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Mathematical approach for sampling plan performance assessment for aflatoxin B1 in pistachios

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ABSTRACT

According to the EU Regulation 165/2010, the level of aflatoxin B1 and total aflatoxins in pistachios must not exceed 8 μg/kg and 10 μg/kg, respectively. Due to the heterogeneous distribution of this contaminant, the performance of sampling plans highly depends on the way they are designed. So, the sampling plan to be used must be assessed thanks to an OC (Operating Characteristic) curve showing both consumer and producer risks. The first risk is the risk of authorizing pistachios for sale while the contamination level is above the threshold; whereas the second risk is the risk of rejecting a lot having a contamination level under the threshold.

The method developed the use of early split contamination levels and incidence in individual pistachios to derive OC curves for various sampling plan designs for aflatoxin B1. Contamination levels in individuals were calculated from small sample contamination levels, thus having a negligible probability of containing more than one contaminated individual. Incidence levels of early split pistachios were derived for each harvest day. Indeed, early split nuts are dubious nuts that were considered to contain all the aflatoxin content found in a global sample. As their percentage increased along the harvesting period, it led to various contamination distributions in 10 kg samples. The EU sampling plan (Regulation 178/2010) was found to give a consumer risk with a probability of acceptance at 5% for a lot mean aflatoxin B1 concentration of 75.34 μg/kg and a producer risk with a probability of acceptance at 95% for a lot mean concentration of 1.62 μg/kg. Finally, the performance of several sampling plan designs was evaluated to demonstrate how to manipulate the number of samples to reduce misclassification of pistachio lots.

1. Introduction

Aflatoxins, toxic metabolites of some *Aspergillus* fungi species, are potent carcinogens which pose serious health hazards to humans and domestic animals because they frequently contaminate agricultural commodities (CAST, 1979; Diener et al., 1987). The primary aflatoxin-producing fungus, *Aspergillus flavus*, is intimately associated with agricultural environments and crops, including tree nuts. *A. flavus* populations are influenced by agriculture and other human activities, but in many cases it is not clear how or why (Cotty, Bayman, Egel, & Elias, 1994). Climate has got a great influence, as aflatoxin contamination is prevalent both in warm humid climates and in irrigated hot deserts, whereas in temperate regions, contamination may be severe during drought (Cotty & Jaime-Garcia, 2007). *A. flavus* is a weak pathogen, as it requires a wound (Ashworth, Rice, McMeans, & Brown, 1971) or natural opening (Klich & Chmielewski, 1985) to invade plant tissues. One type of aflatoxin: aflatoxin B1 (AFB1) is the main aflatoxin found in pistachios, as well as the most toxic. Due to its threat for human health, the European Union (EU) has set a maximum level at 8 μg/kg in pistachios (Regulation 165/2010) in 10 kg laboratory samples (Regulation 178/2010), knowing that total aflatoxins are also regulated, with a maximum level at 10 μg/kg. However, all statistics in this work will be developed for 8 μg/kg and for AFB1 solely. There is a high contamination variability between individual pistachios. Per example, it has been estimated that only one pistachio out of 10,000 to 1,000,000 might be highly contaminated (Schatzki & Pan, 1996), or one out of 25,000 nuts (Sommer, Buchanan, & Fortlage, 1986). Moreover, a single pistachio with an aflatoxin concentration of 60,000 μg/kg can contaminate an aflatoxin free sample of 4.5 kg (approximately 3000 nuts) to 20 μg/kg toxin (Doster & Michailides, 1994). When the hull surrounding the shell is intact at harvest, it enables the protection of the kernel from moulds during growth stage and the nuts are considered to be free from aflatoxin (Doster & Michailides, 1995). In case this hull is ruptured, this protection is not provided anymore, the kernel can be infected by moulds. There are two types of hull ruptures, resulting in either early split or cracked pistachios. In the first case, in the orchard, some nuts have the hull attached to the shell so that the hulls split with the shell (Doster, Michailides, & Goldhamer, 1993). For cracked pistachios, the process is distinct, because the cracking occurs after the shell splits, and the location of the crack is not along the suture where the shell split (Doster & Michailides, 1994). In Californian orchards, it is

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estimated that the incidence of decay by aflatoxin-producing fungi in growth split nuts was substantially lower than that of early-split nuts (Doster & Michailides, 1994). In addition, all early split pistachios do not form at the same period and Doster and Michailides (1995) reported that it is due to the fact that pistachio nuts mature unevenly over a period of time. It was found that the earlier the early splits are formed, the higher the incidence of aflatoxin contamination at harvest in these nuts (Doster & Michailides, 1995). As harvest normally begins in early September and may continue through late October (GAP, 2009), the proportion of early split (ES) nuts depends on the day when harvest occurs. All these aspects must be taken into account to define sampling plans, as this inter-individual variability has got an impact on the accuracy of the contamination level that can be found in a global sample. For this reason, a sampling plan assessment must be set up in order to decide of the appropriate sampling plan, and more precisely of the relevant number of laboratory samples and of the laboratory sample size. The Codex (2009) reported a sampling plan assessment method for aflatoxin in almonds, hazelnuts and pistachios, based on Operating Characteristic (OC) curve computation. The method used variances associated with the test procedure as well as the negative binomial distribution for the distribution of sample contamination levels within a lot. This type of OC curve computation was developed by Whitaker and co-workers (per example Whitaker, Dickens, Monroe, & Wiser, 1972; Whitaker, Slate, Hurley, & Giesbrecht, 2007) on various tree nuts and also for other contaminants and commodities (Whitaker, Saltsman, Ware, & Slate, 2007). An important aspect is that such a method is based on sample data without considering the proportion of contaminated individuals within a sample. For this reason, the objective of the work is to develop a new method, based upon the Monte Carlo technique, for distribution and variability assessment for AFB1 in pistachios, considering prior knowledge of the contamination pattern. The aflatoxin distribution among 10 kg laboratory samples was used to compute Operating Characteristic (OC) curves in order to assess sampling plans and finally to reduce misclassification of pistachio lots. Such curves, enable one to quantify consumer and producer risks. Consumer risk is the probability that a lot having a true concentration above the threshold (unsafe lot) is authorized for sale and consumption. Producer risk is the probability that a lot at a true concentration lower than the threshold (good lot) is rejected for sale.

2. Data used and methods

Previously published results were used in this work to compute AFB1 concentration distribution in laboratory samples. The reason is that although there are AFB1 concentration distributions in the literature, these data are:

- Either calculated on a small number of samples per lot, or
- Calculated on a big number of samples but without separation per lot (i.e. data on a whole harvest year).

In the first case, given the very high variability between samples, it is difficult to obtain a reliable distribution with such a small number of dots. In the second case, it is not possible to obtain distributions for various lots. Nevertheless, in the sampling plan assessment process, it is required to compute reliable laboratory sample concentration distributions for lots having various mean contamination levels. As discussed in the Introduction, the high contamination variability is due to a low contamination incidence and a high contamination level sustainable by a single nut. For this reason we chose to compute laboratory sample concentration distributions for various lot mean concentration levels from both contamination incidence and contamination level data for individual nuts.

Contrarily to previously published methods such as the Codex and Whitaker and co-workers OC curve computation methods (mentioned in the Introduction), the newly developed method does not reflect variability associated with sample preparation and analysis, but only variability associated with sampling. Indeed, the Codex (2009) reported that aflatoxin test procedure consists of sampling, sample preparation and analytical errors. However, studies on almonds, hazelnuts and pistachios reported that the sampling step accounts for 93.27%, 99.43%, and 74.78%, respectively, for a 10 kg laboratory sample, and with a threshold concentration for aflatoxin at 8 μg/kg (Technical Information).

The AFB1 level Probability Density Functions (PDFs) in 10 kg laboratory samples of pistachios were calculated thanks to the observed proportion of dubious nuts and the contamination of these individual dubious nuts thanks to Monte Carlo simulations using @Risk 6 software (Palisade Corp.). These PDFs were obtained for each possible harvest day (from 6 September to 4 October) and were used to compute Operating Characteristic (OC) curves enabling to evaluate various sampling plan designs.

2.1. Observed proportion of dubious nuts

The observed proportions in percentage of dubious nuts that can be found in an open-shell sample were calculated thanks to the equation for total early split (ES) and the equation for shrivelled ES as described hereafter. Finally, the proportions of ES having sound kernel, and of ES having mouldy kernel were derived for each harvest day (6 September to 4 October), as they vary during the harvesting period.

2.1.1. Equation for total early split

Data for the proportion of pistachios with ruptured hulls were obtained from a publication from Panahi and Khezri (2011), who studied the evolution of the proportion of nuts with various characteristics in a completely randomized block design. For each time of sampling, fruit clusters were picked at random of trees, combined and hand sorted according to shell and hull aspect. They compiled data from an observation period of four years from 2001 to 2004 in the field to generate mean values and standard error for four cultivars, among which was a popular Iranian cultivar, named "Kaleh-Ghoochi". They reported that nut harvesting almost occurs between the 6th of September and 6th of October. We used their data from the harvesting period for the following dates: 09/06, 09/13, 09/20, 09/27, and 10/04 and for the "Kaleh-Ghoochi" cultivar on the percentage of ES nuts reported on the total number of nuts. We derived the corresponding percentage of ES nuts on the number of open-shell pistachios thanks to their open-shell pistachio proportion data. The reason for this is that it is this type of high value pistachios that is imported in Europe, whereas closed-shell pistachios result in lower value pistachios. Early-splitting is the only type of hull defect that they reported on open-shell pistachios, as cracked nuts were only reported for closed shell pistachios. Then, we derived a regression equation of the percentage of ES nuts as function of the date of sampling, which was reported as the number of days after September the 6th. This equation was called equation for total ESs, as ESs comprise both normal and shrivelled (or mouldy) early splits.

2.1.2. Equation for shrivelled early split

In addition, a publication from Doster and Michailides (1995) was used to derive the percentage of shrivelled ESs among the total number of ES as function of the period of formation. In their study, they examined hull splitting on trees on three periods per year and wrapped a coloured yarn around the stems of the ESs. They harvested the nuts on 31 August 1992 and 7 September 1993 (beginning of commercial harvest) to report the percentage of nuts with shrivelled hulls among ES pistachios. For each ES formation period reported (before 5 August, 5 to 19 August, 19 to 31 August 1992, and before 12 August, 12 to 26 August, 26 August to 7 September 1993), we calculated a corresponding mean number of days before harvest, with the first period (before 5 August 1992 and before 12 August 1993) set at 28 days before harvest. So, the following periods were calculated as being 19 days (5 to 19 August 1992; 12 to 26 August 1993) and 6 days (19 to 31 August 1992; 26 August to 7 September 1993) before harvest. Then, the mean percentage of