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Research Paper

The effects of various storing temperatures in suppressing *Aspergillus flavus* and mycotoxin contaminations in hermetically packed dried nutmeg (*Myristica fragrans* Houtt) seed

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ABSTRACT

Nutmeg seeds from Indonesia are an important agricultural commodity with various culinary and medicinal uses. However, they often face issues with fungal and aflatoxin contaminations, leading to increased border rejections. To address those issues, a study was conducted to minimize the risk of contamination by adjusting storage temperatures for whole nutmeg seeds without shells. The research involved storing nutmeg seeds at different temperatures (16, 22, and 28 °C) over three periods (0, 30, and 60 days) using hermetically sealed packaging to control *Aspergillus flavus* growth and aflatoxin contamination. The findings revealed that storing the seeds at 16 °C resulted in optimal moisture content (5–6%) and minimal weight loss (below 4%), preventing significant physical attribute degradation. This approach also effectively controlled mold growth (the least colony diameter = 14–20 mm), and subsequently kept aflatoxin B_1 (0.8–4.3 μg/kg), total aflatoxin (1.5–4.5 μg/kg), and ochratoxin A (0.6–1.1 μg/kg) contaminations below the allowable limit for up to 60 days. These findings are significant as they provide a viable solution for long-distance distribution, thereby reducing the risk of border rejections and increasing market value in the nutmeg industry.

1. Introduction

Nutmeg (*Myristica fragrans* Houtt) has been one of the most strategically impacted plantation commodities globally. As an exporter, Indonesia has significantly supplied around 75%, chased by Granada for almost 25%, in a particular case of European country's demand (Dharmaputra et al., 2022; Kabak and Dobson, 2016). Hence, the supply chain of nutmeg cultivated initially in Indonesia has been substantially crucial as one of the most critical raw materials for the food and pharmaceutical industries (Dharmaputra et al., 2018; Punnathara, 2011).

Unfortunately, there is a high risk of fungal and aflatoxin contaminations, which occurred as a severe consequence of inappropriate environmental storage conditions (Rahayu et al., 2020) during distribution and has been primarily a leading cause of many border rejections. Some previous studies have strongly linked these rejections to a significant economic loss of 25% from the sum of global production reaching 2.8 million tons during 2016–2017 (FAOSTAT, 2017; Thanushree et al., 2019). In another study, Moses et al. (2013) mentioned that high correlations between hot temperature and high air humidity were the most conducive factor for triggering the growth of some molds producing mycotoxins such as *Aspergillus* sp., *Fusarium* sp, and *Penicillium* sp. frequently found on several tropical countries.

High concern for food safety caused by aflatoxin contaminations has been regularly governed in many developed countries and urgently needs to be addressed. In general, aflatoxins have been divided into aflatoxins B_1, B_2, G_1, and G_2, and aflatoxin B_1 has been recognized as

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group I, the most carcinogenic agent for humans (IARC 2012). These toxins have been strongly associated with some strategic agricultural commodities, such as corn, peanut, pistachio, pepper, and even nutmeg (Dharmaputra et al., 2013, 2018; Inan et al., 2007).

Hermetic plastic packaging has been widely considered a promising option for eliminating the potent risk of preventing a successive growth of fungal-producing aflatoxins. Also, this package has been proven to be secure, less expensive, and environmentally friendly in managing mycotoxin contamination (Akhila et al., 2022; Kumar and Kalita, 2017). As previously reported, it has formed a new air composition with a minimum oxygen level, significantly affecting suppressing mold and aflatoxins production (Van Long et al., 2016) in particular storage periods.

Based on our knowledge, a study evaluating the impact of temperature fluctuations on the storage of dried nutmeg seeds, which have been separated from the shells and hermetically sealed for a specific period of time, while also addressing measures for controlling fungal growth and mycotoxin contamination within permissible levels over an extended period, is still limited. Through this research, a simulation for storing those commodities needs to be adapted, particularly in some warehouse exporters' facilities, at least to build a series of practical improvements finally dealing with mitigating those risks of contamination and hence, it is going to be significantly impacted on declining notifications of non-compliance.

2. Materials and methods

2.1. Time, site Locations, and nutmeg seeds

This study was conducted at a well-designed research site in Bekasi Regency, West Java, Indonesia, recognized as the Applied Research Institute of Agricultural Quarantine (ARIAQ) from January to December 2022. Thirty kilograms of dried nutmeg seeds without shells (grade ABCD) originating from several local plantations located in Maluku Province were differently supplied by the two national exporter companies. Before being distributed to the final destination, the whole nutmeg seeds were wrapped in a plastic sack; then, they were sent through land transportation using the local vehicle for almost 3 h. All the samples were kept at room temperature for a night.

A series of simulations of storage circumstances and the fungal test colony were technically conducted on a research site of ARIAQ. The determination of aflatoxins and ochratoxin A level was substantially analyzed in a testing laboratory in Jakarta, Special Region of Jakarta, Indonesia, organized by the Directorate of Standardization and Quality Control, Ministry of Trade.

2.2. Initial toxigenic test of Aspergillus flavus isolate

Appearance toxigenic isolates of *Aspergillus flavus* indigenously extracted from those nutmeg seed samples were mainly detected using a selective medium called Aspergillus differentiation medium base M1127 (HIMedia Laboratories Private Limited, Mumbai India). As an initial step, isolation, and purification of those targeted mold isolates on this selective medium were carried out. The next step was filtration using 200 mL liquefied coconut medium, commercially known as "SUN KARA" (PT. Kara Santan Pertama, Jakarta Indonesia), referred to a method developed by Dharmaputra et al. (2018). The final step was determining aflatoxin through a series of quantitative analyses using LC-MS/MS (Dzuman et al., 2014; Garcia-Moraleja et al., 2015).

2.3. Simulations of the three different temperature storage

The dried nutmeg seeds observed in this study were divided into two types of samples. The first samples were initially gained from the national exporter home based in Ambon, Maluku Province (known as type I), and the second ones were supplied from another exporter home based in Cengkareng, Banten Province (recognized as type II). Both samples were hermetically packed, weighing around 500–510 g in each plastic package (Grain Pro, Washington DC, USA). These simulations were initiated by inserting 10 pieces of type I and 30 pieces of type II into three storage rooms (3 x 3 × 2.5 m^3) at temperatures 16, 22 and 28 °C. The daily alterations of store circumstances were monitored from four thermo-hygrometers, HTC-2 (HTC Corporation, Xindian, Taiwan) placed on each stored one.

The whole period for these simulations was set up to be 60 days. To ensure the properness of those critical attribute alterations, periodic observations were conducted from 0, 30–60 days of storage. The two samples were periodically released for further physical and chemical examinations at each time interval.

2.4. Weight loss

This parameter was determined promptly based on a method described by Arslan and Ozcan (2012).

2.5. Moisture content

Moisture content was determined using the ISO 939:2021 method. The amount of water content is entrained by azeotropic distillation using an organic solvent immiscible with water and collected in a graduated tube.

2.6. Colony growth of Aspergillus flavus

A method used in this step referred to Dharmaputra et al. (2013). Colony diameter, as one of two critical parameters, was regularly measured during the incubation period until 5 × 24 h. Another one was the color intensity of the *A. flavus* colony quantitatively measured using a color reader CR 13 (Konica Minolta Sensing Inc, Osaka Japan) as described by Prasetia et al. (2018) once at the end of the storage period was achieved.

2.7. Determination of aflatoxin and ochratoxin A

A modified version of the official AOAC method 991.31 (AOAC, 2005) was used in this study. The multi-mycotoxin (aflatoxins B_1, B_2, G_1, G_2 and ochratoxin A) immunoaffinity columns, Afla Ochra HPLC Colums 25/box Vicam (Waters Cooperation, Milford MA, USA) was used for simultaneous purification before detection step. HPLC equipped with a photochemical reactor and fluorescence detector is used to determine the type and amount of aflatoxin and ochratoxin A in samples.

Aflatoxin content was analyzed utilizing HPLC Model 2690 Alliance and Model 474 fluorescence detector (Waters Cooperation, Milford MA, USA) with an immunoaffinity column, Afla Ochra HPLC Colums 25/box Vicam (Waters Cooperation, Milford MA, USA) based on a method of AOAC 991.31 (Weaver and Trucksess, 2010). Aflatoxins standard solutions were based on four sources of stock – A6636, A9887, A0138, and A0263 (Sigma P-3813, Sigma Aldrich, St. Louis MO, USA). The standard working solutions of aflatoxins were prepared at 0.2, 0.5, 2, 5, 10, and 20 ng/ml. The coefficient of linearity (R^2) was 0.998. Meanwhile, the limit of detection, as well as quantification, was subsequently set up at 0.58 and 0.78 μg/kg.

Whereas on determination of ochratoxin A content was quantified through HPLC (Model 2690 Alliance and Model 474 fluorescence detector (Waters Cooperation, Milford MA, USA) equipped an immunoaffinity column Afla Ochra HPLC Colums 25/box Vicam (Waters Cooperation, Milford MA, USA) (Micromass Quattro Micro triple-quadrupole mass spectrometer with an Agilent 1100 LC MSD Model G1946D Mass (Agilent Technologies Inc, Santa Clara CA, USA)) referred on the standard method of CEN 14132 (2009). Ochratoxin A (Sigma Chemicals, France) standard solutions were clear of benzene and carboxypeptidase (Tozlovanu and Leszkowicz, 2010). The standard

working solutions of ochratoxin A were arranged from 0.2, 0.8, 1, 4–8 ng/ml. The linearity coefficient (R^2) was 0.999. Detecting limit and quantifying one were subsequently designed at 0.58 and 0.8 μg/kg.

2.8. Statistical analysis

A factorial utterly randomized design focused on the temperature of storage, i.e., 16, 22, and 28 °C, and the interval of storing period, i.e., 0, 30, and 60 days, operated in triplicates, were selected in this research. A significant difference gained from the analysis of variance was further analyzed through the Duncan Multiple Range Test (DMRT) at a 95% confidence level using SPSS 20.0 and stated as mean ± SE.

3. Results

3.1. Initial aflatoxin production expressed from the toxigenic assay

Aflatoxin production expressed as the result of enriching *A. flavus* grown in a selective medium is strongly related. It means no significant inhibition emerged after 5 × 24 h incubation at room temperature. Furthermore, the orange color observed behind the Petri dish indicated this pigment is specifically related to the group of aflatoxigenic mold. (Fig. 1a–b).

A confirmatory test conducted to determine the level of aflatoxin B1 on the tested ones was positively linked. This result proved that the samples chosen for this study were naturally contaminated by this mold, which is primarily toxigenic (Table 1). Aflatoxin B_1 and total aflatoxin contained in those two types of samples were subsequently 21.44–23.35 and 22.48–25.39 μg/kg. This contamination was seemingly unavoidable due to the heterogeneity of mold contamination addressed to the origin of the sample gained.

This data showed that low standard errors from those previous assays indicated that the final values of both chemical contaminants were usually coherent. Therefore, those variations were not critical in determining the overall concentration values of aflatoxin B_1 and aflatoxin total, even in each of their individual calculating repeats.

3.2. Temperature and air humidity data during periodical storage

Both temperature and air-humidity fluctuating data must be monitored to ensure the achievement of the critical level of environmental storage. In thirty days of storage, a series of observations of storing temperatures demonstrated that the targeted values were not achieved as a whole. Similar results were also gained in the air-humidity profiles. In the second month of the storage period, these two leading indicator profiles shifted into a close range of the targeted values (Fig. 2 a-b).

Table 1
Aflatoxin B_1 and total contents extracted from the toxigenic *A. flavus* isolate.

Type of samples	Aflatoxin B_1 (ug/kg)	Aflatoxin total (ug/kg)
Type I	21.44 ± 0.031	22.48 ± 0.031
Type II	24.35 ± 0.025	25.39 ± 0.025

Those linearity trends observed were consistent to be gradually lowered. It summarized that a lowering temperature profile was in line with declining air humidity, which was monitored until the end of the storage period. Moreover, these results were also confirmed with R^2-values near one on each of the designed store temperatures, indicating almost no interferences significantly affecting the two critical conditions.

3.3. Moisture content alterations of whole dry nutmeg during storing-time

Environmental conditions have played a key role in determining the moisture content fluctuation of nutmeg. The trends observed on those two types of nutmeg samples stored in three different temperatures have yet to achieve a critical quality, shown by a whole of moisture content assays lower than 10% (Fig. 3 a–b). However, at the end of storage, those samples preserved at 16°C were in the lowest ones, at around 5–6%. This finding was quite stable in maintaining those levels moderately away from the critical value.

The trends between the two types of samples were contradictory. However, for the first sample type, the lowest store temperature significantly impacted the minimalization of the moisture content. Meanwhile, in the second type of sample stored in the lowest one, a stable pattern in maintaining the same observed parameter was distinctly apparent until the end of storage. Variations of sample origin sites, harvesting time, the application of a series of postharvest treatments, and environmental distribution conditions have possibly affected the appearance of those contrasting trends. Therefore, the lowest temperature level used in this study was steadily sufficient due to the minimum impact of fluctuation caused.

3.4. Changes in weight loss occurred during the whole storage period

Interestingly, several mainly formed trends were not linearly associated with the result previously described. There was a consistent effect regarding the inclination of data until the final observation period. This finding was consistently observed on the two types of samples until the end of the storage period (Fig. 4 a-b).

The significant inclinations as briefly a whole were similar until the end of the storage period. However, for the second type of sample, the

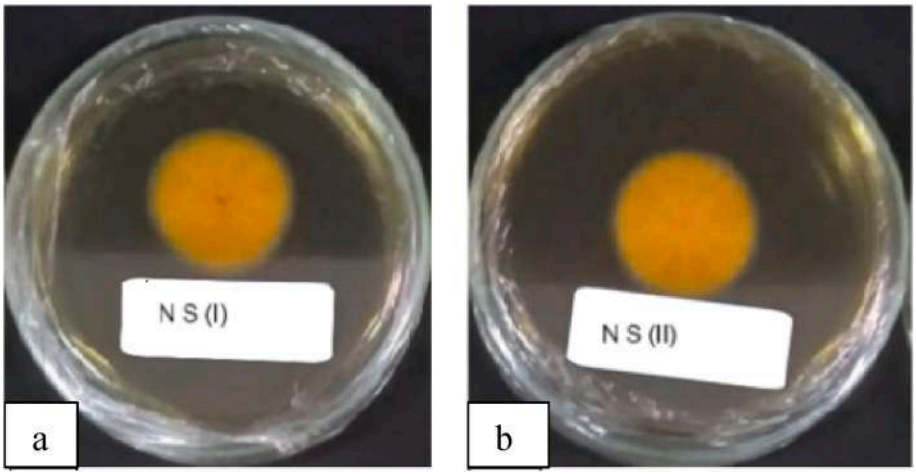

Fig. 1. Initial marks demonstrating a thread of toxin released by *A. flavus* extracted both from a.) type I and b.) type II of whole nutmeg samples.

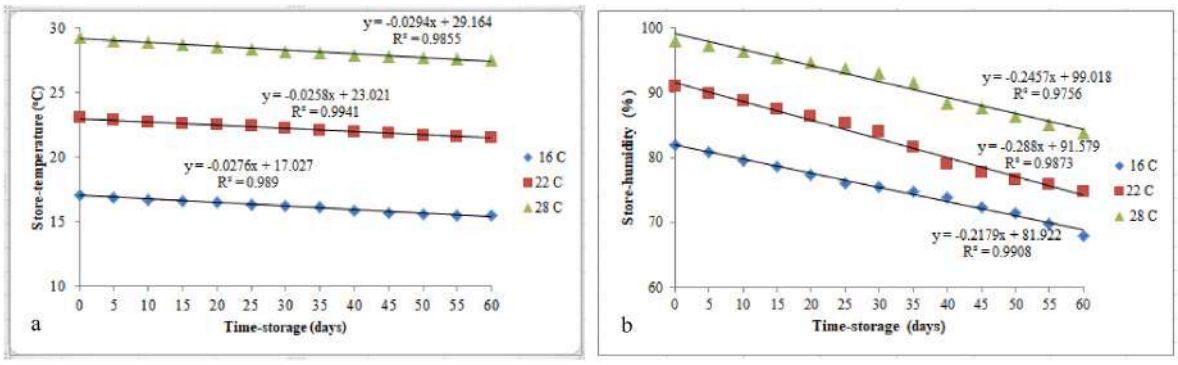

Fig. 2. The linearity model observed two environmental circumstances, temperature (a) and air humidity (b), until the end of the storage period.

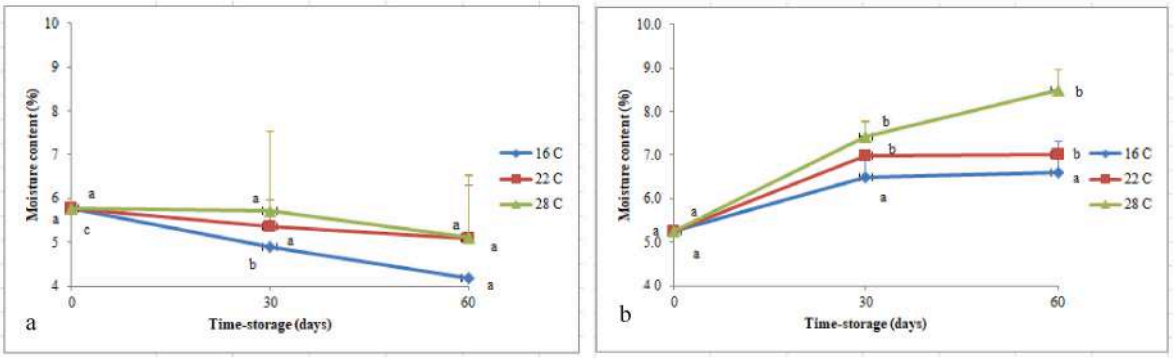

Fig. 3. Moisture content alteration of the type I nutmeg (a.) and the type II one (b.) was stored at three different temperatures. Results are stated as means ± SE. Each lowercase letter expresses significant results between storing-period observations ($p < 0.05$) based on DMRT.

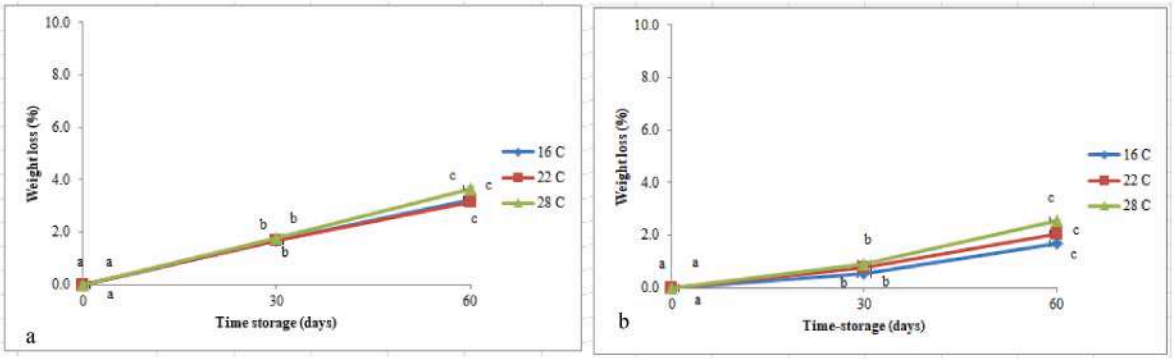

Fig. 4. Weight loss profiles were observed on the type I sample of nutmeg (a.) and the type II sample of nutmeg (b.) reserved in three different temperatures. Results are stated as means ± SE. Each lowercase letter expresses significant results between storing-period observations ($p < 0.05$) based on DMRT.

rate of increase was slightly slower, particularly in the first half of reserved time. Later, this rate was slightly higher for the remaining storage. The gaps that emerged from these inclinations were observed on the second one, indicating that the nutmeg stored at the lowest temperature was properly more affected in suppressing the weight loss, below 4%.

3.5. *Inhibition of Aspergillus flavus colony growth on the specific agar medium*

Aspergillus flavus colony growth has been the leading indicator for evaluating food safety aspects for herbs and spices, including nutmeg. In this study, a growth inhibition rate due to different temperature storage was strongly correlated with the minimum diameter size of mold colony periodically caused by. As mentioned previously, a number of the unique inhibiting profiles were created from (Fig. 5 a-b).

In the first sample type, an exception of the declining trend was significantly monitored on nutmeg stored at 16 °C. Therefore, this lowest temperature mainly influenced the minimization of the growth of *A. flavus*, as proved by the terminal of colony diameter decreased by almost a quarter rather than the highest value upon cultivation in the AFPA agar medium. In the second sample type, the lowest one also had the most influential effect in suppressing colony growth, nearly two-thirds of the highest, even though it fluctuated.

The influence of the three different temperatures significantly ($p < 0.05$) contributed to determining these quantitative color parameters (Table 2). As the previous finding, the growth inhibition of the targeted fungi colony indirectly correlated with the lowest values of the b quantitatively measured on the two types of samples stored at the lowest temperature. Lowering the yellow-orange intensity color was directly

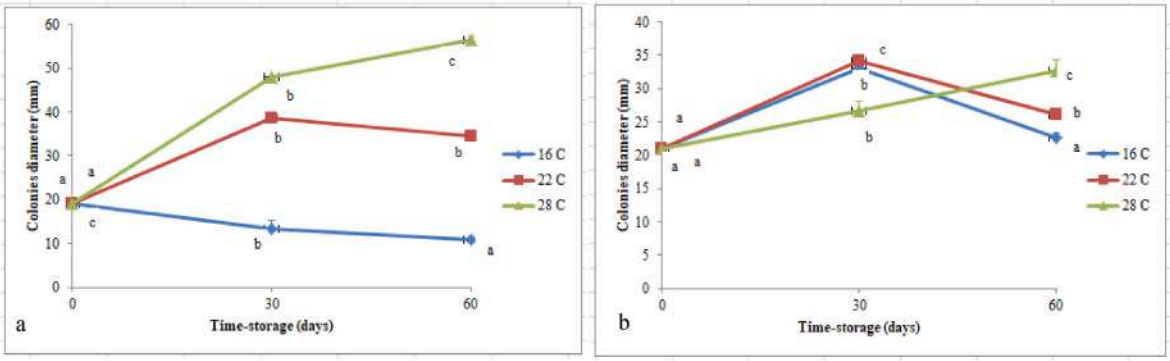

Fig. 5. The development of the colony size of Aspergillus flavus infests the type I nutmeg sample (a.) and the type II nutmeg one (b.), which is affected by three different storage temperatures. Results are stated as means ± SE. Each lowercase letter expresses significant results between storing-period observations ($p < 0.05$) based on DMRT.

Table 2
Colony color of *Aspergillus flavus* infesting on the nutmeg samples after 60 days stored at the three different temperatures.

Type of sample	Reserved-temperature (°C)	Color intensity		
		L	a	b
I	16	51.63 ± 1.0 b	8.07 ± 1.44 a	34.77 ± 1.59 a
	22	50.43 ± 0.91 ab	17.37 ± 2.03 b	45.53 ± 1.10 b
	28	47.3 ± 1.38 a	20.7 ± 0.61 b	39.1 ± 2.65 ab
II	16	52.45 ± 0.8 b	8.87 ± 1.35 a	35.98 ± 1.42 a
	22	51.36 ± 0.75 ab	18.12 ± 1.84 b	46.48 ± 1.06 b
	28	49.1 ± 1.26 a	21.4 ± 0.72 b	40.03 ± 2.23 ab

Remarks: Results are stated as means ± SE. Each lowercase letter expresses significant results ($p < 0.05$) based on DMRT.

related to minimalizing the a-values, as distinctly monitored on those similar samples, indicating that the targeted fungi colony tends to be the least reddish, indirectly linked to the brightest shown by the highest score of the L-values.

3.6. *Effect of three different temperatures of the storage on managing mycotoxin contaminations*

Store temperature has contributed as the main factor in controlling a sharp increase in mycotoxin levels naturally occurring in herbs and spices, including whole dry nutmeg. Therefore, a series of analytical assays on each level of this factor must be complied with (Fig. 6 a-f). Surprisingly, many uncommon profiles were formed.

Several pieces of evidence were contrastingly expressed, specifically in those two types of samples. As partly demonstrated in Fig. 6 (a-b), both aflatoxin B_1 and total aflatoxin contained in the whole type I nutmeg seed samples stored at different temperature levels did not achieve the critical level yet (respectively not exceeding 5 and 10 μg/kg both for aflatoxin B_1 and total aflatoxin yet). In advancing observation, those samples subsequently stored at 16 and 22 °C were not significantly affected ($p \geq 0.05$) by inclinations of the two types of observed

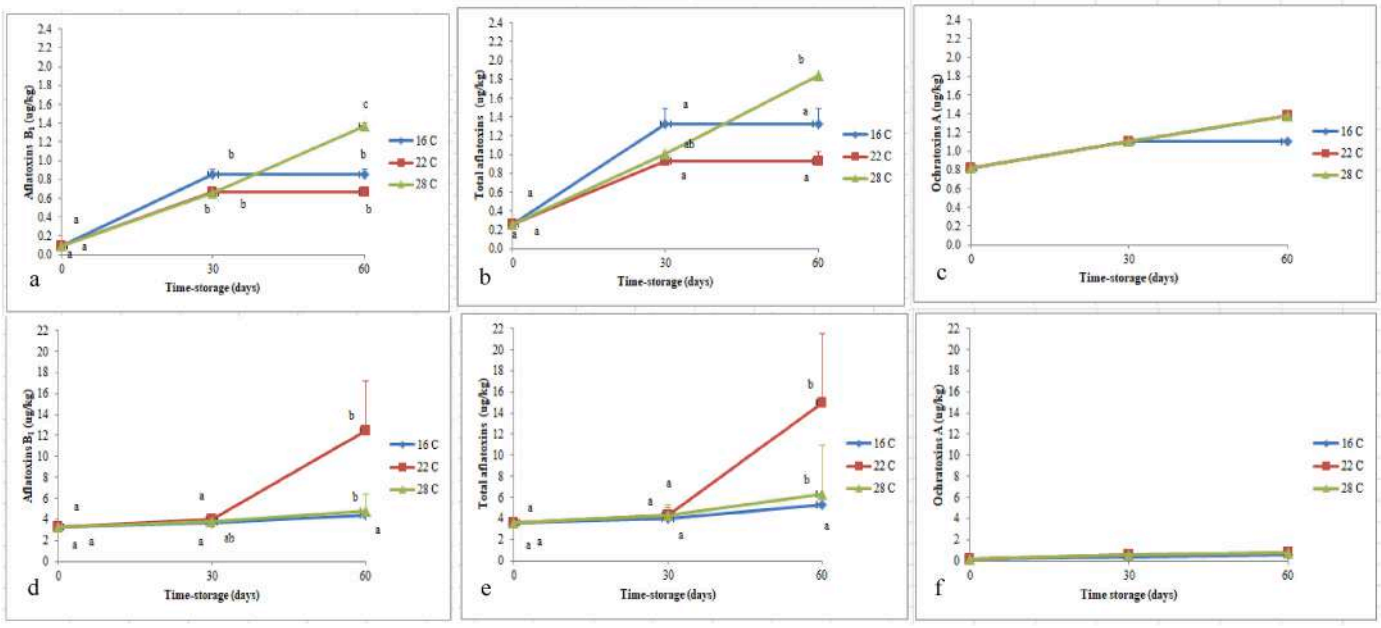

Fig. 6. Influences of the three-reserved temperature on altering mycotoxins content quantified both on the type I (a–c) and the type II (d–f) of nutmeg samples. Results are stated as means ± SE. Each lowercase letter expresses significant results between storing-period observations ($p < 0.05$) based on DMRT.

contaminants. Different results, as shown in Fig. 6(c), revealed that only particular samples stored at 16 °C did not show a significant rise ($p \geq 0.05$) in terms of ochratoxin A level until the end of the storage period.

Nevertheless, some contrast profiles were finally expressed on the type II nutmeg seed samples stored at three different temperatures. As briefly shown in Fig. 6 (d-e), those samples stored at 22 and 28 °C were in significant inclinations ($p < 0.05$) of aflatoxin B_1 and total aflatoxin levels. Hence, those contaminations achieved a critical level at the terminal storing time, except for samples preserved at 16 °C, successfully kept at 4.3 and 4.5 μg/kg for those two toxins. A vice versa result was quite obvious in terms of ochratoxin A level, as shown in Fig. 6(f), no significant differences ($p > 0.05$) were observed among whole samples, indicating this critical level of contamination (not exceeding 15 μg/kg yet) was not achieved yet. Therefore, it summarized a strong correlation between minimalizing the targeted fungi growth and suppressing a drastic increase in mycotoxins content, particularly as seemingly monitored at the lowest storing temperature.

4. Discussion

As widely known to be the most prospective plantation commodity, nutmeg has been cultivated in most tropical regions, including Indonesia, Sri Lanka, and Brazil, for almost centuries (Valente et al., 2015). Unfortunately, due to the extended range of humidity, most of these commodities have been severely infested by many microbial contaminations, including several storage molds having high sensitivities to oxygen availability and free moisture content utilized (Asghar et al., 2022; Norlia et al., 2020; Redfern et al., 2012). Those two components have been proven to be the entry point for massive fungal growth, particularly on the material surface (Das et al., 2012; Fonseca et al., 2002; Reiter et al., 2010).

Recognized as one of the critical post-harvest fungi, *A. flavus* has widely grown at the specific ranges of warm temperatures and medium humidity (Dharmaputra et al., 2022; Egbuta et al., 2015; Wang et al., 2022). It has been scientifically proven through some previous studies mentioning that *A. flavus* has optimally grown between 28 and 35°C, and hence, this fungal species has massively enriched its hosts in a_w = 0.95–0.99 (Fleurat-Lessard 2017; Mannaa and Kim, 2017; Sorenson et al. 1967). Therefore, mycotoxins, produced as the secondary metabolites, have been coherently in line with the environmental circumstances highly favorable (Dharmaputra et al., 2015; El-Desouky et al., 2012; Muga et al., 2019).

Oval shaping orange pigment seen in the reverse colony (Fig. 1) has distinctly indicated aflatoxigenic attributed to *A. flavus* and *A. parasiticus* (Nair et al., 2014) moderately reported to contaminating spices and their derivative products (Barakat and Swaileh, 2022). This phenomenon has substantially been a rising serious threat in terms of cross-contamination of aflatoxins on food products to extreme levels frequently occurring due to improper condition of storage (Frisvad and Larsen, 2015). Hence, re-designing of minimum temperature storage equally balanced with a marginal decrease in relative humidity will be available as a potential option, particularly in terms of minimalizing this fungal growth and aflatoxin contamination (Abdel-Hadi et al., 2012; Gallo et al., 2016). Our results have confirmed that the lower the temperature level, the less relative humidity covered at the end of storage (Fig. 2). Hence, those correlations positively reflected what was achieved in suppressing the colony diameter of this mold observed (Fig. 5) and in minimalizing the intensity of chrome colors absorbed from those fungi studied (Table 2).

Improper storage conditions have severe consequences for highly impacting microbiological quality, mainly affecting an increase of moisture content, influentially affecting both the proximate composition and the sensory attributes (Bhattacharya and Raha, 2002; Opio and Photchanachai, 2018; Zorzete et al., 2013). This scientific evidence has positively related to our finding, as it severely impacted those samples stored at 28°C, compared to other samples kept at 16 and 22°C (Figs. 3 and 4). These results have indicated that the room temperature was not properly fitted for the long-term storage period for this commodity.

Interestingly, mycotoxin contaminations have been frequently related to some inclinations of moisture content and air humidity drastically carried on at the end of storage time. These inclinations have presumably related to several reports mentioning that the optimum condition of *A. flavus* in producing aflatoxins occurred at a certain level of temperature ranging from 25°C to 35°C, mainly triggered by severe environmental moist (Hassane et al., 2017; Schindler et al., 1967; Schmidt-Heydt et al., 2010). A unique result reported by Pratiwi et al. (2015) mentioned that high contamination of this toxin on stored soybeans occurred at 20 and 80% relative humidity (RH). Our study distinctly figured out that only a lot of samples stored at 16 °C (Fig. 6) fully complied with those official requirements regulated in EC 1881/2006. Further study shall take place to re-assay how effective these schemes of simulated trials are compared to other nutmeg products with different physic-chemical characteristics, i.e. shrivel and broken wormy punky (BWP) ones. Hence, a detailed recommendation for designing a nutmeg distribution storage guideline would be properly and comprehensively formed.

5. Conclusion

Managing storage conditions has significantly suppressed the growth of *A.* flavus and minimized the contamination of mycotoxins produced by this mold. Overall, dry nutmeg kernel kept at 16°C successfully avoided the sharp rise of weight loss and moisture content, positively linked to a much more controlled mold colony. Hence, the production of mycotoxins was achieved below the maximum allowable limit. Therefore, this storage temperature was recommended for long-term distributed storage.

CRediT authorship contribution statement

Hendra Adi Prasetia: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Lenny Panjaitan:** Visualization, Validation, Project administration, Investigation, Funding acquisition, Data curation. **Bambang Hesti Susilo:** Visualization, Validation, Resources, Project administration, Conceptualization. **Slamet Budiawan:** Visualization, Resources, Investigation, Data curation. **Budhi Suherman:** Visualization, Resources, Investigation, Formal analysis, Data curation. **Nurul Dwi Handayani:** Visualization, Resources, Methodology, Investigation, Data curation, Conceptualization. **Mutia Riefka Fauziaty:** Resources, Project administration, Investigation, Funding acquisition, Data curation. **Salbiah:** Visualization, Resources, Investigation, Data curation. **Okky Setyawati Dharmaputra:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Conceptualization. **Dian Herawati:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Conceptualization. **Santi Ambarwati:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Conceptualization. **Antarjo Dikin:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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