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Risk management towards food safety objective achievement regarding to mycotoxins in pistachio: The sampling and measurement uncertainty issue

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ABSTRACT

The emerging risk management metrics, FSO, PO and PC, were applied to the aflatoxins (AFs) and ochratoxin A (OTA) determination in 8 commercial lots of pistachio. In order to determine the sampling uncertainty, two sampling plans (EU official sampling and company plan) in quadruplicate, and two analytical methods (ELISA and HPLC), were considered in parallel. The combination of EU official sampling plan and HPLC proved to be the most appropriate option. The major variability was associated with the subfraction selection and, therefore, increasing the number of the analysed subfractions could be an alternative for reducing uncertainty. AFs were present in all lots, mainly AFB1 and AFB2, while OTA was never detected. The effect of toasting on AFs presence in pistachio (a performance criteria, PC) was evaluated in order to achieve a given PO, taking into account the FSO, i.e., the EC limits. Percentages of AFs reduction were 87.62% ± 11.89, 81.05% ± 15.51 and 86.74% ± 11.31 for AFB1, AFB2 and total AFs, respectively. Given an initial AFB_1 and AFBs level ≤12 μg/kg and ≤15 μg/kg, respectively, the toasting would ensure the AFB1 and AFs legal limits compliance before human consumption (FSO).

1. Introduction

The Food Safety Objective (FSO) for a hazard is the maximum frequency and/or concentration of the hazard in a food at the time of consumption, and is preceded by the Performance Objective (PO), which is the maximum frequency and/or concentration of the hazard in a food at a specified step in the food chain before the time of consumption (ICMSF, 2002), that still provides or contributes to the achievement of an FSO or Appropriate Level of Protection (ALOP), as applicable. In the case of chemical hazards such as mycotoxins, the limits set by a country for mycotoxins in foods can be logically considered also to have the status of an FSO.

Nuts present low a_W and, due to their intrinsic characteristics, fungi are the major microbiological contaminants. Some of these moulds are mycotoxigenic, thus high levels of mycotoxins have frequently been reported in nuts from the orchards and from the market (Bayman, Baker, Doster, Michailides, & Mahoney, 2002; Fernane, Cano-Sancho, Sanchis, Marin, & Ramos, 2010). In pistachios, the dominant mycobiota are *Aspergillus* section *Nigri*, *Aspergillus flavus* and *Penicillium* spp. (Denizel, Jarvis, & Rolfe, 1976; Fernane, Sanchis, Marín, & Ramos, 2010). Several studies have reported that *Aspergillus* spp. causes decay in pistachio nuts at different parts of the world, such as California (USA) (Doster & Michailides, 1994), Iran (Mojtahedi, Rabie, & Lubben, 1979), and Turkey (Denizel et al., 1976). The most important mycotoxins found in pistachio are aflatoxins (AFs), including aflatoxin B_1 (AFB1), aflatoxin B_2 (AFB2), aflatoxin G_1 (AFG1) and aflatoxin G_2 (AFG2) and ochratoxin A (OTA). The International Agency for Research on Cancer (IARC) classified AFs in group 1, as human carcinogens, and OTA in group 2B, as a possible human carcinogen (IARC, 2002, pp. 171–300).

On the regulatory side, legally binding, EU-wide maximum levels (MLs) for mycotoxins in food have been introduced by the European Commission and updated subsequently. Nowadays the Comission of the European Communities (EC) has established maximum levels of mycotoxins in nuts to be subjected to sorting, or other physical treatment. These established values should led processing companies to accept only those raw material batches which allow compliance with the final PO of the company in the final product. Regarding pistachio, toasting is the main way, together with physical separation, to reduce the levels of mycotoxins. Removal of highly contaminated pistachio nuts by sorting decreases AFs contamination by 2–4 times in processed pistachios compared to non-processed pistachios (Schatzki & Pan, 1996). However, conflicting results have been published about the effect of the heat treatment in AFs in pistachios (Ariño et al., 2009; Yazdanpanah, Mohammadi, Abouhossain, & Cheraghali, 2005).

In order to determine mycotoxin presence in foods, sampling and analysis are needed, despite a large variability and uncertainty

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is associated with these procedures. Mycotoxin determination is a multistage process and consists of three distinct phases: sampling, sample preparation and analysis. It is assumed that the total uncertainty associated with the AFs test procedure is the sum of the uncertainty associated with the three steps (Whitaker et al., 2006). However most variability is due to sampling; in hazelnuts and almonds this step accounts for 96.2–99.4% of the total variability, respectively (Ozay et al., 2006; Whitaker et al., 2006). Also the Codex Alimentarius (CAC) proposed formula for calculating variances associated with the aflatoxin test procedure for hazelnuts, almonds and pistachio (CAC, 2008).

Sampling and subsampling procedures should be designed according to the mycotoxin distribution. Mycotoxins are heterogeneously distributed, and the general rule is that the bigger individual particles or seeds the greater the sampling problems. Cucullu, Lee, Mayne, and Goldblatt (1966) reported that most individual peanuts have zero AFs concentration, but occasionally a peanut may have an extremely high concentration of AFs. Other studies also showed the heterogeneous distribution of AFs in other substrates such as cottonseed, pistachios and corn (Cucullu, Lee, & Pons, 1977; Johanson et al, 2000; Schatzki, 1995; Shotwell, Goulden, & Hesseltine, 1974). A common practice to reduce the heterogeneity of mycotoxins in commodities when sampling is through the formation of aggregate samples, thus, the sample should be an accumulation of many small portions taken from many different locations (Parker, Bauwin, & Ryan, 1982). However, as a result of this practice, the spatial information, variability and distribution of the mycotoxin is lost (Rivas Casado, Parsons, Weightman, Magan, & Origgi, 2009).

For these reasons, harmonisation process for mycotoxin establishing maximum limits and sampling plans are necessary to protect consumer health and facilitate international trading. The Codex Committee on Contaminants in Foods (CCCF) has established maximum levels of AFs and sampling plans, where two 10 kg laboratory samples are needed in the case of Ready to Eat (RTE) lots, both containing less than 10 $\mu g\ kg^{-1}$ AFs. In the case of further processing products (DFP); a single 20 kg laboratory sample taken from a lot must result in less than 15 $\mu g\ kg^{-1}$ AFs in order to be accepted (CAC, 2008).

EU maximum limits for AFs in nuts have been recently changed (Commission Regulation 1881/2006 amended by Regulation 165/2010) after European Food Safety Agency (EFSA) reviewed the maximum limits and intake assessment for tree nuts concluding that there was no additional consumer concern at 4, 8, 10 or 15 $\mu g\ kg^{-1}$ AFs (EFSA, 2007) in the context of exposure from all other sources and previous pertinent exposure assessments, Regarding to OTA, although its occurrence in nuts has been reported in several studies, even by the Rapid Alert System for Food and Feed (RASFF, 2011), its presence has always been significantly lower than AFs, and indeed maximum levels have not been set by the European Commission in nuts. Consequently, taking into account the developments by the CAC and considering the recently established European maximum levels for mycotoxins in pistachios, the sampling procedure for tree nuts in Regulation (EC) No 401/2006 was afterwards amended (EC No 178/2010), maintaining the number of incremental samples but decreasing the weight of the incremental sample to 10 kg for lots higher than 15 t and between 1 and 10 kg for lots equal or less than 15 t. Also the number of laboratory samples from an aggregate sample decreased in lots higher than 5 t, Finally, when it is not possible to carry out the sampling method described above because of unacceptable commercial consequences resulting from damage to the lot (because of packaging forms, means of transport, etc.), an alternative method of sampling could be applied provided that it is as representative as possible and is fully described and documented.

However, while these plans aim to harmonize official sampling regimes, they have been criticized for the unrealistic need of workforce. In fact on the European cereal trading sector, 73–77% of the companies prefer their own sampling method for their quality control programs instead of the official method (Siegel & Babuscio, 2011). Commission Regulation 401/2006 allows the use of alternative sampling methods in cases of unacceptable commercial consequences or practical unfeasibility of the official method but only in case of quality control (Comission of the European Communities, 2006a).

Another important consideration in risk management is the analytical method used. Although the uncertainty is not as high as in the sampling, sensitive and reliable methods are required for mycotoxin detection. Companies require simple, fast and cheap methods, and ELISA and HPLC are the most demanded respectively for internal and external analyses (Siegel & Babuscio, 2011).

A recent review work (García-Cela, Ramos, Sanchis, & Marin, 2012) highlighted the lack of existing information regarding performance criteria (PC) in pistachio processing as a key aspect in AFs risk management. The aim of this study was to evaluate the PC or effect of toasting on mycotoxin in pistachio, in order to achieve a given PO. PC cannot be described if methods to assess sampling uncertainty are not in place, thus, in parallel, the impact of sampling and measurement uncertainty was also evaluated.

2. Materials and methods

2.1. Samples and sampling plans

Eight lots ($n = 8$) of pistachio, weighted more than 15 t each, were sampled using two different sampling plans before and after industrial toasting. Industrial toasting included two main steps: pre-toasting ($\cong$135 °C) and toasting ($\cong$165 °C) during a total time of 20 min. However, the temperatures could change in a range of 6 °C depending on the initial characteristics of the product.

2.1.1. Sampling plan A

Sampling of raw and toasted pistachio was made according to Commission Regulation (EC) No. 178/2010, and more specifically according to D.2. point, “method of sampling for groundnuts (peanuts), other oilseeds, apricot kernels and tree nuts”. Twenty kg aggregate samples were obtained from 100 elemental samples of 200 g of each sampled lot. The composite samples were mixed and divided into two equal sub-samples of 10 kg (subsample) before grinding. To that purpose a Romer Analytical Sampling Mill (RAS® Mill, Coring-System Diagnostix GmbH, Gernsheim, Germany) was used. The RAS® Mill was specifically developed for products that are difficult to grind due to their hardness together with a high moisture and/or high oil content, like pistachios. Samples were kept at 4 °C until analysis. After grinding, six sub-fractions of 50 g were taken from each sub-sample. Sub-fractions were stored at 4 °C until analysis. Finally, two sub-fractions (10 g) were analysed in different days to account for the variability between days in duplicate (Fig. 1a). The whole sampling plan was performed in quadruplicate.

2.1.2. Sampling plan B

Sampling of raw and toasted pistachio was made in this case following the current sampling plan used for the quality control of a Spanish nut processing company. Aggregate samples of 5 kg were obtained by pooling 20 elemental samples of 250 g from each sampled lot. The aggregate samples were mixed and 250 g (subsample) were taken and ground. After grinding, 10 g of each subsample were analysed in duplicate (Fig. 1b). This sampling plan also was performed in quadruplicate.