Recent progress of the effect of environmental factors on *Aspergillus flavus* growth and aflatoxins production on foods

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Abstract

The contamination of *Aspergillus flavus* and subsequent aflatoxins (AFs) has been considered as one of the most serious food safety problems due to their acute and chronic adverse effects on humans and animals. This review collects the available information from recent years on the effect of the major environmental factors such as water activity (a_w), temperature, CO_2, and pH on the fungal growth, the expression of AFs-related genes, and AFs production by *A. flavus* on foods. In particular, the relationship between the relative expression of key regulatory (*aflR* and *aflS*) and structural genes (*aflD*, *aflO*, *aflQ*, etc.) and AFs production under different environmental conditions are collected and discussed. The information collected in this review can be used to design control strategies of *A. flavus* and AFs contamination in practical applications, primarily during storage and processing. These data suggest that integrating various post-harvest methods with synergistic functions may be more efficient for the control of *A. flavus* growth and AFs production, although the individual environmental factors alone have an impact.

Key words: *Aspergillus flavus*; aflatoxin; water activity; temperature; CO_2.

Introduction

The moldy contamination of staple foods such as cereals has been regarded as one of the most serious food safety problems due to their acute and chronic negative effects on humans and animals (Medina et al., 2014). Certain molds such as *Aspergillus flavus* and *Aspergillus parasiticus* grown in corn, peanuts, and nuts produce aflatoxins (AFs). AFs have been classified as carcinogens in Group 1 by the International Agency for Research on Cancer (IARC, 1993, 2002). They are estimated to induce up to 25% of the total worldwide cases of hepatocellular carcinoma (HCC), the most common form of liver cancer (Liu et al., 2012; Wu, 2014). Moreover, AFs also inevitably cause acute intoxication, immune suppression, and growth retardation in children (Groopman et al., 2008). Among AFs, aflatoxin B₁ (AFB₁) was the most toxic one. The main source of exposure to AFs is the ingestion of contaminated food and feed. Therefore, the high threat of AFs to the health of humans and animals has resulted in strict legislative limits in most countries and regions of the world for AFs and AFB₁ in a wide range of foodstuffs and feeds (Commission of the European Communities, 2010; US Food and Drug Administration, 2010).

*Aspergillus flavus* is the predominant fungal species contaminating foodstuffs and feeds and producing AFs worldwide. It is also the main contaminant during the food storage since its ability to produce AFs and its potential to persist as a pathogen and saprophyte...
in the food supply before and after harvest (Labouar et al., 2015). Aspergillus flavus is a xerophilic fungus that has developed physiological mechanisms that adapt to environmental stress factors like low water activity ($a_w$), allowing them to compete and often dominate other fungal communities (Nierman, 2008; Medina et al., 2015). The competitive advantage is because its metabolic plasticity allows it to produce a range of extracellular hydrolyses, secondary metabolites, and volatiles (Magan and Aldred, 2007). Therefore, preventing and controlling the fungal growth and AFs production by A. flavus are essential and urgent to ensure the food safety and security in the world.

To eliminate AFs contamination in food chain, the most useful and cost-effective strategy is to create an adverse environment for A. flavus growth and AFs production. The growth of A. flavus at the phenotypic level and the AFs production were observed to be associated with several environmental factors such as $a_w$, temperature, storage time, composition of the substrate, carbon and nitrogen source, pH, light, content of oxygen ($O_2$) and carbon dioxide ($CO_2$), loss of grains' integrity caused by insects or mechanical/thermal damage, and the interaction between fungal species that share the same ecological environment (Dantigny and Vaamonde, 2005; Vaamonde et al., 2006; Astoreca et al., 2014; Medina et al., 2015). Regarding abiotic factors, $a_w$ and temperature and their interactions have been demonstrated to be the most aetiological determinants in regulating fungal growth and secondary metabolites production (Mannaa and Kim, 2017; Medina et al., 2017). As an aerobic fungus, A. flavus growth and subsequent AFs production are highly influenced by $CO_2$, with certain levels (Peleg et al., 1988; Mousa et al., 2016). AFs are biosynthesized by 29 genes located in a 75 Kb gene region of A. flavus (Mousa et al., 2013; Mannaa and Kim, 2018) investigated the effects of different relative humidities (RHs; 12, 44, 76, and 98%) and temperatures (10, 20, 30, and 40°C) on major grain fungal populations including A. flavus and some other fungi. They indicated that the populations of all tested fungi in inoculated rice grains were significantly enhanced by both increased RH and temperature. In addition, multiple linear regression analysis revealed that one unit increase of temperature resulted in greater effects than that of RH on fungal populations.

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Effect of $a_w$ and temperature on A. flavus growth, gene expression, and AFs production on grains

Plenty of literatures have reported that the two key environmental parameters, $a_w$ and temperature, play important roles in modulating the A. flavus growth and AFs production on foods (O'Brian et al., 2007; Schmidt-Heydt et al., 2009, 2010; Yu et al., 2011; Marroquin-Cardona et al., 2014; Bai et al., 2015). Among foods, grains are the most susceptible to the infection of A. flavus and the subsequent AFs contamination (Guo et al., 1998). Regarding AFs production, various grains have a different sensitivity to $a_w$ and temperature. Moreover, on the same grain, the optimal conditions for AFs production were significantly influenced by the A. flavus strains, incubation time and status of grains, etc.

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Mousa et al. (2013) conducted a study to model the radial growth rate and to assess AFs production by A. flavus as a function of $a_w$ = 0.82–0.92 and temperature 12–42°C on polished and brown rice. They found that the brown rice is more susceptible to fungal invasion and AFs production than polished rice. The optimum conditions for A. flavus growth and AFs production in brown rice were both at 0.92 $a_w$ and 30°C. Fungal growth and AFs were undetectable at 0.82 $a_w$ on polished rice while they were both detected at this $a_w$ under 25–35°C on brown rice. At the temperature range of 25–30°C, the minimum $a_w$ values for AFs production on polished rice and brown rice were 0.84 and 0.82, respectively. The highest levels of AFs were observed at the highest $a_w$ 0.90–0.92 at 20°C after 21 days of incubation on both types of rice. However, at 7 and 14 days of incubation, the highest level of AFs was observed at $a_w$ 0.92 and 30°C on polished rice. On brown rice, the optimal condition for AFs production at 7 and 14 days of incubation was $a_w$ 0.92 × 25°C and $a_w$ 0.92 × 30°C, respectively. When temperature reached to 40°C, only a small amount of AFs were detected during the 3 weeks of incubation, and both types of rice were only detectable at 0.92 $a_w$.

Similarly, Choi et al. (2015) showed that brown Korean rice was sensitive to A. flavus growth and AF production compared with rough Korean rice and white Korean rice. Regardless of the degree of milling of Korean rice, the optimum growth rate during 120 day storage was at 85% RH/30°C and 97% RH/21°C. The highest population of A. flavus and highest amount of AFB, were observed at 97% RH/21°C and on inoculated brown rice. Trace amounts of AFB were detected during 10 day storage at 85% RH/21°C and in all three non-inoculated types of rice.
Lv et al. (2019) investigated the effect of aw (0.92–0.96) and temperature (28–37°C) on the fungal growth and AFB1 production by *A. flavus* on polished rice and paddy. The results indicated that AFB1 production on polished rice can occur over a wider range of temperature × aw levels than that on paddy. *Aspergillus flavus* grew better at 0.92–0.96 aw and 28–37°C on polished rice, and the highest level of AFB1 was observed at 33°C and 0.96 aw after 7 days of incubation. In comparison to the optimal temperature of 33°C, all tested genes of *A. flavus* on polished rice were significantly up-regulated at 25°C under 0.96 aw. Compared with aw 0.96, most of structural genes of pathway were significantly down-regulated at aw 0.90 and 0.99 under 33°C, although two regulatory genes (aflR and aflS) was up-regulated at aw 0.90. For the *A. flavus* growth on paddy, the optimal temperature and aw were 37°C and 0.94 within the tested range, respectively. Moreover, the highest concentration of AFB1 on *A. flavus* on polished rice and paddy. The results indicated that AFB1 needed minimum aw levels (≤0.91) and growth on CEM. They found the AFB1 production occurred by *A. flavus* isolated from corn on Czapek yeast agar (CYA) and corn extract agar (CEM). They found the AFB1 production occurred more favourably on CYA while the maximum levels of CPA were observed on CEM. The minimum level of aw for both toxins production was 0.83 with the tested aw. The maximum amount of AFB1 was observed at 0.96 aw and 30°C after 21 days of incubation, regardless of the isolates and media. Although they belong to three different chemotypes: chemotype I (AFB1&4& aflR&CYP1A), chemotype III (AFB1&CYP1A& aflR&4), and chemotype IV (AFB1& aflR&CYP1A&4), respectively, the three isolates do not differ in the response to the environmental factors (aw and temperature). Moreover, the limiting and optimum conditions for AFB1 and CPA production were similar on both media.

Bermejo et al. (2017) investigated the impact that interactions between aw and temperature may have on growth, the expression of aflR, and AFB1 production by *A. flavus* on a maize-based medium. They found that there were some differences between lag phases and growth rates of *A. flavus*. The optimum condition for *A. flavus* growth on maize was 0.99 aw and 30°C and the maximum AFB1 production on maize was observed at 0.98 aw and 30°C. *Aspergillus flavus* growth was completely inhibited at 0.90 aw and 20°C. Both aw and temperature significantly influenced the relative expression of aflR gene and AFB1 production. However, the influences on AFB1 production were not consistent with the effects on gene expression and growth. These results suggested that the aflR expression was not a good indicator of AFB1 production alone. Therefore, further molecular studies of other AFs biosynthetic genes should be conducted. Based on the research by Kružl et al. (2019), the optimal conditions for AFB1 biosynthesis were observed at 30°C in the temperature ranges of 15–37°C and the aw levels at (0.85–0.99) in the inoculated shell-less and shelled cereals. Moreover, the shell has a higher amount of AFB1 in hull-less grain than the dehulled grains and the AFB1 content in the hull was even 10–170 times higher than the grain, indicating the shell has a protective effect.

**Effect of aw and temperature on *A. flavus* growth, gene expression, and AFs production on peanuts**

Peanut (*Arachis hypogea* L.) is a globally important economic and oilseed crop worldwide. However, peanuts contamination with AFs and aflatoxigenic *A. flavus* is regarded as the most serious problem in the world (Williams et al., 2004; Liu et al., 2017). Liu et al. (2017) investigated the effect of aw (0.85–0.99) and temperature (15–42°C) on fungal growth, the expression of AF biosynthetic genes on un- autoclaved peanut kernels. The results indicated that AFB1 production in peanut kernels can occur over a wider range of aw levels at (0.85–0.99) and ≤20°C. The optimal conditions for *A. flavus* growth on peanuts were observed at 0.98 aw and 37°C. The highest amount of AFB1 was observed at 0.96 aw and 28°C.

Moreover, the expression of AF-related and growth-related genes was significantly modulated by aw and temperature. At 0.92 aw, 16 of the 25 genes had the highest expression levels at 28°C, whereas 9 genes had the highest expression levels at 37°C. In addition, all AF biosynthetic pathway genes were down-regulated at 42°C compared with 37°C. Compared with 0.99 aw, all the pathway genes and laeA were up-expressed at aw of 0.96 under 28°C. In particular, the ratio of aflStaflR was positively correlated with the AFB1 production. The expressions of laeA and braI were positively associated with AFB1 production and fungal growth respectively.

**Effect of aw and temperature on *A. flavus* growth and AFs production on tree nuts**

Tree nuts are commodities with moderate to high risk of AFs contamination because they are produced at environmental conditions favouring fungal growth and AFs production by aflatoxigenic fungi especially *A. flavus* (Arrus et al., 2005; Gallo et al., 2016). The incidence of AFs contamination in nuts is low; however, their levels vary widely and can produce high levels in a small number of nuts (Campbell et al., 2003).

For the pistachio nuts, the highest amount of radial growth rate of *A. flavus* and AFB1 production was observed at 0.93 aw and 30°C using a full factorial design with different moisture content levels (10, 15, 20, 25, and 30%) and incubation temperatures (10, 15, 20, 25, 30, 37, and 42°C) (Marín et al., 2012). They also found that the limited AFs levels in pistachio nuts by European Commission would be surpassed in a period as short as 1 month if pistachio nuts reach 20°C, unless moisture content is ≤10%. However, this conclusion is not accurate because it was drawn from a single *A. flavus* strain. As we all know, the level of mycotoxins produced is highly variable even if only considering mycotoxin-producing strains in a species (Hua et al., 2012).

Gallo et al. (2016) evaluated the effects of different combinations of aw (0.90, 0.93, 0.96, and 0.99 aw) and temperature (20, 28, and 37°C) on *A. flavus* growth, AFB1 production, and expression of the two regulatory genes and two structural genes on an almond medium solidified with agar. The maximum amount of fungal biomass and AFB1 production was observed at 0.96 aw and 28°C. At the driest tested conditions (0.90 and 0.93 aw), no fungal growth and AFB1 production were observed at 20°C. At 20 and 37°C, the yield of AFB1 was reduced by 70%–90% or completely suppressed, depending on aw. Both regulatory genes (aflR and aflS) showed high
expression at maximum (28°C) and minimal (20 and 37°C) AFB₁ production. In contrast, the two structural genes (aflD and aflO) showed high expression only at the maximum AFB₁ production (28°C and 0.96–0.99 a₀). Based on this, temperature appears to be a key factor affecting AFs production, which was strictly correlated with the expression of the structural genes (aflD and aflO), but not to that of the two regulatory genes. This result suggests that some post-transcriptional regulatory processes are involved in modulating AFs biosynthesis.

Prencipe et al. (2018) evaluated the drying temperatures (from 30 to 50°C) on A. flavus growth and AFs production in chestnuts and indicated that the optimal temperature for fungal growth was 30°C, whereas the highest concentrations of AFB₁ and AFB₂ were obtained at 40°C. At this temperature, A. flavus was under suboptimal conditions for growth (0.78 a₀), but AFs biosynthesis was under the optimal conditions. When the drying temperatures reached 45–50°C, AFs production was completely inhibited. Drying at 45°C for 7 days (0.64 a₀) could be a promising strategy to effectively control both A. flavus growth and AFs production.

Effect of a₀ and temperature on A. flavus growth, gene expression, and AFs production on other foods

Recently, there are some researches that reported the effect of a₀ and temperature on the fungal growth and AFs production by A. flavus on other foods. In whole black peppercorns (Piper nigrum L.), the growth and AFs production of three A. flavus isolates and one A. parasiticus isolate were investigated using a full factorial design with seven a₀ levels (0.826–0.984) and three temperatures (22, 30, and 37°C) (Yogendrarajah et al., 2016). Among secondary models, the extended Gibson model was the best model to describe the combined effect of a₀ and temperature on the growth rate of both fungal species in peppercorns. The highest population of A. flavus occurred at 0.92 a₀ and 30°C, and the maximum yield of AFB₁ was also observed under this condition based on diverse secondary models. The estimated minimum a₀ and temperature for the growth of A. flavus were 0.73–0.76 and 11–16°C, respectively. High variability in AFs production of different aflatoxigenic species limited the modelling of AFs production. Based on the research of Yogendrarajah et al. (2016), the limiting a₀ and temperature should be considered to prevent the aflatoxigenic fungal species growth and AFs production in food during storage.

Peromingo et al. (2016) evaluated that the interaction between a₀ (0.85, 0.90, and 0.95) and temperature (10, 15, 20, and 25°C) may occur on lag phases prior to growth, growth rates, and AFs production by two strains of each A. parasiticus and A. flavus on cured meat over 12 days. Aspergillus flavus CBS 573.65 had shorter lag phases than A. parasiticus CECT 2 688; however, the growth rates of the two strains were similar. The optimum growth and AFs production occurred at 0.95 a₀ and 25°C. At 10°C and all tested a₀, no growth occurred for both species. Both species produced AFs when the a₀ and temperature were a₀ ≥ 0.90 and ≥15°C, respectively. Although similar AFB₁ production characteristics were found between the two species, the concentration of this toxin produced by A. flavus was much higher than that of A. parasiticus.

To elucidate the relationship between the relative expression of AFs-related genes and AFs production, Peromingo et al. (2017) evaluated the effect of different a₀ and temperatures on the temporal relative expression of three genes in AFs biosynthesis cluster and their correlation with AFs production on dry-cured ham-based medium by A. flavus and A. parasiticus. In general, the expressions of the regulatory aflR and aflS genes were similar and much lower than the expression of the structural aflB gene. The expression of aflR and aflS genes in A. flavus increased over a decrease of a₀ regardless of temperature. Regarding A. parasiticus, the highest and lowest expression values of both regulatory genes were observed at 0.95 a₀ and 0.85 a₀, respectively. In contrast, the expression of aflB gene in both species was stimulated at low temperature and a₀. Furthermore, a strong correlation between the relative expression of aflR and aflS gene and AFs production was obtained under environmental conditions which simulate dry-cured ham ripening.

Effect of other environmental factors on A. flavus growth and AFs production on food

In addition to a₀ and temperature, there are more environmental parameters that have been investigated for the influences on the growth and AFs production by A. flavus in many studies. It has been reported that pH, CO₂ level, and light treatment also exhibit significant effects on fungal growth and AFs production (Schmidt-Heydt et al., 2008; Oms-Oliu et al., 2010; Castellari et al., 2015).

Regarding pH, Casquete et al. (2017) investigated the effect of pH (5.0, 5.5, and 6.0), a₀ (0.90, 0.95, and 0.99), and temperature (15, 20, 25, and 30°C) on the lag phases, growth, and AFs production of three aflatoxigenic A. flavus strains (CQ7, CQ8, and CQ103) on cheese-based medium. The results showed that the behaviour of A. flavus strains was affected by pH, a₀, and temperature; however, A. flavus growth was less affected by pH than the others. The CQ7 strain exhibited maximum growth at pH 5.5, 0.99 a₀, and 25°C. However, for the CQ8 and CQ103 strains, there was no difference between pH 5.5 and 6.0. The maximum AFs production on the cheese-based medium occurred at pH 5.0, 0.95 a₀, and 25 or 30°C, depending on the strain. Kosegarten et al. (2017) also indicated that an increase in pH value between 3.5 and 6.5 resulted in an increase in the growth of A. flavus.

For CO₂ level, Mousa et al. (2016) illustrated that the growth of A. flavus and AFs production was highly influenced by the CO₂ level (20%–80%) on paddy. In general, fungal growth rates and AFs production were negatively correlated with CO₂, whereas the lag phase durations were positively correlated with CO₂. However, the highest tested CO₂ level (80%) could not completely inhibit the fungal growth. Under 0.98 a₀, 20% and 80% CO₂ caused at least 59% and 88% reduction in growth and 47% and 97% reduction in AFs production, respectively. In addition, a significant inhibition of growth was observed at 75% CO₂ at both 0.95 and 0.92 a₀ on agar medium. The population of A. flavus isolated from grains was inhibited at up to 75% CO₂ (Giorni et al., 2008; Giorni et al. (2008) also has reported that the moisture maize treated with 25% CO₂ was sufficient to effectively reduce the development of A. flavus, but at least 50% CO₂ was required to significantly reduce the synthesis of AFs. Therefore, controlling pH and CO₂ levels during manufacturing and storage is an effective control strategy to avoid the contamination of A. flavus and subsequent AFs (Chulze, 2010).

Discussion and Conclusions

These previous studies have indicated that several environmental factors play important roles in regulating fungal growth, the expression of AF biosynthetic genes, and AFs production by A. flavus and A. parasiticus. Especially, a₀ and temperature are limiting factors during storage (Liu et al., 2017). Therefore, this review summarizes the different ranges of a₀ and temperature and the optimal condition for A. flavus growth and AFs production on different food substrate
and formula media (Figures 1 and 2). As shown in the two figures, the optimal conditions of $a_w \times$ temperature for A. flavus growth and AFs production vary on different foods or formula medium (Lv et al., 2019). On yeast extract sucrose (YES) medium, the suitable temperatures for AFB1 production were 25–30°C at 0.99 $a_w$, whereas the range changed to 30–35°C at 0.95 $a_w$ (Abdel-Hadi et al., 2012). On un-autoclaved peanut kernels, the suitable temperatures for AFB1 production were 0.92–0.96 $a_w$ and 25–33°C, and the highest level of AFB1 was observed at 0.96 $a_w$ and 28°C (Liu et al., 2017). Similar findings were obtained on almond medium; the maximum fungal growth and AFB1 production were observed at 0.96 $a_w$ and 28°C (Gallo et al., 2016). On un-autoclaved polished rice, high production of AFB1 was observed at 0.90–0.99 $a_w$ and 25–33°C, and the maximum amount of AFB1, was observed at 0.96 $a_w$ and 33°C (Lv et al., 2019). Moreover, these results suggest that the fungal growth and AFs production on foods can occur over a wider range of $a_w \times$ temperature than on formula medium. The diversity of optimal conditions may be due to the differences in media structure and nutrient availability (Ahmad et al., 2013; Mousa et al., 2013).

Compared with the investigations published before 2015, the recent publications pay more attention to the correlation between the expression of AFs biosynthetic genes and AFs production. The researchers attempt to get a good indicator of possible AFs contamination on foods by early detecting the expression of AFs-related genes. As the key regulatory genes in AFs biosynthesis gene cluster, $aflR$ and $aflS$ were highlighted. On YES medium, the down-regulation of $aflR$ and $aflS$ induced the inhibition of AFs production (Yu et al., 2011). And the decrease in the expression ratio of $aflS/aflR$ leads to transcription inactivation of AFs cluster. Similar findings were obtained on un-autoclaved peanut kernels (Liu et al., 2017). At 42°C, the lower ratio of $aflS/aflR$ resulted in lower AFB1 production compared with 28 and 37°C.

In contrast, some literatures have reported that the $aflR$ expression is not consistent with AFs production at several conditions. On a maize-based medium, the influences of $a_w$ and temperature on AFB1 production were different with the expression of $aflR$ gene (Bernaldez et al., 2017). On polished rice, the down-regulation of all the tested AFs structural genes at $a_w$ 0.96 resulted in a low level of AFB1 production compared with $a_w$ 0.90, although $aflR$ and $aflS$ were both up-regulated (Lv et al., 2019). Similar findings were reported by O’Brian et al. (2007), who obtained an opposite relationship between AFs biosynthesis and the expression of $aflR$ and $aflS$. AFs production was completely inhibited at 37°C although both regulatory genes were highly expression in liquid A and M media.
Schmidt-Heydt et al. (2009) indicated that the transcription level of aflS was high at >37°C under almost all aw ranges tested, but the amount of AFs production was low on YES medium. These results suggest that some other molecular mechanisms, such as post-transcriptional mechanisms, were involved in modulating the transcriptional level of AFs structural genes and the subsequent AFs production.

Compared with the two regulatory genes aflR and aflS, the transcriptional levels of some structural genes seem to have more strong correlation to AFs production. Gallo et al. (2016) indicated that the effect of environmental factors on AFs biosynthesis had a better correlation to the transcriptional activation/inactivation of structural genes (aflD and aflO) than the two regulatory genes. Similarly, Abdel-Hadi et al. (2010) also obtained a good correlation between aflD expression and AFs production by A. flavus in raw peanuts under different aw levels. In addition, the significant differences between the relative expression of aflD at different temperatures (28, 37, and 42°C) were observed, resulting in the significant differences in AFs production on peanut kernels (Liu et al., 2017). On YES medium, the expression profile of aflD, aflO, and aflP was consistently correlated with the ability of AFs production (Scherm et al., 2005). On polished rice, the structural genes (aflM, aflN, aflO, aflP, aflQ, aflU, and nadA) involved in the middle and late stages of AFs biosynthesis did not show any clear trends in their expression on PDA and CYA at 20%, 50%, and 75% relative air moisture (Lv et al., 2019). The lower expression of these structural genes led to the decrease of AFs production.

Besides modulating aw and temperature, modified atmosphere packaging is regarded as a promising food preservation technique because it integrates the control microbial activity and insects, which tend to maintain the quality of the products and extend the shelf life with minimal application of chemicals (Taniwaki et al., 2009; Mousa et al., 2016). CO2 was proved to retard the fungal growth and inhibit AFs production by A. flavus. Taniwaki et al. (2009) indicated that no fungal growth of A. flavus was observed at 40% and 60% CO2 when O2 level was <0.5% while growth was observed on PDA and CYA at 20% CO2. However, even at the lowest CO2 level (20%) studied, the retarding effect of CO2 on the growth rate of A. flavus was significant on paddy (Mousa et al., 2016). Overall, 55%–100% reduction in fungal growth was achieved with CO2 treatment. On moistened maize, modified atmospheres with 25%–30% CO2 only contributed to 30%–35% reduction in A. flavus growth while as the concentrations of CO2 increased up to 75%, the reduction of above 50% was obtained (Giorni et al., 2008). The more reduction of fungal growth on paddy than maize with CO2 treatment may be due to the more resistance of paddy’s physical structure to the invading fungi (Mousa et al., 2016).

Besides fungal growth, AFs production was also significantly inhibited by modified atmosphere with CO2. Mousa et al. (2016) found that the reduction in AFs production on paddy with CO2 treatment (20%–80%) was in the range of 65.8%–98.0%, 70.4%–94.6%, and 72.9%–96.8% at 0.98, 0.95, and 0.92 aw, respectively. However, no significant reduction in AFs production on maize was observed with modified atmosphere enriched with 25% CO2 and balanced with N2 (Giorni et al., 2008). With the application of 50% and 75% CO2, 46% and 58% overall reduction in AFs production was observed, respectively. Additionally, on PDA or CYA enclosed with a modified atmosphere with 20% CO2 and <0.5% residual oxygen, no AFs production was observed for 30 days (Giorni et al., 2008). It is important to note that the residual O2 may be a limiting factor for fungal growth and AFs production under modified atmosphere packaging (Taniwaki et al., 2009).

The pH is also an important factor in determining A. flavus growth and AFs production. Klich (2007) indicated that the increase in pH (4.6) was related to the increase of A. flavus growth, and the expression of the AFs pathway gene was also affected by the pH of the medium. In some foods such as fermented food, salted food, and canned food, fungal growth and AFs production can be controlled by adjusting pH values. However, there are some limitations on the practical application of adjusting pH in most foods, especially the grains and spices listed in this paper.

In conclusion, the low aw and temperature or the increase in the CO2 level alone is not effective for complete control of A. flavus and AFs production. Integrating various post-harvest methods with synergistic functions may be more efficient for the complete inhibition of A. flavus growth and AFs production during storage and processing of foods. For example, reducing aw is the prerequisite step to prevent A. flavus infection and AFs production, and controlling aw and temperature and increasing the level of CO2 in the atmosphere are useful strategies during storage. Good Agricultural Practices (GAP) and Good Processing Practices (GPP) represent primary preventive measures against A. flavus and the subsequent AFs. Integrating the two principles with the Hazard Analysis and Critical Control Point (HACCP) can be used efficiently (Mousa et al., 2016). A better knowledge of the environmental factors governing fungal growth and AFs production provided in the above-mentioned recent researches would help in establishing optimal guidelines in GAP and GPP, preventive measures, and critical limits in HACCP plans.

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Conflict of Interest

None declared.

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