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Modelling the probability of growth and aflatoxin B_1 production of *Aspergillus flavus* under changing temperature conditions in pistachio nuts

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Abstract

The aim of this work was to use probability models for the prediction of growth and aflatoxin production by *Aspergillus flavus* as a strategy to mitigate the aflatoxin presence in pistachio nuts during postharvest. Logistic models, with temperature and time as explanatory variables, were fitted to the probability of growth and aflatoxin B_1 (AFB1) production under constant temperature levels, afterwards they were used to predict probabilities under non-isothermal scenarios. The models obtained showed levels of concordance from 80 to 100% in most of the cases. Moreover, the presence of AFB1 in pistachio nuts could be correctly predicted through AFB1 models developed in agar medium or through growth models in pistachio nuts. These findings can support decision making, at transport and storage level, and could be used by producers and processors to predict the time for AFB1 production by *A. flavus* in pistachio nuts in postharvest.

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Keywords: Predictive mycology; *Aspergillus flavus*; food safety; pistachio; temperature; non-isothermal conditions; probability model

1. Introduction

Pistachio nut (*Pistacia vera* L.) is one of the most popular tree nuts in the world, and is subjected to infection by a variety of microorganisms that can cause foodborne illness, spoilage or toxic effect on human[1]. Within these microorganisms, *Aspergillus flavus* and *Aspergillus parasiticus*, weak opportunistic plant pathogenic fungi[2], are the

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most relevant species. Both species can produce aflatoxins (AFs), secondary metabolites produced by various strains[3]. AFs are the most important mycotoxins, and the AFB1 is listed as a carcinogen of group I by the International Agency for Research of Cancer, and due to their hepatocarcinogenic potential, AFs are highly regulated (European Commission Regulation 165/2010). The maximum limits for AFB1 are 12 ㎍/kg for pistachios to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs, and 8 ㎍/kg for pistachios intended for direct human consumption or use as an ingredient in foodstuffs. According to the RASFF (EU Rapid Alert System for Food and Feed) in 2014 there have been 125 notifications related with AFs in nuts, nut products and seeds from Iran, China and Turkey. From the food safety point of view, only mycotoxins entail a hazard, while yeast and moulds themselves may cause food spoilage but are not harmful to humans.

Nut infections may occur along all the food chain, but are more common to occur during preharvest; nevertheless it might occur in the subsequent steps (storage, manufacturing, transport and packaging), if minimum preventive measures are not established. During postharvest, fungal growth should not occur if the freshly harvested nuts are dried as soon as possible to 6% of moisture content and then cool stored. However, shipping of nuts is not always carried out under cool conditions, as this is economically costly. It is noticeable that the temperature fluctuations during transport and retail storage can affect the quality and food safety. Increases in temperature and humidity within the bulk of pistachio nuts during transport and storage may allow fungal growth and mycotoxin production. In this way, it is important to control temperature and humidity during transport and do not allow the pistachio bulk to reach a temperature which jeopardizes the safety of the product.

The specific objectives of the present study were to: i) model the probability of growth/AF production of *A. flavus* under non-isothermal conditions; ii) validate the derived models on AFB1 data generated directly in pistachio nuts under non-isothermal conditions.

2. Material and methods

2.1. Experimental design and data generation

A full factorial design was developed, where factors involved were temperature and medium. Regarding medium, the whole experiment was carried out in both pistachio extract agar (PEA) and pistachio nuts. Regarding temperature, nine profiles were tested: five static temperatures (15, 17.5, 20, 22.5 and 25 ºC), plus four different scenarios of dynamic temperature levels (upward shift (US), downward shift (DS), upward ramp (UR) and downward ramp (DR)). These temperature levels were chosen based on the levels which may be encountered during shipping of pistachios at room temperature. Both the static and changing temperatures were kept for a 42 days period. a_w was initially adjusted to 0.87, corresponding to about 15% moisture content. The experiments were carried out with a minimum of ten replicates per treatment.

A. flavus suspensions were point-inoculated on the center of each Petri dish, on both PEA and pistachio nuts, under aseptic conditions, and incubated in computer controlled incubators set at the conditions designed for this study. PEA and nuts Petri dishes were daily checked for visible growth, using a binocular magnifier for easy viewing in the case of pistachios nuts.

Once positive growth had been recorded, 10/12 existing Petri plates per treatment were taken from incubation at different time points for AFB1 analysis, always when colonies were in the range 4-20 mm diameter. Extraction of the AFs from the agar was carried out with methanol and filtered. For pistachio nuts, the moldy ones were extracted with acetonitrile:water. Diluted extracts were passed through immunoaffinity columns (Easi-extract Aflatoxin immunoaffinity columns, R-Biopharm Rhône) and the eluate was dried in a nitrogen stream. All extracts were resuspended and injected in the HPLC system (Waters, Milford, MA, USA).

2.2. Model fitting

A logistic model was used to model the probability of growth and AFB1 production of *A. flavus* as a function of time under static conditions, using R statistical software (R Development Core Team, www.R-project.org, v 2.14.1), with the glm function. The percentage of plates with growth was calculated as P_G = plates with growth/total plates.