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Assessing aflatoxin B1 distribution and variability in pistachios: Validation of a Monte Carlo modeling method and comparison to the Codex method

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Aflatoxin B1 (AFB1) has genotoxic carcinogenic effects with no threshold. This mycotoxin, produced by *Aspergillus* fungi species, can be found at a very low incidence in pistachios. More precisely, the contaminant is heterogeneously distributed. Moreover, it is usually found at high concentration levels in contaminated individual nut kernels. For this reason, it is crucial to assess AFB1 distribution and variability accurately in pistachio samples, in order to establish informed sampling schemes for health protection purposes. A modeling method using Monte Carlo simulations for AFB1 distribution in pistachio samples, developed in previous work, required validation. For this purpose, the simulation was adjusted to the distribution observed in 30 kg samples as calculated from the RASFF (Rapid Alert System for Food and Feed) levels reported by Germany for Iranian pistachio imports from September 2002 to September 2004, for the lots tested. As a final result, when the simulated distribution in 10 kg samples was computed from the prior simulation, it was very similar to the distribution observed in representative samples for the same sample size from the RASFF. The simulation method was then compared to another method, for AFB1 distribution and variability assessment, used by the Codex to design aflatoxin sampling plans for tree nuts. Indeed, the distribution observed in 30 kg samples from the RASFF was considered as an input distribution. The output distribution for 10 kg representative samples, as computed using the method, was close to the observed distribution. Results of both simulation methods were very similar. Finally, an extensive comparison of the principles of both methods was performed.

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1. Introduction

In previous work, a method for aflatoxin B1 (AFB1) distribution and variability assessment in representative pistachio samples was developed (Wesolek & Roudot, 2014). This method was later modified and adapted to assess sampling plan performance (Wesolek, Ramirez-Martinez, & Roudot, 2014). This simulation method uses the Monte Carlo technique, thus enabling distributions to be combined. It therefore takes into account the whole range of variability of the variables. The specificity of the model is that it is based on the distributions of sample contamination levels in various pistachio categories with visual defects (Wesolek & Roudot, 2014; Wesolek et al., 2014). These defects appear while pistachios are still in the orchard and are due to abnormal features during pistachio growth. The categories considered in the model are early split pistachios with sound kernels, cracked pistachios with sound kernels and mouldy pistachios (Wesolek & Roudot, 2014). Although visible most of the time, these pistachio categories cannot be totally eliminated from pistachio lots, even after the visual sorting step during processing. Indeed, this step, which aims to detect and remove stained nuts from the lot, is not 100% efficient, as some stained pistachios have very light colored stains or are not detected by the operators. The final objective of this modeling method is to enable sampling plans to be derived for AFB1 level assessment in pistachio lots, which will accurately predict the risk of misclassifying lots. Although official sampling plans have already been developed, they could possibly be improved in order to reduce lots rejected at destination. Indeed, some differences are observed between AFB1 levels obtained by importing countries vs. levels obtained by exporting countries for

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the same pistachio lots as described hereafter. The European Union (EU) has defined a maximum concentration of 8 μg/kg for ready-to-eat pistachios (Regulation 165/2010) in 10 kg samples (Regulation 178/2010), based on a specific sampling plan. Pistachio consignments imported from Iran have to be accompanied by the results of official sampling and analysis, and by a health certificate issued by the Iranian Ministry of Health since Decision 97/830/EC, finally amended by the current Regulation 1152/2009/EC. However, despite this control in Iran, some lots are still rejected at the point of entry into the EU after AFB1 analyses have been carried out. Indeed, the EU Rapid Alert System for Food and Feed (RASFF) still reports a high number of alerts due to unacceptable levels of aflatoxin contamination in pistachios – mostly from Iran.

The aim of this study is to further validate the modeling method, given that prior work has already demonstrated that the model is robust (Wesolek & Roudot, 2014). Real contamination data from the RASFF were used for the validation. First, the model was adjusted to the real distribution of 30 kg samples for lot-to-lot variation, and then the corresponding distribution for 10 kg samples was computed and compared to real data for all the lots tested, composed of three 10 kg representative samples per lot. Moreover, the same process was carried out for another simulation method, which is currently widely used to design sampling plans, and applied by the Codex (Codex, 2009). The consistency of both simulation methods' output with real data was assessed in order to determine which method best fits the data under study.

2. Data used and methods

2.1. Contamination data used

AFB1 contamination levels in pistachio samples from Iran are tested at the point of entry into the EU. If the contamination level of one of the 10 kg representative samples is above the legal threshold, then the pistachio consignment, which is usually about 25 tons, is rejected. Rejects are reported through the RASFF (Rapid Alert System for Food and Feed) portal and are publicly available. In this study, AFB1 contamination levels in 10 kg representative samples from rejected lots, reported by Germany for Iranian pistachio imports from September 2002 to September 2004, were used. Germany is by far the main importing country among the EU-15 countries. Indeed, we calculated from data from the Iran Pistachio Association (2002 and 2003 reports) that for crop years 2002 and 2003 Germany imported 75% and 71%, respectively, of total EU-15 imports of Iranian pistachios. As Germany is also a major exporter, this country implements transshipments of pistachios (USDA, 2003). Moreover, in Germany, the sampling process in use, for pistachio imports, meets the requirements of the European Commission legislation, as reported by the Food and Veterinary Office (FVO, 2002). For the period under study (September 2002 to September 2004), the Commission Directive 98/53/EC stated that, for a 25-ton consignment, three equal samples of 10 kg had to be analyzed. For this reason, we only used RASFF portal data consisting of three contamination values for each case of rejected consignment reported.

2.2. Monte Carlo modeling method

2.2.1. General principle

The Monte Carlo simulation method used in this study was developed in previous work (Wesolek & Roudot, 2014), using the @Risk software. However, the method was adapted to enable the method validation as described in the following paragraphs. The contamination level distributions in contaminated individual nuts, which are part of a global sample, as well as the incidence of contaminated nuts were used to derive the contamination distribution of representative samples. The method derived contamination levels and the incidence of contaminated nuts from samples that are small enough to contain at most one contaminated pistachio.

2.2.2. Initial 30 kg sample simulation

The modeling method developed previously used the Monte Carlo technique, and comprised many steps (Wesolek & Roudot, 2014). The distributions of contamination levels in individual pistachio nuts corresponding to each category (early split sound kernel; cracked sound kernel; mouldy) were fitted to lognormal distributions. These distributions were then used to calculate 30 kg sample contamination levels for a given number of contaminated individual nuts per sample. Differences between good and bad lots were taken into account with lots split into 3 quality lot classes.

The contamination Probability Density Function (PDF) in 30 kg samples from the 1st quality lot class gave a mean AFB1 level of 0.503 μg/kg (standard deviation of 1.15 μg/kg). For the 2nd quality lot class, the mean level was 3.57 μg/kg (standard deviation of 18.92 μg/kg), whereas for the 3rd quality lot class, the mean value was equal to 49.67 μg/kg (standard deviation of 129.40 μg/kg).

As the simulation was to be compared to contamination levels for 30 kg samples from lots rejected at the point of entry into the EU, which are expected to have a mean contamination level above 2 μg/kg, we considered that the probability for the 1st quality lot class was 0.

Based on this assumption, only the 2 lowest quality lot classes were combined: 2nd and 3rd quality lot classes. Our simulation adjustment parameters were the binomial probabilities to put the two quality lot classes together. These parameters were chosen in such a way that the resulting final PDF for contamination levels in 30 kg samples was adjusted to the contamination distribution for 30 kg representative samples reported through the RASFF. This latest distribution was obtained from the mean of the three contamination results reported for 10 kg representative samples for each rejection at the German point of entry.

2.2.3. Simulation transformation for 10 kg samples

The same simulation was transposed to 10 kg samples. The difference between this simulation and the one for 30 kg samples was the number of individual pistachios in the sample. Thus, the number of contaminated individual nuts in the sample varied accordingly.

2.3. For the method used in the Codex

2.3.1. General principle

The aflatoxin B1 (AFB1) distribution and variability assessment in pistachio samples for the Codex was extracted from a method for sampling plan validation (Codex, 2009). In this method, the calculation steps were similar to the method developed by Whitaker (Whitaker, Slate, Adams, & Birmingham, 2010; Whitaker, Slate, Hurley, & Giesbrecht, 2007; Whitaker et al., 2006). The Codex reported that sample concentration levels from a lot fitted the negative binomial distribution and gave an equation of the variance as function of the concentration level of the lot. Both the theoretical distribution and the prediction of concentration variability between representative samples of the same lot were used to calculate the probabilities of acceptance of lots for the sampling plan tested.

2.3.2. Adjustment for our study

The Codex method gave an equation of the variance as function of the lot's mean concentration, and split the variance into Sample

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