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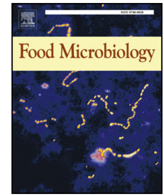

Production of aflatoxin B_1 and B_2 by *Aspergillus flavus* in inoculated wheat using typical craft beer malting conditions

Danieli Cristina Schabo[a,c], Ligia Manoel Martins[b], Janeeyre Ferreira Maciel[c], Beatriz Thie Iamanaka[b], Marta Hiromi Taniwaki[b], Donald William Schaffner[d], Marciane Magnani[c,*]

[a] *Federal Institute of Education, Science and Technology of Rondônia, Campus Colorado do Oeste, BR 435, Km 63, Colorado Do Oeste, RO, 76993-000, Brazil*
[b] *Center for Science and Food Quality, Food Technology Institute, Avenue Brazil, 2880, Campinas, SP, 13070-178, Brazil*
[c] *Laboratory of Microbial Processes in Foods, Department of Food Engineering, Center of Technology, Federal University of Paraíba, Campus I, João Pessoa, PB, 58051-900, Brazil*
[d] *Department of Food Science, Rutgers, The State University of New Jersey, 65 Dudley Road, New Brunswick, NJ, 08901, USA*

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ABSTRACT

The production of aflatoxin (AF) B_1 and B_2 was determined during malting of wheat grains artificially contaminated with a toxigenic *A. flavus* strain (CCDCA 11553) isolated from craft beer raw material. Malting was performed in three steps (steeping, germination and kilning) following standard Central European Commission for Brewing Analysis procedures. AFB_1 and AFB_2 were quantified in eleven samples collected during the three malting steps and in malted wheat. Both, AFB_1 and AFB_2 were produced at the beginning of steeping and detected in all samples. The levels of AFB_1 ranged from 229.35 to 455.66 μg/kg, and from 5.65 to 13.05 μg/kg for AFB_2. The AFB_2 increased during steeping, while no changes were observed in AFB_1. Otherwise, AFB_1 decreased during germination and AFB_2 did not change. AFB_1 and AFB_2 increased after 16 h of kilning at 50 °C and decreased at the end of kilning, when the temperature reached 80 °C. The levels of AFB_1 wheat malt were lower than those detected in wheat grains during steeping; however, levels of both AFB_1 (240.46 μg/kg) and AFB_2 (6.36 μg/kg) in *Aspergillus flavus* inoculated wheat malt exceeded the limits imposed by the regulatory agencies for cereals and derived products.

1. Introduction

Beer is the second most popular alcoholic beverage consumed worldwide (WHO, 2018). The consumption of craft beer has recently increased even in countries not traditionally known for beer consumption (AssoBirra, 2017; Brewers Association, 2018a; Gómez-Corona et al., 2016). Craft beer is defined as a product manufactured on small scale using traditional methods. Breweries that focus on craft beer production generally prioritize high quality products with characteristic sensory properties rather than on large scale production (Brewers Association, 2018b; Gómez-Corona et al., 2016). Craft beer may include malted or unprocessed cereals such as wheat, barley, rice, rye, maize, oats and sorghum in addition to the basic beer ingredients: malted barley, hops, yeast and water (Jin et al., 2018a; Pascari et al., 2018). Although barley malt is the main raw material traditionally used for beer brewing, wheat beers made using at least 50% of wheat malt are popular in a variety of countries (Faltermaier et al., 2014; Mascia et al., 2014).

The increased consumption of craft beer has resulted in an emergence of small-scale breweries which in turn has led to potential food safety concerns because of a lack of standardization. The main food safety concerns have been related to the quality of the raw material since craft beer is non-filtered and unpasteurized and is thus more subject to microbial contamination (Piacentini et al., 2015). Grains (e.g. wheat, barley) used as raw materials can also be infected with toxigenic fungi in the field or during storage and mycotoxins can be produced under favorable conditions (Neme and Mohammed, 2017). *Aspergillus* or *Penicillium* species are the predominate contaminants of cereals (Pitt et al., 2013) and may include aflatoxin (AF) or ochratoxin A (OTA) and citrinin (CIT) producers (Neme and Mohammed, 2017).

A. flavus is one of the most prevalent potentially toxigenic species isolated from cereals used as raw material in craft beer brewing (Jedidi et al., 2018). Isolates of this species with the ability to produce AFB_1 and AFB_2 have been isolated from barley (Gonzalez Pereyra et al., 2011;

* Corresponding author.
E-mail addresses: magnani2@gmail.com, magnani2@pq.cnpq.br (M. Magnani).

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Maenetje and Dutton, 2007; Tabuc et al., 2009) and wheat (Al-Wadai et al., 2013; Jedidi et al., 2018; Riba et al., 2010). The contamination of grains with AFB_1 producers receives major attention because of the toxicity and thermostability of this mycotoxin (IARC, 2012; Ostry et al., 2017), which is classified as a class 1 toxin by the International Agency for Research on Cancer (IARC). The detection of AFB_1, as well as AFB_2, has been reported in pasteurized beers in several countries (Pagkali et al., 2018; Peters et al., 2017).

The production of AFs by *A. flavus* occurs in grains containing > 80% moisture (Pitt et al., 2013) and the toxin survives at temperatures > 40 °C (Zhang et al., 2018). These characteristics are of interest during malting because grains are exposed to high moistures during steeping and germination and subsequently dried over an increasing temperature gradient (Habler et al., 2016). Craft beer brewing temperatures can reach 100 °C (Pascari et al., 2018), while AFB_1 and AFB_2 are only destroyed at temperatures approaching 160 °C (Raters and Matissek, 2008).

Despite reports of wheat contamination with toxigenic *A. flavus* or AFs at levels exceeding those imposed by the European Union in cereal derived products (2 μg/kg for AFB_1 and 4 μg/kg for total AF) (The Commission of the European Communities, 2010), by the US Food and Drug Administration in foods (20 μg/kg for total AF) (FDA, 2011) or by the Brazilian Regulations in cereals (5 μg/kg for total AF) (Brasil, 2011), the knowledge about the production and stability of AFs during malting for use in craft beer remains unclear. Most studies of mycotoxins produced during malting or detected in beers have focused on those produced by *Fusarium* species such as trichothecenes (including deoxynivalenol toxins, nivalenol, T-2 and HT-2), zearalenone (ZEA) and fumonisins B_1 and B_2 (FB_1, FB_2) (Habler et al., 2016; Hu et al., 2014; Jin et al., 2018a, 2018b; Mastanjević et al., 2018).

In the present study filamentous fungi of the genera *Penicillium* and *Aspergillus* isolated from raw materials used in wheat craft beer brewing were evaluated for the mycotoxin production potential. A toxigenic *A. flavus* strain, isolated from wheat grains, was inoculated into wheat grains to assess the production and stability of AFB_1 and AFB_2 during the eleven steps used in craft beer production.

2. Material and methods

2.1. Sampling of raw material for filamentous fungi isolation

Samples of malt and grains of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) were collected from August to November 2017. A total of 40 samples (approximately 2 kg each) comprising barley grains (n = 16), wheat grains (n = 10), barley malt (n = 10) and wheat malt (n = 4) were evaluated. Grain samples were obtained from storage of farmers or beer stores in the South of Brazil, while samples of malt were obtained from craft breweries or home brewers located in the South, North and Center-west regions of the country (Table 1).

2.2. Isolation, identification of Aspergillus and Penicillium and toxigenic potential of isolates

Samples (about 100 g) were subsampled (50 g) and surface disinfected in a sodium hypochlorite solution (0.4%) for 2 min (Samson et al., 2004). Afterwards, a total of 150 grains (thirty particles per plate) was directly placed onto Dichloran Rose Bengal Chloramphenicol Agar (DRBC) media (Merck, Darmstadt, Germany) (Pitt and Hocking, 2009). The plates were incubated for 5–7 days at 25 °C, and visually verified for fungal growth.

Representative colonies of putative toxigenic fungi (only *Aspergillus* section *Flavi* and section *Nigri* and some *Penicillium*) were purified onto Malt Extract Agar (MEA) (Himedia, Mumbai, India) and then three points were inoculated on MEA and Czapek Yeast Extract Agar (CYA) (Himedia, Mumbai, India) for identification based on macroscopic (colony growth, colony diameter) and microscopic characters using the appropriate identification keys of Klich and Pitt (1988), Pitt (2000) and Pitt and Hocking (2009). All identified isolates were preserved in silica gel following procedures described by Perkins (1962) and stored at 4 °C in the Laboratory of Microbial Processes in Foods, Department of Food Engineering, Federal University of Paraíba.

The potential to produce toxins from the isolates was tested according to Filtenborg et al. (1983). After incubation on Yeast Extract Sucrose Agar (YES) (Himedia, Mumbai, India) at 25 °C for 7 days, fungal extracts were taken as plugs and placed on Thin Layer Chromatography (TLC) plates. A mixture toluene: ethyl acetate: 90% formic acid: chloroform (7:5:2:5, v/v/v/v) was used as mobile phase and the bands were visualized under UV light at 365 nm. Standards of a mixture of AFB_1, AFB_2, AFG_1 and AFG_2, ochratoxin A (OTA) and citrinin (CIT) (Sigma-Aldrich, St. Louis, USA) were used for comparison.

2.3. Malting of artificially contaminated wheat

2.3.1. Test strain and inoculum preparation

The test strain included in experimental malting was *A. flavus* isolated from wheat grains (WG) sample number 06-A, collected in southern Brazil and initially described as *A. flavus* WG06-A (Table). The strain was characterized as AFB_1 and AFB_2 producer and the confirmation of the identity of the isolate (> 99% similarity) was performed by sequencing the ITS region (fragments of ITS1-5.8 S-ITS2) according Magnani et al. (2005). The strain was deposited in the Public Culture Collection of Microorganisms of the Food Science Department at Federal University of Lavras, Brazil and officially coded as *A. flavus* CCDCA 11553.

To obtain the inoculum, *A. flavus* CCDCA 11553 was cultivated on MEA at 25 °C for 7 days to produce spores. Spores were collected by scraping the mycelium using a spatula, sterile distilled water and 0.1% of Tween 80 (Labsynth, Diadema, Brazil). The mixture was filtered using sterile gauze to retain the mycelium and hyphal fragments. The concentration of spores in the suspension was standardized using a Neubauer chamber for a final concentration of 10^6 spores/mL (Wigmann et al., 2016).

2.3.2. Wheat grain inoculation

Wheat (*Triticum aestivum* L.) grains (around 1 kg) were purchased in a local brewing store of the city of João Pessoa, Paraíba state, Brazil. To ensure that the grains were free of the AFs, samples were tested for the presence of AFs following the procedures described in item 2.4 and no AFs were detected. Before the experimental malting, the wheat grains were disinfected by immersion in 1% sodium hypochlorite for 10 min and rinsed twice with sterile distilled water (Fiori et al., 2014). The artificial contamination was performed by adding the *A. flavus* CCDCA 11553 spore suspension (10^6 spores/mL) to the wheat grains. The concentration of the inoculum was selected for the experimental malting based on previous study (Habler et al., 2016).

2.3.3. Experimental malting process using A. flavus inoculated wheat grains

The malting process was performed according to the standard method proposed by the Central European Commission of Brewing Analysis (MEBAK) (Anger, 2006) comprising three main steps: steeping, germination and kilning (Table 2; Supplementary Fig. 1). The wheat grains immersed in the spore suspension of *A. flavus* CCDCA 11553 was considered the beginning of the steeping. There were three days of steeping (5-h wet period following a 19-h aeration period, 4-h wet period with a subsequent 20-h aeration period and maintenance of 45% steeping degree in grains by spraying water when needed) and 4 days of germination in perforated germination boxes to ensure aeration inside climate-controlled chambers (14.5 ± 0.5 °C and 95–98% relative humidity) with twice daily turning of grains to avoid microclimate clumps. The germinated wheat grains (green malt) were then kilned 16 h at 50 °C followed by 1 h at 60 °C, 1 h at 70 °C and finally 5 h at

Table 1
Fungal species isolated from raw material for beer brewing collected in distinct regions of Brazil and corresponding toxigenic profile.

Sample number	Raw material	Origin of sample[a]	Strain isolated	Mycotoxin production[b]
1–8	Barley grains	Store	–	–
9–16	Barley grains	Farmer	–	–
17–18	Wheat grains	Farmer	–	–
19	Wheat grains	Farmer	*A.* section *Nigri* uniseriate (WG03-A)	OTA (−)
20–22	Wheat grains	Farmer	–	–
23	Wheat grains	Farmer	*A. flavus* (WG06-A)	AFB_1 (+) and AFB_2 (+)
24–26	Wheat grains	Store	–	–
27	Barley malt	Craft brewery	*A.* section *Nigri* biseriate (BM01-K)	OTA (−)
28	Barley malt	Craft brewery	*A. tamarii* (BM02-G)	–
	Barley malt	Craft brewery	*Penicillium* sp. (BM02-A)	–
	Barley malt	Craft brewery	*P. citrinum* (BM02-B)	CIT (+)
	Barley malt	Craft brewery	*P. citrinum* (BM02-C)	CIT (+)
	Barley malt	Craft brewery	*P. citrinum* (BM02-D)	CIT (+)
	Barley malt	Craft brewery	*P. citrinum* (BM02-E)	CIT (+)
	Barley malt	Craft brewery	*Penicillium* sp. (BM02-F)	–
29–33	Barley malt	Home brewer	–	–
34	Barley malt	Home brewer	*A.* section *Nigri* uniseriate (BM06-B)	OTA (−)
	Barley malt	Home brewer	*A.* section *Nigri* uniseriate (BM06-C)	OTA (−)
	Barley malt	Home brewer	*P. citrinum* (BM06-A)	CIT (+)
35–36	Barley malt	Craft brewery	–	–
37	Wheat malt	Craft brewery	*A.* section *Nigri* uniseriate (WM01-C)	OTA (−)
38–40	Wheat malt	Home brewer	–	–

[a] The name of the places has been preserved.
[b] *OTA (−)* non-ochratoxin A producer, *CIT (+)* citrinin producer, *AFB_1 (+)* aflatoxin B_1 producer, *AFB_2 (+)* aflatoxin B_2 producer, - not isolated or not tested.

Table 2
Experimental conditions and sampling time of wheat grains artificially contaminated with *Aspergillus flavus A. flavus* CCDCA 11553 during standard malting.

Malting step	Experimental condition	Sampling time within step (h)	Sample code
Before malting	Wheat grains before malting	–	WG
Steeping	5 h wet stage followed by a 19 h air stage	24	SI
	4 h wet stage	28	SII
	20 h air stage	48	SIII
	24 h air stage	72	SIV
Germination	24 h air stage	24	GI
	24 h air stage	48	GII
	24 h air stage	72	GIII
	24 h air stage	96	GIV
Kilning	16 h at 50 °C	16	KI
	1 h at 60 °C, 1 h at 70 °C, and 5 h at 80 °C	23	KII
Wheat malt	Cold malt with rootlets removed	1	KIII

80 °C. Rootlets were removed from the wheat malt at the end of kilning. Approximately 50 g samples were collected in duplicate at pre-defined intervals for detection of AFB_1 and AFB_2 (Table 2).

2.4. Aflatoxin quantification in malting samples

2.4.1. Clean-up

Samples (~50 g) were finely ground using a laboratory mill (IKA A11 basic, Sigma-Aldrich, St. Louis, USA). An aliquot (12.5 g) was extracted with 50 mL of methanol: water solution (8:2, v/v) and NaCl (1.25 g). Suspensions were blended (30 min) at high speed (10,000 rpm) using a horizontal shaker (New Brunswick Scientific Company, USA). The homogenized solution was filtered through quantitative filter paper (Nalgon, Germany) and glass microfiber filter (Vicam, Milford, MA, USA). The filtrate (10 mL) was diluted in phosphate buffered saline (PBS; pH 7.0) (60 mL) and applied to an Aflatest WB immunoaffinity column (Vicam, Milford, MA, USA) at a flow rate of 2–3 mL/min. The column was washed with distilled water (30 mL) and aflatoxins eluted with methanol (1250 μL) and Milli-Q water (1750 μL) into an amber vial. The eluate was filtered using pre-cleaned PVDF syringe filter 13 mm × 0.22 μm (Pall Corporation, USA) prior to HPLC injection.

2.4.2. Chromatographic conditions

Aflatoxin quantification were carried out on wheat and malt by high performance liquid chromatography (HPLC) technique, based on Stroka et al. (2000). Analyses were performed in an Agilent Model 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA), with a fluorescence detector set at 362 nm (λ_{ex}) and 455 nm (λ_{em}), using a C18 column. The mobile phase used was water: acetonitrile: methanol (6:2:3, v/v/v), containing KBr (119 mg/L) and nitric acid (4 M, 350 μL/L), and the flow rate was 1 mL/min. A mix of AFB_1, AFB_2, AFG_1 and AFG_2 standards was used to construct a six-point calibration curve of peak areas versus concentration (μg/mL). The injection volume was 20 μL for both standard solution and sample extracts. An isocratic and reverse phase system associated with a Kobra Cell electrochemical reactor (R-Biopharm, Darmstadt, Germany) connected to a current of 100 μA for post-column derivatization of aflatoxins B_1 and G_1 was used.

The detection limit (LOD) and quantification limit (LOQ) for AFB_1 and AFB_2 were determined according to recommendations of Eurachem guides (Magnusson et al., 2015), and were 0.03 and 0.09 μg/kg for AFB_1 and 0.02 and 0.07 μg/kg for AFB_2, respectively. The concentrations of AFB_1 and AFB_2 in each sample collected during malting are presented as "dry basis", considering the moisture content of samples determined by official gravimetric analysis (AOAC, 2016).

2.5. Statistical analysis

All analyses were performed in duplicate in two independent experiments and the data was expressed as mean ± standard deviation. Statistical analyses were performed to determine significant differences ($\rho < 0.05$) between the levels of AFB_1 or AFB_2 detected in each sample collected during malting process using ANOVA, followed by post-hoc Tukey's test. All statistical analyses were performed using R software (R Core Team, 2017).

3. Results and discussion

3.1. Identification and toxigenic potential of Aspergillus and Penicillium isolates from craft beer brewing raw material

Fifteen percent (6/40) of the raw samples evaluated were infected with *Aspergillus* spp. and/or *Penicillium* spp. (Table 1). One third (2/6) were wheat grains, 50% barley malt (3/6) and 16.66% wheat malt (1/6). *Aspergillus* spp. or *Penicillium* spp. were not detected in any samples of barley grains. These results agree with previous reports that these genera are prevalent in foodstuffs and feedstuffs, may contaminate grains in the field, during harvesting or storage (Al-Wadai et al., 2013; Jedidi et al., 2017, 2018).

Five of the seven isolates of *Penicillium* spp. were identified as *P. citrinum* classified as CIT producers (Table 1). The isolation of this species was previously reported in wheat from Argentina (Comerio et al., 1998) and wheat and barley from southeastern Romania (Tabuc et al., 2009). *P. citrinum* is the main producer of CIT (Bragulat et al., 2008; Pitt and Hocking, 2009), however we found no previous studies which reported the isolation of CIT producers from barley malt. CIT has not been subject to regulatory action (Bragulat et al., 2008) since information on its toxicity is limited to little evidence in experimental animals (EFSA, 2012). In the present study we did not explored the production of CIT during malting because it is thermolabile and its levels will be reduced in beer, particularly by the wort boiling step where temperatures exceed 100 °C for about 1 h (Lhotská et al., 2016).

Seven *Aspergillus* spp. were isolated, five belonging to section *Nigri* and two belonging to section *Flavi* (Table 1). Four isolates of the section *Nigri* strains were classified as *A.* section *Nigri* uniseriate and 1 as A section *Nigri* biseriate, one isolate of those belonging to *A.* section *Flavi* was identified as *A. tamarii* and 1 as A *flavus*. None of five isolates from the section *Nigri* showed capability to produce OTA (Table 1). *A. flavus* WG06-A (further coded as *A. flavus* CCDCA 11553) was classified as AFB_1 and AFB_2 producer (Table 1).

Aspergillus belonging to section *Nigri* and section *Flavi* were previously isolated from barley malt samples collected in South Africa (Maenetje and Dutton, 2007) and Argentina (Gonzalez Pereyra et al., 2011). The incidence of *Aspergillus* and *Penicillium* isolates in malt may be related to contamination in the field (Maenetje and Dutton, 2007) and non-elimination of fungi in contaminated grains during malting (Mastanjević et al., 2018) or cross-contamination during storage (Schwarz, 2017).

The isolation of aflatoxigenic *A. flavus* has been previously reported in wheat grains from Saudi Arabia (Al-Wadai et al., 2013), from Lebanese cultivated wheat (Joubrane et al., 2011) and in Algerian wheat (Riba et al., 2010). We are not aware of any prior studies on the effects of malting conditions on aflatoxins production, or stability, using naturally or artificially contaminated wheat, despite the prevalence of potentially toxigenic isolates of this species in cereals used to produce craft beer (Jedidi et al., 2018). Since aflatoxins are thermostable, once formed in the malting wheat they can eventually contaminate the final product.

3.2. Production and stability of aflatoxins during wheat malting steps

AFB_1 and AFB_2 were detected in all samples collected during the standard malting process in concentrations ranging from 229.35 to 455.66 μg/kg for AFB_1 and 5.65–13.05 μg/kg for AFB_2 (Fig. 1; Supplementary Table 1). These findings show that the standard procedure currently proposed for malting of grains may favor the production of AFB_1 and AFB_2. The higher levels of AFB_1 produced compared to AFB_2 have been explained by the efficient synthesis and dominant biochemical pathway of AFB_1 compared to other AFs (Xie et al., 2018).

A total of 386.04 μg/kg and 5.65 μg/kg of AFB_1 and AFB_2 were produced after 24 h of steeping (SI) (Fig. 1; Supplementary Table 1). No changes ($\rho \geq 0.05$) in levels of AFB_1 were observed in samples collected after 28 h (SII), 48 h (SIII) or 72 h (SIV) of steeping. Otherwise, levels of AFB_2 increased ($\rho < 0.05$) approximately 50% after 28 h (SII) and no further changes were observed until the end of steeping (Fig. 1; Supplementary Table 1).

The production of AFB_1 and AFB_2 during the first 24 h of steeping is associated with the increased moisture content of grains, resulting from water immersion and aeration at 15 °C, which are conditions favorable for production of AFB_1 and AFB_2 by *A. flavus* (Mastanjević et al., 2018). Although the optimal temperature for growth of *A. flavus* range from to 25–30 °C at > 34% moisture content (~a_w 0.99) (Giorni et al., 2007), the growth can occur at temperatures as low as 11–16 °C (Yogendrarajah et al., 2016), and as high as 42 °C (Luo et al., 2018). Mousa et al. (2013) suggest that the stress imposed by temperatures lower than those optimal for growth (as used during steeping) can induce higher toxin production.

Although information regarding the effects of malting conditions on AF production are limited, Oliveira et al. (2012) reported that *Fusarium culmurum* could produce deoxynivalenol (DON) in barley grains after 24 h of steeping. Even if the AFB_1 concentration has not changed significantly after the first 24 h of steeping, AFB_2 increased significantly during the second 4 h wet stage (SII) (Fig. 1; Supplementary Table 1). This is in contrast to previous reports of decline up to 100% of DON in barley grains during steeping (Habler et al., 2016; Lancova et al., 2008), which is likely due to the fact that DON is water soluble and removed in the steep water (Lancova et al., 2008; Schwarz, 2017). Otherwise, AFs are only slightly soluble in water and able to be internalized in grains (IARC, 2012; Shephard, 2018).

No changes in levels of AFB_1 production were observed up to 48 h of germination (GII); however, a decrease ($\rho < 0.05$) of approximately 25% was observed after 72 h of germination (GIII) and 21% after 96 h of germination (GIV) compared to those detected at end of the steeping (SIV) (Fig. 1; Supplementary Table 1). AFB_2 levels detected in wheat grains at the end of steeping did not otherwise change during the germination.

Germination involves activation of enzymes needed to access the cellular reserves of starch and proteins (Pascari et al., 2018) and the decrease in AFB_1 levels may be attributed to enzymatic degradation or reaction with the components of wheat starch degradation (Verheecke et al., 2016). Biological degradation of AFs is also possible and should not be discarded (Verheecke et al., 2016).

Levels AFB_1 and AFB_2 increased ($\rho < 0.05$) by 55.4% and 78.5%, respectively after 16 h of kilning at 50 °C (KI) compared to those detected at the end of germination (GIV). AFB_1 levels at this step (KI) were similar to those detected at the beginning of steeping (SI), while levels of AFB_2 were the highest ($\rho < 0.05$) detected during the malting. At the beginning of kilning grains are dried at 50 °C for 16 h, and moisture content of the grains is reduced to 10–12%. The temperature increase (from 15 to 50 °C) could trigger further production of mycotoxins since fungal metabolism is still active at the high (45%) initial moisture in the grain (Schwarz, 2017; Wolf-Hall, 2007).

When the temperature reached 80 °C at the end of kilning (KII) levels of both AFB_1 and AFB_2 decreased ($\rho < 0.05$) by 56.2% and 122.9%, respectively, compared to those detected at the beginning (KI) of kilning (Fig. 1; Supplementary Table 1). This decrease was probably due to degradation at higher temperature (60–80 °C) (Schwarz, 2017). Although AFs inactivation occurs at 160 °C and above (Raters and Matissek, 2008) some degradation occurs at lower temperatures and longer times (Arzandeh and Jinap, 2011; Lee et al., 2015).

No additional changes were observed in levels of AFB_1 or AFB_2 after removal of rootlets (KIII). Because rootlets removed from malted grains have been proposed for animal feed (Pinotti et al., 2016), further studies focusing on fate of AFB_1 or AFB_2 over the wheat malting process would be important to clarify the possible risks of mycotoxins contamination in this by-product.

Throughout the malting process AFB_1 decreased ($\rho < 0.05$) by

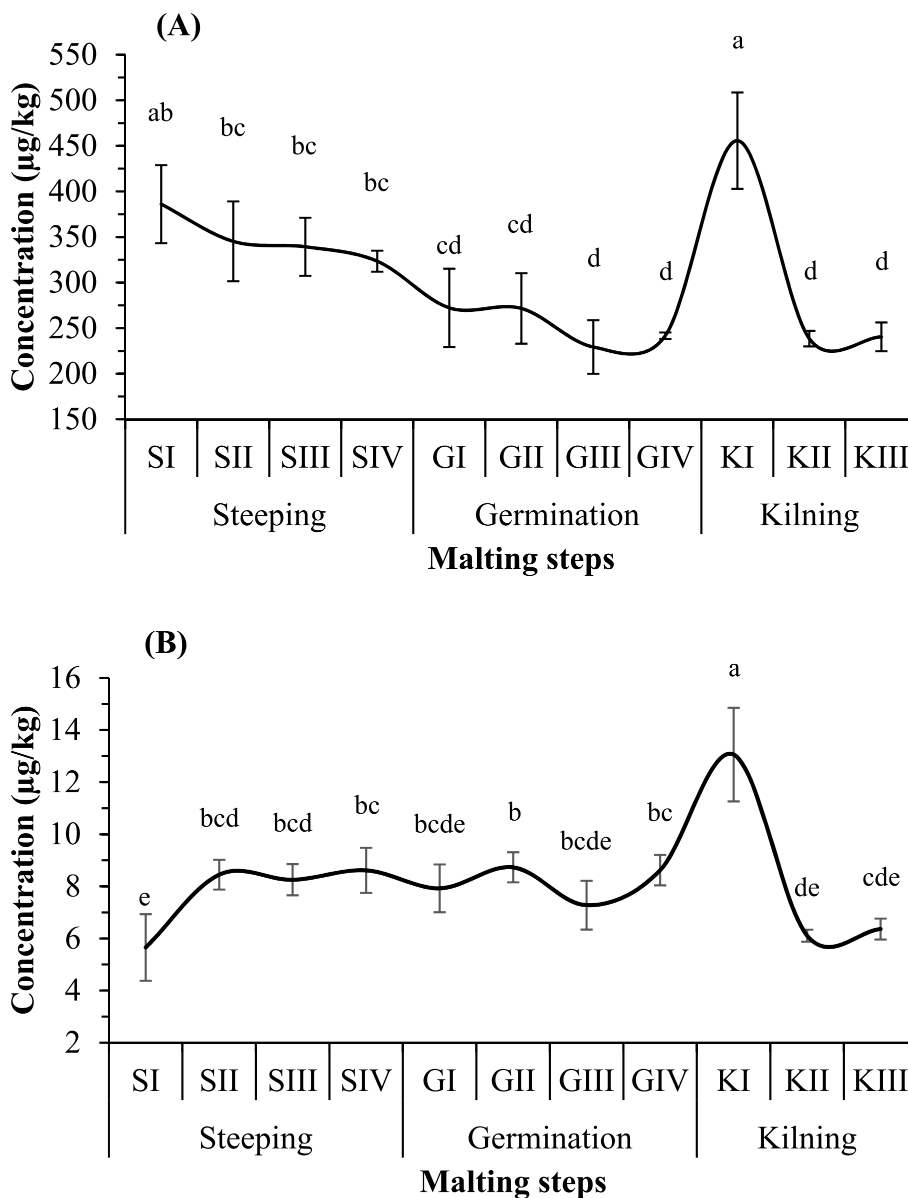

Fig. 1. Levels (µg/kg) of aflatoxins detected in each sample collected during standard malting process of wheat grains: (A) Aflatoxin B_1; (B) and Aflatoxin B_2.

37.7%, while AFB_2 did not change ($\rho \geq 0.05$) compared to the beginning of steeping (SI). The levels of AFB_1 and AFB_2 detected in the wheat malt, obtained at the end of experimental malting process, were of 240.46 and 6.36 µg/kg, respectively.

4. Conclusions

Our results show that standard malting conditions allow AFB_1 and AFB_2 production by *A. flavus* during the steeping and kilning steps. The AFs levels varied during the experimental malt process and, although decreases were observed in the levels of AFB_1, both, AFB_1 and AFB_2 were still present in the wheat malt at concentrations higher than those accepted by the European Union in cereal derived products, US Food and Drug Administration in foods and Brazilian Legislation in cereals or their derived products. These findings clearly indicate that typical craft beer malting conditions will allow significant aflatoxin production if grains are contaminated with aflatoxigenic *A. flavus* and evoke attention for the potential contamination of derived craft beers. Further studies evaluating the fate of AFB_1 and AFB_2 during production of wheat craft beer using the contaminated wheat malt will help to assess the risks of exposure to these toxins from wheat craft beer consumption.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2020.103456.

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