_w and temperature inside each sorption container remained constant throughout the entire duration of the experiment.

Packaging and Storage of Nutmeg Kernels

As much as 200 g of nutmeg kernels (moisture content ± 10%) were placed in a plastic netting sack. The sack was then hung in sorption containers (3 sacks per container). The kernels were stored in sorption containers with different a_w. The duration of storage was 0, 15, and 30 days. Three replicates (*i,e.,* = 3 sorption containers) were used for each a_w and storage duration. The sorption containers were placed on a wooden table at room temperature (± 28 °C).

Sampling Method for Obtaining a Working Sample

We collected samples of nutmeg kernels from each sack. The kernels from each sack in the three sorption containers were collected for each storage duration. Each sample was then ground for 30 seconds (25 000 rpm) using a Mill Powder RT-04 no. Serie 980923 (Mill Powder Tech. Co LTD, Taiwan), mixed homogenously and manually divided into 3 parts to obtain working samples to determine moisture content, fungal population and reserved samples.

Determination of Kernel Moisture Content and Fungal Population

Moisture content of the kernels was determined based on wet basis using a distillation method according to Standard Nasional Indonesia (SNI) (1993). All fungal species from each sample were isolated and quantified using a serial dilution method, followed by the pour-plate method on Dichloran 18% Glycerol Agar (DG18) (10 g/L glucose, 5 g/L peptone, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4.7H_2O$, 220 g/L glycerol, 15 g/L bacto agar, 2.0 mg/L dichloran and 100 mg/L chloramphenicol (Pitt and Hocking, 2009). The plates were incubated for 7 days at room temperature (± 28 °C). Fungal species was determined based on the procedure of Pitt and Hocking (2009).

Statistical Analysis

We employed a completely randomized design factorial. The factors were water activities and storage duration. We analyzed the observed data using analysis of variance (ANOVA) for statistically significant differences, followed by Duncan's multiple range test at the 5% probability level. We used the Statistical Analysis System (SAS) software (version 9.1 3) statistical package (SAS Institute Inc. North Carolina).

RESULTS AND DISCUSSION

Equilibrium Kernels Moisture Content

In order to prevent fungal deterioration during storage, the highest moisture content of nutmeg kernel should be 10% (Standard Nasional Indonesia, 1993). However, environmental factors particularly a_w affect the physical properties of the kernels. In storage, dry kernels are hygroscopic and tend to absorb or release water from and into surrounding air, this process continues until the kernels reach equilibrium with the moisture in the atmosphere (Daniel *et al.*, 2012; Jyoti and Malik, 2013). Moisture equilibrium of the kernels in the sorption containers was indicated by their attaining a constant weight after a certain storage duration. This equilibrium was attained after different amounts of time depending on a_w (saturated salts solutions used). The shortest moisture equilibrium of the kernels occurred at $a_w = 0.75$ after 10 days of storage; no significant differences ($p < 0.05$) in kernels weight were observed when the storage duration was 15 or 30 days. Previous studies demonstrated that the growth of storage fungi is inhibited at $a_w \leq 0.75\%$ and completely stopped at $a_w \leq 62\%$ (Jyoti and Malik, 2013). The longest equilibrium occurred at $a_w = 0.97$ after 20 days of storage. The kernels minimum weight occurred at $a_w = 0.75$ (202.4 g) and the maximum weight gain occurred at $a_w = 0.97$ (210.6 g). Fungal population on storage kernels was affected significantly ($p < 0.05$) by a_w and equilibrium kernel moisture content in the sorption containers. The relationship between a_w and equilibrium kernels moisture content during storage was shown in (Figure 1).

Fungal Population

We isolated 13 fungal species and one unidentified filamentous fungus (isolate A) from kernels at various water activities over the course of 30 days of storage.
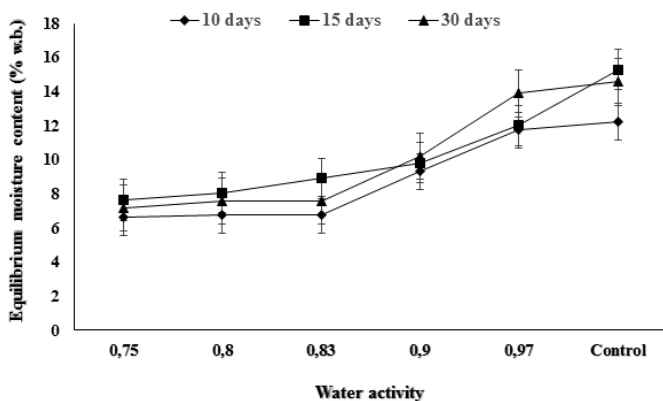

Figure 1. Moisture content of nutmeg kernels after different duration of storage for various water activities at 29 °C, (control, $a_w = 0.99$)

These species included *Aspergillus candidus, A. flavus, A. niger, A. tamarii, A. wentii, Eurotium chevalieri, Fusarium semitectum, F. verticillioides, Penicillium citrinum, P. islandicum, Penicillium* sp*., Cladosporium cladosporioides, Syncephalastrum racemosum*, and isolate A.

Water activity, kernel moisture, and storage duration determined fungal growth on nutmeg kernel. The significantly difference of a_w 0.75-0.83 and 0.90 ($p < 0.05$) affect total fungal populations. *Cladosporium* and *Fusarium* were most prevalent at the beginning of storage. *Aspergillus* spp. grew after several days of storage. Elshafie *et al.* (2002) reported that fungal infection occurred either in the field or while the ripe nutmegs were in contact with the soil during harvesting. The presence of fungi at the beginning of the storage revealed that the fungal infection might have occurred during pre-harvest and harvesting. The process of infection continue while the water activity and kernel moisture maintained fungal growth.

The effect of water activity and storage duration on total fungal population by can be seen in the data presented in Table 1. Each fungal species grow on certain a_w range. During storage, increasing of a_w and moisture content followed by the increased of fungal population. However, at $a_w = 0.75$, 0.80, and 0.83 low fungal populations were found. The population increased significantly ($p < 0.05$) for kernels stored at $a_w \geq 0.90$. *Cladosporium cladosporioides* was the predominant species during the first period of storage. A relatively small population of this species (0.2×10 CFUg^{-1}) was present at $a_w = 0.75$; the population then grow abundantly at $a_w = 0.97$ (1.7×10^5 CFU g^{-1}). However, the population decreased after 30 days of storage and was replaced by *Penicillium, Aspergillus* and *Eurotium*. Bensch *et al.* (2010) observed that conidia of *C. cladosporioides* were aerial and phyllosphere. We assumed that the fungal infection occurred before fell to the ground. Similar to *C. cladosporioides,* other species such as *Fusarium verticillioides* and *F. semitectum* were found during in the first period of storage at a_w 0.80 and 0.97.

Among storage fungi, *P. citrinum* was considered as the most common fungal infection during storage. The species was found in all a_w levels. At the beginning of storage population of *P. citrinum* was the highest (2.6×10^5 CFU g^{-1}). Toma and Abdulla (2013), El-Gali (2014) reported that *Penicillium* spp. and *Aspergillus* spp. are the most predominant fungi that associated with spices. Conidia of *Penicillium* spp. and *Aspergillus* spp. are easily grow in all kinds of substrates and dispersed in the air, therefore they grow abundantly in environment (Bennet, 2010; Krijgsheld *et al.*, 2012).

Eurotium chevalieri was the third most frequent found throughout storage duration. Its population increased with the increased of storage duration. Although *E. chevalieri* was able to grow at low and wide range a_w, its highest population

Table. 1. Total fungal population on nutmeg kernels after different storage duration at various water activities

Fungal species and isolate	Storage duration (day) / water activity / fungal population (CFU g⁻¹ w.b.)																	
	0						15						30					
	0.75	0.80	0.83	0.90	0.97	C	0.75	0.80	0.83	0.90	0.97	C	0.75	0.80	0.83	0.90	0.97	C
1. *Aspergillus candidus*	0	0	0	0	0	0	0	0	0	0.3×10	0	0	0	0	0	0	0	0
2. *A. flavus*	0	0	0	0	0	0	0	0	0	0.3×10	0	0	0	0	0	0	0	0
3. *A. niger*	0	0	0	0	0	2.0×10^3	0.2×10	0.1×10	0.2×10	0	0	3.8×10^3	3.5×10^2	0	0.2×10	0.1×10	0.2×10	7.3×10^3
4. *A. wentii*	0	0	0	0	0	1.0×10^2	0.5×10	0.1×10	0.3×10	0.1×10	0	1.0×10^3	2.7×10	0	0.3×10	0.1×10	0.4×10	1.3×10^3
5. *A. tamarii*	0	0	0	0	2×10^3	2.6×10^3	1.0×10	0	0.4×10	0	2.9×10^3	1.0×10^4	1.4×10	0	1.0×10	0	1.3×10	3.3×10^3
6. *Cladosporium cladosporioides*	0.2×10	0.4×10	0.3×10	0.9×10	1.7×10^5	5.0×10^2	0.5×10	0.6×10	0.3×10	4.0×10	1.0×10	0	1.4×10	3.0×10	0	0	0	0
7. *Eurotium chevalieri*	0.2×10	0.2×10	0	3.6×10^2	9.8×10^3	1.6×10^4	1.6×10	0.2×10	0.4×10	9.5×10^2	7.5×10^3	3.6×10^4	1.0×10	0.4×10	1.3×10	1.1×10^4	1.2×10^4	5.9×10^3
8. *Fusarium semitectum*	0	0	0	0	2.3×10^2	0	0	0	0	0	0	0	0	0	0	0	0	0
9. *F. verticillioides*	0	0.1×10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10. *Penicillium citrinum*	0.1×10	1.4×10	1.2×10	1.0×10	2.6×10^5	2.6×10^3	0.3×10	0.5×10	0	0.1×10	2.0×10^4	1.6×10^4	0	0.1×10	0	1.2×10^2	2.4×10^4	20×10^4
11. *P. islandicum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.0×10^2	8.6×10^2
12. *Penicillium* sp.	0	0	0	0	6.2×10^4	0	0	0	0	0	0	0	0	0	0	0	0	0
13. *Syncephalastrum racemosum*	0.1×10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14. Isolate A	0	0.2×10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0.6×10	2.3×10	1.5×10	3.8×10^2	5.0×10^5	2.4×10^4	4.1×10	1.5×10	1.6×10	9.1×10^2	3.0×10^4	6.7×10^4	1.0×10^2	3.5×10	2.8×10	1.1×10^4	3.5×10^4	2.2×10^5

CFU = colony forming unit, C = control (a_w = 0.99)

occurred at a$_w$ = 0.97. *Syncephalastrum racemosum* was found at the beginning of storage at a$_w$ = 0.75. Its population was 0.1×10 CFU g^{-1}. Pitt and Hocking (2009) reported that *S. racemosum* was tolerant species that able grow at low a$_w$. The ability of *S. racemosum* to grow in glucose medium at minimum a$_w$ = 0.84 was reported by Hocking and Miscamble (1995). The presence of the species was the same a$_w$ with that of *C. cladosporioides*, *E. chevalieri*, and *P. citrinum*. It was approved that *S. racemosum* was xerophilic and able to grow at low a$_w$ (≤ 0.85). Five species of *Aspergillus* were isolated from the kernels after 15 and 30 days of storage. As xerophilic fungi, most species of the *Aspergillus* found at a$_w$ ≤ 0.85. The ability of xerophilic fungi to prevent water loss from their cell due to accumulation of polyol such as glycerol, arabitol, erythritol and mannitol was reported by George *et al.* (2004). Previous study showed that minimum a$_w$ for *A. candidus* was at 0.75 (Pitt and Hocking, 2009). Gibson *et al.* (1994) reported the minimum a$_w$ for *A. flavus* was at 0.81. Among *Aspergillus* isolated at 30 days of storage at a$_w$ = 0.75, *A. niger* was the highest population (3.5×10^2 CFU g^{-1}). Relationship between seed moisture content during storage, a$_w$ and fungal growth was presented on Figures 2, 3, and 4.

Cladosporoium cladosporioides was one of the species that was able to grow in wide a$_w$ range. The species was found particularly in high seed moisture content. and present in each storage duration (Figure 2). The other species such as *F. verticillioides* and *F. semitectum* were found at a$_w$ = 0.80 and 0.97. As a field fungi, genus *Fusarium* were found only at the beginning of storage while seed moisture content still high. However, the fungus becoming inactive or probably dormant after 15 days of storage. The other species such as *Eurotium chevalieri* and *P. citrinum* were found at a$_w$ = 0.75-0.97. At the beginning of storage *Aspergillus niger*, *A. tamarii* and *wentii* were found only in control (a$_w$ = 0.99), however, after 15 days (Figure 3) and 30 days (Figure 4), these species found at low moisture content and able to grow at a$_w$ range from 0.75 to 0.97.
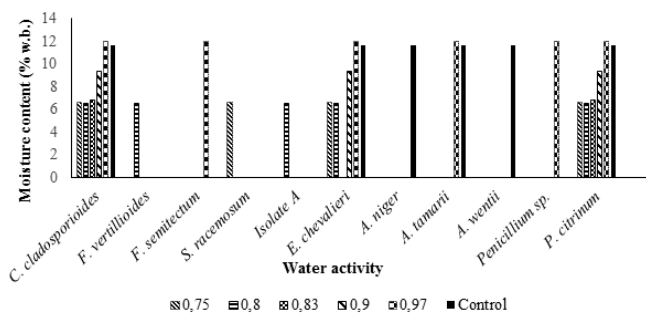

Figure 2. Interaction between water activity, moisture content and fungal population of nutmeg kernels at the beginning of storage
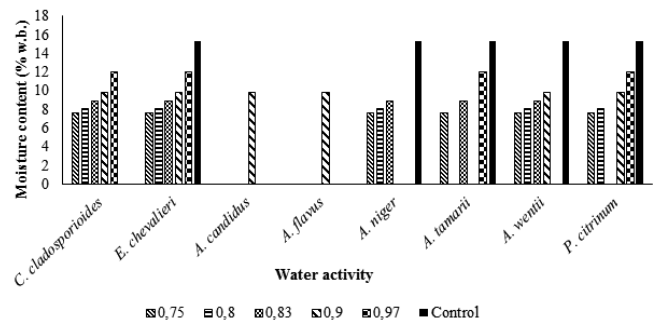

Figure 3. Interaction between water activity, moisture content and fungal population of nutmeg kernels after 15 days of storage
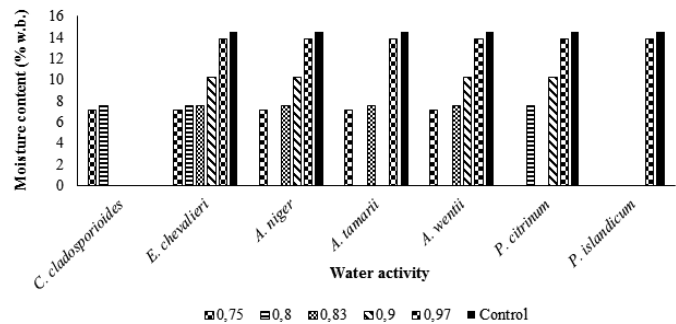

Figure 4. Interaction between water activity, moisture content and fungal population of nutmeg kernels after 30 days of storage

Each fungal species grows on its required a$_w$. Previous studies reported that critical tolerable minimum of most fungi and yeast at a$_w$ = 0.62 (Sjarief,1983). Pitt (1975) observed that several species *Aspergillus*, *Eurotium*, and *Penicillium* were xerophilic and were able to grow at minimum a$_w$ such as *A. candidus* (0.75), *A. flavus* (0.78), *A. niger* (0.77), *A. wentii* (0.84), *E. chevalieri* (0.71), *P. citrinum* (0.80), and *P. islandicum* (0.83). Aihara *et al.* (2002) reported that growth inhibition were occurred on *Cladosporium. sphaerospermum* and *C. cladosporioides* at minimum a$_w$ requirement. This results is consistent with finding of Pratiwi *et al.* (2015) who stated that the smallest diameter colony and cell mass of *A. flavus* BIO 2237 was found while the fungus grow at low a$_w$.

It was assumed that the propagules of *C. cladosporioides*, *F. semitectum*, *F. verticillioides*, *P. citrinum*, *E. chevalieri*, and *Aspergillus* spp. were in contact on seed while the seeds were still in the field and resist on the seeds during drying and storage. Therefore, seed deterioration caused by fungal infection cannot be prevented (Balesevic-Tubic *et al.*, 2005). Controlling a$_w$ during storage might prevent fungi to grow (Azis *et al.*, 1998). Postharvest handling methods particularly drying and storing at a$_w$ = 0.75-0.83, and maintaining low moisture content of the nutmeg during storage were able to prevent the growth of spoilage fungi.

CONCLUSION

Nutmeg kernels harvested by picking from the ground were infected by field and storage fungi. *Cladosporium cladosporioides*, *P. citrinum*, and *E. chevalieri* were the most frequent fungi found and present at a wide range of a_w during storage. Total fungal population increased with the increase a_w and kernel moisture. Controlling proper condition by maintaining low a_w during storage is required to minimize fungal population.

REFERENCES

Aihara, M., Tanaka, T., Ohta, T. and Takatori, K. (2002). Effect of temperature and water activity on the growth of *Cladosporium sphaerospermum* and *Cladosporium cladosporioides*. *Biocontrol Science* **7**(3): 193–196.

Aziz, N.H., Youssef, Y.A., El-Fouly, M.Z. and L.A. Moussa. (1998). Contamination of some common medicinal plant samples and spices by fungi and their mycotoxin. *Botanical Bulletin Acad Sinica* **39**(4): 279–285.

Balesevic-Tubic. S., Malenèiæ, D., Tatiæ M. and Miladinovic, J. (2005). Influencing of aging process on biochemical changes in sunflower seed. *Helia* **28**(42): 107–114.

Bennett, J.W. (2010). *Aspergillus Molecular Biology and Genomics*, edited by Masayuki Machida and Katsuya Gomi. Caister Academic Press, United Kingdom.

Bensch, K., Groenewald, J.Z., Dijksterhuis, J., Starink-Willemse, M., Andersen, B., Summerell, B.A., Shin, H.D., Dugan, F.M., Schroers, H.J., Braun, U. and Crous, P.W. (2010). Species and ecological diversity within the *Cladosporium cladosporioides* complex (Davidiellaceae, Capnodiales). *Studies in Mycology* **67**: 1–94.

Campbell, B.C., Molyneux, R.J. and Schatzki, T.F. (2013). Current research on reducing pre- and postharvest aflatoxin contamination of US almond, pistachio and walnut. *Journal of Toxicology* **22**: 225–266.

Chiou, R.Y.Y., Koehler, P.E. and Beuchat, L.R. (1984). Hygroscopic characteristics of peanut components and their influence on growth and aflatoxin production by *Aspergillus parasiticus*. *Journal of Food Protection* **47**: 791–794.

Codex Alimentarius Commission (2014). Joint FAO/WHO Food Standards Programme Codex Committee on Spices and Culinary Herbs, First Session. Koichi, India 11–14 February 2014.

Daniel, I.O., Oyekale, K.O., Ajala, M.O., Sanni, L.O., Okelana, M.A., Adetumbi, J.A., Akintobi, D.A.C. and Adebisi, M.A. (2012). Moisture sorption in commercial hybrid maize (*Zea mays* L.) seeds during storage at ambient tropical conditions. *Research Journal of Seed Science* **5**(1): 32–37.

Dharmaputra, O.S., Ambarwati, S., Retnowati, I. and Nurfadila, N. (2015). Fungal infection and aflatoxin contamination in stored nutmeg (*Myristica frargrans*) kernels at various stages of delivery chain in North Sulawesi Province. *Biotropia* **22**(2): 129–139.

Directorate General of Estate Crops (2013). Tree Corp Estate Statistics of Indonesia 2012-2014. Spices and Beverage Crops. Ministry of Agriculture. Jakarta, Indonesia.

El-Gali (2014). Spices in original form. *Global Journal of Scientific Researches* **2**(3): 83–88.

Elshafie, A E., Al-Rashdi, T.A., Al-Bahri, S.N. and Bakheit, C.S. (2002). Fungi and aflatoxins associated with spices in the Sultanate of Oman. *Mycopathologia* **155**(3): 155–160.

George, J., Ruijter, G., Visser, J. and Rinzema, A. (2004). Polyol accumulation by *Aspergillus oryzae* at low water activity in solid-state fermentation. *Microbiology* **150**: 1095–1101.

Gibson, A.M., Baranyi, J., Pitt, J.I., Eyles, M.J. and Roberts, T.A. (1994). Predicting fungal growth: the effect of water activity on *Aspergillus flavus* and related species. *International Journal of Food Microbiology* **23**: 419–431.

Hocking, A.D. and Miscamble, B.F. (1995). Water relations of some Zygomycetes isolated from food. *Mycological Research* **99**(9): 1113–1118.

Hussain, A., Sohail, M. and Shafqatullah (2012). Aflatoxin contamination of spices sold in different markets of Peshawar. *Journal Chemical Society of Pakistan* **34**(5): 1052–1055.

Jayas, D.S. and White, N.D.G. (2003). Storage and drying of grain in Canada: low cost approaches. *Food Control* **14**(4): 255–261.

Jyoti and Malik, C.P. (2013). Seed deterioration: A review. *International Journal of Life Sciences Biotechnology and Pharma Research* **2**(3): 374–385.

Kitic, D., Denise, C., Jardim, P., Favetto, G.J., Resnik, S.L. and Chirife, J. (1986). Theoretical prediction of the water activity of standard saturated solutions at various temperatures. *Journal of Food Science* **51**(4): 1037–1041.

Kneifel, Wolfgang, Berger and Erika (1994). Microbial criteria of random samples of spices and herbs retailed on the Austrian market. *Journal of Food Protection* **10**: 854–941.

Krijgsheld, P., Bleichrodt, R., van Veluw, G.J., Wang, F., Müller, W.H., Dijksterhuis and J. Wösten, H.A.B. (2012). Development in *Aspergillus*. *Studies in Mycology* **74**: 1–29.

Martins, M.L., Martins, H.M. and Bernardo, F. (2001). Aflatoxins in spices marketed in Portugal. *Food Additives and Contaminants* **18**: 315–319.

Pitt, J.I. (1975). Xerophilic fungi and the spoilage of foods of plant organisms. *In*: Duckworth, R.B. *Water Relations of Foods*, p. 273–307. Academic Press, London.

Pitt, J.I. and Hocking, A.D. (2009). *Fungi and Food Spoilage.* 3rd edn. Springer, New York.

Pratiwi, C., Rahayu, W.P., Lioe, H.H., Herawati, D., Broto, D. and Ambarwati, S. (2015). The effect of temperature and relative humidity for *Aspergillus flavus* BIO 2237 growth and aflatoxin production on soybeans. *International Food Research Journal* **22**(1): 82–87.

Syarief, R. (1983). *Influence des conditions hydrothermiques de stokage sur les qualites microbiologiques et technologiques du grain.* PhD dissertation, Universite de Nantes, French.

Škrinjar, M.M., Janković, V.V., Vesković, S.M., Moračanin and Vukojević, J.B. (2012). Xerophilic moulds isolated from spices used in meat industry as potential producers of mycotoxins. *Proceedings National Science Matica Srpska Novi Sad* **123**: 7–16.

Standar Nasional Indonesia (SNI) 01-0006 (1993). *Biji Pala.* Badan Standardisasi Nasional.

Stankovic, N., Komic, L. and Kocic, B. (2006). Microbiological correctness of spices on sale in health food stores and supermarket in Nis. *Acta Fac Med Naiss* **23**(2): 79–84.

Toma, F.M. and Abdulla, N.Q.F. (2013). Isolation and identification of fungi from spices and medicinal plants. *Research Journal of Environmental and Earth Sciences* **5**(3): 131–138.

Varga, J., Frisvad, J.C. and Samson, R.A. (2009). A reappraisal of fungi producing aflatoxins. *World Mycotoxin Journal* **2**(3): 263–277.