Effect of drying temperatures and exposure times on aspergillus flavus growth and aflatoxin production on artificially inoculated hazelnuts

Research Paper

Running title: Hazelnut drying and effect on viability and ability of *A. flavus* to produce aflatoxins

Effect of drying temperature and exposure times on *Aspergillus flavus* growth and aflatoxin production on artificially inoculated hazelnuts

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ABSTRACT

Aspergillus flavus may colonise hazelnuts and produce aflatoxins in field and during storage. The main purpose of this study was to investigate the influence of drying temperature and exposure times on the viability and ability of *A. flavus* to produce aflatoxins during the drying process and storage. Hazelnuts were inoculated with *A. flavus* and dried at different temperatures to reach 6% moisture content and a_w 0.71, a commercial requirement to avoid fungal development and aflatoxin contamination. Hazelnuts were dried at 30, 35, 40, 45, and 50 °C and subsequently stored at 25 °C for 14 days. After drying at 30, 35 and 40 °C, an increased number of *A. flavus* was evident, with the highest concentration at 35 °C (6.1 ± 2.4 x10^6 *A. flavus* CFU/g). At these temperatures, aflatoxins were detected only at 30 °C and 35 °C. Aflatoxins, however, were higher after drying at 30 °C with a concentration of 1.93 ± 0.77 μg/g for aflatoxin B1 (AFB1) and 0.11 ± 0.04 μg/g for aflatoxin B2 (AFB2). After 14 days of storage, the highest *A. flavus* concentration and the highest level of mycotoxins were detected in samples treated at 35 °C (8.2 ± 2.1 x10^7 *A. flavus* CFU/g, 9.30 ± 1.58 μg/g and 0.89 ± 0.08 μg/g for AFB1 and AFB2, respectively). In hazelnuts dried at 45 °C or 50 °C no aflatoxins were found both after drying and storage, and a reduction of *A. flavus* viable conidia was observed, suggesting that a shorter and warmer drying is essential to guarantee the nut safety. The lowest temperature that guarantees the lack of aflatoxins should be selected to maintain the organoleptic quality of hazelnuts. Therefore, 45°C should be the recommended drying temperature to limit *A. flavus* growth and aflatoxin contamination on hazelnuts.

HIGHLIGHTS

- Hazelnuts were artificially inoculated with an aflatoxigenic *A. flavus* strain
- Hazelnuts were dried at different temperatures to reach 6% of moisture content
- Shorter drying time is the key point to guarantee the nut safety
- Drying between 30-40 °C led to *A. flavus* growth and aflatoxin production
- 45°C should be the recommended drying temperature on hazelnuts

Hazelnut (*Corylus avellana* L.) is mainly cultivated in warm temperate areas, such as Turkey which is the first world producer, covering approximately 65% of the world production (675,000 tons in 2017), followed by Italy (approximately 13%), Azerbaijan, the United States, and China (*9*, *15*). The abundance of nutrients in hazelnuts, such as lipids and carbohydrates, makes them susceptible to decay and mould development (*10*, *30*). Several factors can affect fungal contamination on hazelnut, such as the variety, the composition and the presence of damages on shells caused by insects and environmental or processing conditions. Besides, other factors can favour fungal development, including temperature and humidity in the field, methods and time of harvest, processing of hazelnuts (e.g. dehulling and drying methods), time and storage conditions (*8*, *29*).

Most fungi isolated from nuts, including hazelnuts, belong to *Aspergillus* and *Penicillium* genera (*12*, *14*, *16*, *28*, *34*, *35*, *37*, *42*, *45*). These ascomycetes are often present in the field, in the soil and in food matrices as saprophytes. Due to their tolerance to low humidity and temperature, they may remain latent during pre-harvest and develop later during storage.

Aspergillus and *Penicillium* spp. are characterized by their ability to produce several secondary metabolites, including mycotoxins. In particular, *Aspergillus* section *Flavi* may produce aflatoxins, which are highly toxic and carcinogenic compounds. The most important mycotoxins are aflatoxins B1 (AFB1) and B2 (AFB2), mainly produced by *A. flavus* and *A. parasiticus*, and aflatoxin G1 (AFG1) and G2 (AFG2), synthesized by *A. parasiticus* (*31*, *33*, *34*). The European Commission (*6*) imposed maximum levels of aflatoxins in foodstuffs, including nuts. For hazelnuts intended for direct consumption, limits are fixed at 5 µg/kg for AFB1 and 10 µg/kg for total aflatoxins. Aflatoxins are very stable compounds which are not degraded during roasting or other thermal treatments (*41*, *43*, *44*). For this reason, it is very important to prevent conditions favourable to aflatoxin production both in field and during the postharvest phase (*28*, *29*).

The most common technique to reduce fungal growth and prevent mycotoxins in nuts is drying, preferably within 72 hours after harvesting, to a moisture content (5 −8% depending on nuts) that corresponds to a low water activity, a_w (*4*, *13*). The European Commission (*5*) established the maximum moisture content for nuts in 12% for shelled hazelnuts and 7% for dehulled nuts. Commercially, hazelnuts are dried until 10% and 6% moisture content for shelled and dehulled hazelnuts, respectively (*49*). In Turkey, nuts are conventionally dried outdoor for a period ranging from 4 days to 2–3 weeks depending on the weather conditions, before being stored at room temperature for 12 months (*3*, *47*). This prolonged and uncontrolled drying process usually promotes fungal contaminations that can result in fungal growth and aflatoxin production during storage. The use of drying machines is preferable because of the speed and homogeneity in reaching a low moisture content (*28*, *48*). Air temperature, relative humidity and time can be controlled with drying machines. Drying temperatures commonly used by growers range from 30 to 40 °C (*19*, *28*, *49*). Nevertheless, air drying machines could result in physical damage, such as shell-cracks that could reduce the quality and safety of the kernels (*50*). Moreover, several studies highlighted the influence of drying processes on hazelnuts quality, including lipid oxidation, fatty acid profile, enzymatic activity and kernel browning (*20*, *21*, *22*, *27*, *29*, *47*, *48*, *49*). Previously, Ozay *et al.* (*28*) evaluated the effect of drying parameters on aflatoxin contamination on hazelnuts. The influence of drying techniques both on fungal growth and on aflatoxin production has not been investigated on hazelnuts so far.

The aim of this study was to investigate the effect of two significant drying parameters, temperature and drying time, on *A. flavus* viability and aflatoxin production on shelled hazelnuts. The time to reach the commercial threshold of 6% of moisture content at 30 °C, 35 °C, 40 °C, 45 °C and 50 °C was determined. Fungal growth and aflatoxin concentration were determined on hazelnuts inoculated with a conidial suspension of *A. flavus*, able to produce

aflatoxins *in vitro* and *in vivo*. After drying, hazelnuts were stored at 25 °C for 14 days to simulate commercially adopted practices.

MATERIALS AND METHODS

Hazelnuts and sample preparation. Fresh shelled hazelnuts 'Tonda Gentile Romana' (30 kg), provided by Soremartec Italia s.r.l., were harvested in Viterbo (Latium, Central Italy) in 2018. Hazelnuts were stored with shells in sealed polypropylene plastic bags at 4 °C for later use. Hazelnuts used for drying assays were dehulled one day before the experiment and kept in plastic bags at 4 °C overnight. Raw hazelnuts were analysed for absence of AFs (AFG1, AFG2, AFB1 and AFB2). Sampling of hazelnut was done according with the Commission Regulation (EU) No. 178/2010 (*7*): 15 incremental samples were collected randomly from the lot and then mixed thoroughly to achieve a well homogenized aggregate sample of about 3 kg.

Determination of moisture content, water activity and drying conditions. The drying experiments were performed in a drying and heating chamber (Binder M115-230V) while, after drying, an environmental testing chamber (Panasonic MLR-352H-PE) was used for storing hazelnuts at 25 °C for 14 days. Moisture content of nuts was determined by weighing samples before and after drying at 103 °C for 14 hours (*36*). Water activity (a_w) was measured by using an electronic hygrometer AquaLab Series 3TE (Decagon Devices Inc., Pullman, WA, USA), which adopted the chilled-mirror technique at 25 ◦C.

In order to mimic the moisture conditions favouring the fungal development, hazelnuts were soaked with sterile water for 2-14 hours. Hazelnuts were, later, inoculated with *A. flavus*, and fungal growth was visually observed after 7 days at 25 °C. Fourteen hours of soaking were adopted in the subsequent experiments.

To determine the time needed to reach approximately 6% of moisture content in healthy hydrated hazelnuts, they were dried in drying chamber using 5 temperatures (30 °C, 35 °C, 40 °C, 45 °C and 50 °C). Nuts were placed in aluminium trays on a paper towel and were covered with non-woven fabric sheet to promote drying. After 8 −72 hours their moisture content was determined. Furthermore, the water activity of hazelnuts dried at 6% of moisture content was determined.

For each treatment, the analyses were performed in three biological replicates and two technical replicates of 15 hazelnuts. A negative control was added. The analyses were performed twice.

Fungal strain and hazelnut inoculation. An aflatoxigenic strain of *Aspergillus flavus*, AFSP4, was used to inoculate hazelnuts in all experiments (*34*). The strain was grown on Potato Dextrose Agar (PDA, Merck, Darmstadt, Germany) at 30 °C for seven days. In order to collect the spores, 5 mL of sterile deionized water containing 0.1% Tween (Merck, Darmstadt, Germany) were added to each plate and the colony surface was gently scraped. Conidia were pelleted using a centrifuge at 4,452 x *g* for 10 min and resuspended in deionized water with 0.1% Tween and their number was counted with Bürker chamber and adjusted to a final concentration of $1x10^7$ spores/mL.

Before spore inoculation, hazelnuts were surface disinfected by immersion in a 1% sodium hypochlorite solution for 10 min and rinsed in sterile water for 10 min. Then, hazelnuts were soaked for 14 hours and dried for 10 min on paper towel under a laminar-flow hood. Hazelnuts were inoculated by stirring (ARE Hot Plate Stirrer, VELP Scientifica, Velate, Italy) in 200 mL of *A. flavus* spore suspension for 20 min, and then dried on paper towel in laminar-flow hood for 10 min. Inoculated and untreated hazelnuts were used as control.

Effect of drying on *Aspergillus flavus* growth. To evaluate the effect of drying temperatures on *A. flavus* growth, hazelnuts were dried at 30 °C, 35 °C, 40 °C, 45 °C and 50 °C. The relative humidity during drying was 60±5 %. For each drying condition, three biological replicates were used together with one negative control (hazelnuts not inoculated and dried at the same conditions). Biological replicates and the negative control were placed in separate aluminium trays.

A. flavus CFU/g hazelnuts and aflatoxins content were determined immediately after drying and after 14 days of storage at 25 °C and 70±5 % relative humidity. To count the number of viable *A. flavus* CFU/g, each technical replicate of 15 nuts was washed in a beaker placed on a stirrer with 100 mL of sterile water containing 0.1% Tween for 60 min. Serial dilutions of the washing water were plated on Yeast Extract Sucrose Agar (YES Agar) and on PDA. Agar plates were kept at 30 °C for 3 days and fungal colonies were visually counted, and the numbers of CFU/g nuts were calculated, by considering the weight of each sample.

Effect of drying on aflatoxin production. The chemical analyses were performed immediately after drying and after 14 days of storage at 25 °C in three biological replicates and two technical replicates. Each technical replicate consisted of 15 hazelnuts. Each replicate was placed into a 50 mL polypropylene tube and 30 mL of acetone/water (60/40 v/v) were added. The mixture was vortexed at high speed for 1 min and aflatoxins were extracted by sonication for 30 min. An aliquot (10 mL) of centrifuged extract (4,452 x *g* for 15 min) was filtered through a Whatman No. 4 filter and then the analytes were extracted twice with 10 mL ethyl acetate. The organic layer was evaporated to dryness and recovered with 1 mL of methanol/water (30/70 v/v).

Aflatoxin analysis was carried out using an Agilent 1100 series Quaternary pump LC system (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 1100 series

fluorescence detector (Agilent Technologies) set for excitation wavelength of 360 nm, and emission wavelength of 425 nm (quantification) and 455 nm (monitoring). The AFB1 signal enhancement was performed through an electrochemical bromine derivatization cell (Kobra Cell, R-Biopharm, Darmstadt, Germany). Analytes were separated using a reversed phase Eclipse Plus C18 (3.5 µm, 4.6 x 100 mm, Agilent Technologies) column at 25 °C. The isocratic mobile phase comprised water:methanol:acetonitrile (40:32:28, v/v/v) containing potassium bromide (119 mg/L) and 4 M nitric acid (350 µL/L) at flow rate of 1 mL/min for 15 min. The injection volume into the HPLC system was 100 µL. Retention times of 5.6, 7.0, 8.5, and 10.9 min were registered for AFG2, AFG1, AFB2, and AFB1, respectively.

AFs were quantified by external calibration curve. The standard solutions were prepared by diluting mixture of AFB1, AFB2, AFG1 and AFG2 using concentrations ranging from 2 to 500 ng/mL. Standards of the aflatoxins were purchased from Sigma-Aldrich (St. Louis, MO, USA) in crystallized form.

The limit of detection (LOD) of the method was 0.42 ng/g, 0.49 ng/g, 0.33 ng/g and 0.25 ng/g for AFG2, AFG1, AFB2, and AFB1, respectively. The recovery of AFs was determined at three concentrations in the hazelnut matrix. The blank samples were spiked with the standards of AFB1, AFB2, AFG1 and AFG2 at low (5 µg/kg), middle (20 µg/kg) and high concentration (50 µg/kg) in three replicates. The average recoveries for AFB1, AFB2, AFG1 and AFG2 were 90.3 ± 3.6%, 89.6 ± 2.2%, 94.6 ± 1.1% and 98.1 ± 5.4%, respectively. The calculated limits of quantification (LOQ) were 2.10, 1.71, 0.99, and 1.00 µg/kg for AFB1, AFB2, AFG1, and AFG2, respectively.

Statistical analysis. Statistical analyses were performed using IBM SPSS statistics software Inc. version 25 (Chicago, IL, USA), for variance analysis (one-way analysis of

variance) using the Duncan test with $P \leq 0.05$. Data on *A. flavus* CFU/g hazelnuts were logarithmically transformed before statistical analysis.

RESULTS

Effect of drying time on moisture content and water activity. The average moisture content of fresh shelled hazelnuts used in this study was 13.2 ± 0.7%, while dehulled hazelnuts had an average moisture content of 8.5 ± 1.2%, corresponding to a water activity of 0.82. Freshly harvested hazelnuts were already at a low moisture content before drying, due to the dry cropping season. As it was not possible to obtain consistent results about fungal development in fresh hazelnuts inoculated with *A. flavus* and stored at 25 °C for 14 days (data not shown), hazelnuts were soaked in water for 2 to 14 hours to increase the moisture content and consequently the water activity prior to drying (Table S1). After 14 days of storage at 25 °C, fungal growth was observed on hazelnuts soaked for at least 8 hours (Fig. S1). For the drying assay, hazelnuts were soaked for 14 hours to reach 35.9 ± 1.5% moisture content and a_w of 0.94 ± 0.02 to create conditions suitable for *A. flavus* growth.

To calculate the time required to reach about 6−7% moisture content using different drying temperatures, the moisture was determined 8 to 72 hours after the treatments (Table 1). We observed that at 30 °C, 35 °C, 40 °C, 45 °C and 50 °C, there was a need of 72 h, 33 h, 28 h, 23 h and 20 h respectively, to dry hazelnuts. The water activity of samples dried at these conditions was calculated (Table 1), after the drying treatment hazelnuts had a final a_w of 0.71± 0.05.

Effect of drying temperatures on *A. flavus* growth. *A. flavus* was determined as CFU/g in the samples dried at different temperatures. A reduction of *A. flavus* growth was observed with the increase of temperature (Fig. 1). Fungal growth was higher in hazelnuts dried

between 30 °C and 40 °C. In hazelnuts dried at 35 °C, *A. flavus* reached 6.1 x 10^6 CFU/g, while at 45 °C and 50 °C, *A. flavus* growth was significantly lower compared to the samples dried at 30 °C, 35 °C and 40 °C and to the inoculated untreated hazelnuts (3.9 x 10^4 CFU/g) (Fig. 1A). No CFU were found on uninoculated nuts used as negative controls (data not shown).

Similarly, after 14 days of storage at 25 °C, hazelnuts dried at 45 °C and 50 °C showed a lower number of *A. flavus* (CFU/g) compared to control and samples dried at lower temperatures (Fig. 1B). Hazelnuts dried between 30 °C and 40 °C exhibited a significant increase of fungal growth after the storage period, with the highest *A. flavus* concentration at 35°C (8.2 x 10^7 CFU/g) (Fig. 1B).

Effect of drying temperatures on aflatoxin production. No natural aflatoxin contamination was detected on raw hazelnuts used in this study. Aflatoxin production was evaluated in hazelnuts after drying at different temperatures and after 14 days of storage at 25 °C. The strain of *A.flavus* used in this study produces AFB1 and AFB2 (*34*).

As shown in Fig. 2A, production of both toxins (AFB1, AFB2) started already during drying at 30 °C with a concentration of 1.93 ± 0.77 μg/g for AFB1 and 0.11 ± 0.04 μg/g for AFB2. A lower toxin concentration was measured at 35 °C. At 40 °C, 45 °C or 50 °C no aflatoxins were detected immediately after drying.

Fourteen days after the drying treatment, aflatoxin production was observed in the samples dried at 30 °C, 35 °C and 40 °C (Fig. 2B). The highest level of mycotoxins detected was at 35 °C (9.30 ± 1.58 μg/g and 0.89 ± 0.08 μg/g for AFB1 and AFB2, respectively). Similar aflatoxin production was found for the samples dried at 30 °C for both time points tested.

For samples dried at 40 °C, aflatoxin production was found only after 14 days of storage (Fig. 2B), with a concentration of 1.57 ± 0.62 μg/g for AFB1 and 0.15 ± 0.06 μg/g for AFB2.

No aflatoxins were found in samples dried at 45 °C and 50 °C, both after drying and after storage.

DISCUSSION

Hazelnuts can be contaminated with mycotoxigenic fungi at various steps from harvesting to the final product, especially after dehulling, and drying is commonly used to reduce the moisture content of the nut, to avoid the fungal growth. Due to the dry climate conditions in the field before harvest, shelled hazelnuts used in this work had a moisture content of 13.2%, which is similar to the values measured in Oregon hazelnuts (*49*), but lower compared to those measured (about 25%) in hazelnuts used in other studies (*22*, *28*, *46*, *47*). This low amount of water corresponds to a water activity of 0.82, that is not favourable for fungal development and mycotoxin production, because close to the minimum a_w that ranges from 0.80 to 0.83 for fungal growth and is 0.85 for aflatoxin production (*1*, *11*, *24*). To investigate the effect of drying temperatures and drying time on fungal growth and aflatoxin production, the development of *A. flavus* was promoted by increasing moisture content and consequently water activity of hazelnuts. Hydrated hazelnuts with final a_w of 0.94 are suitable for *A. flavus* growth (*11*), and this was confirmed in our work by *A. flavus* growth on hazelnuts.

Hazelnuts were dried at increasing temperatures to determine the time required to reach 6% of moisture content, which is commercially adopted to prevent fungal growth and mycotoxin occurrence (*49*). As expected, higher temperatures required a shorter time of drying, however, at 30 °C, three days of drying were necessary (*27*). The drying times tested were similar to those commercially used for hazelnuts (*28*, *47*).

Significant differences were observed in *A. flavus* growth among different drying treatments: a higher growth (measured as CFU/g) of *A. flavus* was found in the samples dried between 30 °C and 40 °C. Hazelnuts treated at 35 °C showed the highest fungal growth and

aflatoxin production. These results were in agreement with those reported in literature, showing that the optimal temperature for *A. flavus* growth ranges from 30 °C to 35 °C (*41*), but could vary depending on the nature and composition of the substrate and the fungal strain studied (*10*, *11*, *17*). Indeed, in peanuts and sorghum the optimal temperature for fungal growth was 37 °C (*17*, *18*). In soybean and in dried Brazil nuts, however, the highest fungal growth was observed at 30 °C (*32*). On rice, the optimal temperature was predicted to be between 30 and 32 °C (*26*) and on chestnuts (*36*) the highest fungal colonization was observed after 7 days of drying at 30 − 35 °C, while at 45 and 50 °C no mould growth was detected. Similar results were observed in the present study on hazelnuts, however drying times were modified according to the temperature of drying used in order to reach the same moisture content. Our data discourage the use of 30 or 35 °C to dry hazelnuts, because these temperatures are favourable for *A. flavus* growth and because longer times are required to reach a moisture content unfavourable for fungal growth. At 45 and 50 °C, a significant reduction of *A. flavus* growth on hazelnuts was achieved. These high temperatures are therefore suitable to reduce the viability of fungal spores on hazelnuts, avoiding mould growth during storage.

Mycotoxin production is often promoted under suboptimal growth conditions as an adaptive response (*36*, *39*, *40*). For example, in chestnuts, drying at 40 °C resulted in a significant reduction of fungal growth but also in a higher production of aflatoxins (*36*). Moreover, aflatoxin production is generally blocked at temperatures above 42 °C, as confirmed by negative regulation of genes involved in their biosynthesis (*18*). As expected, *A. flavus* growth was dramatically reduced at 45 °C or 50 °C and the remaining spores were unable to produce aflatoxins. These results were consistent with those obtained by other researchers which showed that fungal development occurred in a wider range of a_w and temperatures, compared to aflatoxin production (*1*, *11*, *24*, *25*, *38*). Our results confirm that higher drying temperature are suitable to reduce aflatoxin contamination. The use of high temperature for

drying is also connected with a lower time of the process, which is a benefit from the industrial point of view.

Interestingly, *A. flavus* was able to produce aflatoxins after drying at 30 °C, and, to a lower extent, at 35 °C. This could be explained by the longer time for drying until 6% moisture (*2*). At 30 °C, 72 hours of drying were used, while, at 35 °C 33 h were sufficient.

After 14 days of storage at 25 °C, an increased aflatoxin production was observed for hazelnuts previously dried at 35 °C and 40 °C. In these conditions, an increase of *A. flavus* growth was evident. These results highlight the need to control also storage conditions, such as humidity and temperature, to avoid fungal contamination and proliferation. Indeed, during storage, a modification of these two parameters can cause a rapid increase in water activity and a proliferation of filamentous fungi (*8*, *14*, *23*, *30*), which could result in aflatoxin production.

In conclusion, drying temperatures from 30 °C to 40 °C associated to longer times of drying can promote fungal development and aflatoxin production, while 45 °C and 50 °C seem to be optimal temperatures for drying hazelnuts, because of the reduction of *A. flavus* and the lack of aflatoxins both after drying and storage. Wang at al. (*49*) and Turan and Islam (*48*) suggest to dry hazelnuts with higher temperature (43-49 °C and 45 °C, respectively). For other nuts, the best drying temperatures were found to be above 40 °C: 42 °C for peanuts (*18*) and 45 °C for chestnuts (*36*). In this work, hazelnuts were artificially inoculated, while fungal growth and aflatoxin production were promoted by adjusting the moisture content, as a result, high level of aflatoxins were produced especially between 30 °C and 40 °C. Despite the change of the natural conditions, drying at 45 °C and 50 °C led to a significant reduction of *A. flavus* and to inhibition of aflatoxins production. Further studies are required to confirm that the adoption of these temperatures on non-inoculated nuts, results in the improvement of safety of nuts (both in terms of reduction of aflatoxin contamination and *A. flavus* presence) and in the maintenance of the nutritional qualities of nuts.

In previous reports, the use of temperatures above 40°C permitted to maintain the chemical characteristics of hazelnuts (*22*, *27*, *49*). For instance, the use of drying machines at 45 °C to dry Turkish hazelnuts cv. Levant provided products with a better oxidative stability over 12 month of storage at room temperature (*47*). Instead, it is not advisable to dry hazelnuts at temperatures higher than 50 °C because the frequency of rancidity reactions increases with a damage in hazelnut quality (*49*). By considering that an increase of the temperature of drying could affect the organoleptic quality of hazelnut, the lowest temperature that guarantees the absence of aflatoxins should be selected. Therefore, 45°C should be the recommended drying temperature to limit *A. flavus* growth and aflatoxin contamination on hazelnuts.

This is the first study concerning the effect of drying temperatures both on *A.flavus* growth and AFs production on hazelnuts. The information gained could be used to implement drying strategies to avoid sun drying and to limit the development of aflatoxigenic fungi and of aflatoxins production. Moreover, the control of the environmental parameters during drying and storage is essential to develop appropriate practices to maintain healthy and safe food products.

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SUPPLEMENTARY MATERIALS

Supplemental material associated with this article can be found online at: [URL to be completed by the publisher].

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FIGURE LEGENDS

FIGURE 1. Log_{10} of CFU of *A. flavus* per gram of hazelnuts counted both after drying at different temperatures to reach approximately 6% of moisture content (A) and after 14 days of storage at room temperature (B). Control refers to inoculated samples undried and not stored and used directly to count inoculated *A. flavus* spores. Values followed by the same letter at the same time are not statistically different by Duncan's multiple range test ($P \leq 0.05$). The statistical analysis was performed considering separately values obtained after drying and after storage.

FIGURE 2. Concentration (μg/g) of AFB1 and AFB2 present in inoculated hazelnuts dried at different temperatures to reach 6% of moisture content. (A) shows data from hazelnuts collected immediately after drying; (B) shows data collected from hazelnuts stored for 14 days at 25 °C. N.d. = non detected. Values followed by the same letter at the same time are not statistically different by Duncan's multiple range test ($P \leq 0.05$). The statistical analysis was performed considering separately values obtained after drying and after storage.

TABLE 1. Moisture content (%) of hydrated hazelnuts dried at different temperatures for 8−72 hours. The time points used to reach around 6% of moisture content are in bold and for them the water activity was measured (in brackets). Values are expressed as mean values ± SD (3 replicates of 15 nuts per experiment, 2 repetitions of the experiment).

Drying Time (h)	30 °C	35 °C	40 °C	45 °C	50 °C
8	31.8 ± 2.4	18.7 ± 0.3	17.6 ± 0.2	15.1 ± 0.4	14.8 ± 0.1
20	-	-	-	-	**6.2 ± 0.1** **(0.71 ± 0.06)**
23	27.8 ± 1.0	9.2 ± 0.7	7.8 ± 0.7	**5.6 ± 0.5** **(0.67 ± 0.04)**	4.3 ± 0.3
28	26.0 ± 0.6	7.9 ± 0.4	**6.6 ± 0.4** **(0.74 ± 0.03)**	4.6 ± 0.3	-
33	23.5 ± 0.3	**6.5 ± 0.2** **(0.73 ± 0.03)**	5.2 ± 0.1	3.6 ± 0.1	-
50	15.4 ± 2.9	4.2 ± 0.1	3.4 ± 0.1	2.5 ± 0.1	-
56	12.6 ± 0.1	3.8 ± 0.1	3.1 ± 0.1	2.3 ± 0.0	-
72	**6.4 ± 0.3** **(0.72 ± 0.03)**	-	-	-	-