UNCERTAINTY ASSOCIATED WITH SAMPLING AND EFFECT OF SAMPLING PLAN IN AFLATOXIN ANALYSIS OF DRIED FIGS

Hayrettin Ozer[1)], *Hatice Imge Oktay Basegmez* [2)], *Thomas B. Whitaker*[3)], *Andrew B. Slate* [4)]
[1)]TÜBİTAK MarmaraResearch Center,Food Institute,P.O Box 21, 41470 Gebze-Kocaeli, Turkey – hayrettin.ozer@tubitak.gov.tr
[2)]TÜBİTAK Marmara Research Center, Food Institute, P.O Box 21, 41470 Gebze-Kocaeli, Turkey – imge.oktay@tubitak.gov.tr
[3)]North Carolina State University, Biological and Agricultural Engineering Department, Box 7625, Raleigh, NC 27695-7625, USA – whitaker@ncsu.edu
[4)]North Carolina State University, Biological and Agricultural Engineering Department, Box 7625, Raleigh, NC 27695-7625, USA – andyslat@gmail.com

Abstract– Aflatoxin test procedure is a multi-stage process and generally consists of three steps; sampling, sample preparation and analytical steps. Because of the uncertainty associated with each step, the true aflatoxin concentration of a bulk dried fig lot can't be determined with 100% certainty. The sources of error in the aflatoxin test procedure should be determined so that the errors can be effectively reduced.

Studies were developed to measure the variability and distribution among replicated sample aflatoxin test results taken from the same aflatoxin-contaminated lot of dried figs so that a model could be developed to evaluate the risk of misclassifying lots of aflatoxin sampling plan designs for dried figs.

Keywords: *negative binomial distribution, operating characteristic curves, buyer's risk, and seller's risk*

1. INTRODUCTION

As the worlds leading producer of figs, Turkey exports approximately 90% of their dried fig production. European Union member states are the major importers of dried figs from Turkey and account for about 75% of the market share. Like many other commodities, dried figs are susceptible to the growth of aflatoxin producing moulds. Because aflatoxins are toxic and carcinogenic compounds [2, 4], regulatory limits for aflatoxins and other mycotoxins have been established for food and feed products in about 100 countries [1]. Aflatoxin limits vary widely from country to country. The current situation where aflatoxin limits vary from country to country makes international trade difficult. Exporters must be aware of each importer's aflatoxin limits and official sampling plan designs when they sample dried figs at origin. Because of the

high cost of having lots rejected at destination, exporters try to determine if the product meets the importing country's aflatoxin regulations before shipping to the importer. With so much variation among regulatory limits worldwide, the Codex Committee on Contaminants in Foods (CCCF) began efforts in 2006 to harmonized aflatoxin limits and sampling plans for dried figs [5].

An aflatoxin-sampling plan is defined by an accept/reject limit and a specific aflatoxin test procedure. The accept/reject limit is a threshold concentration that is used to classify lots into "acceptable" and "unacceptable" categories and is usually equal to (but not required) a defined maximum level (ML) established by customers and/or regulatory agencies. The aflatoxin test procedure for granular commodities usually consists of three steps, sampling, sample preparation, and analysis. Because of the variability associated with each step of the aflatoxin test procedure, the true aflatoxin concentration in a bulk lot cannot be determined with 100% certainty.

Because of the variability associated with each step of the aflatoxin test procedure, there is a chance that some lots will be misclassified by a sampling plan. Two types of errors are usually made when using a sampling plan to classify lots based upon the

estimated lot's aflatoxin concentration. The first type of misclassification occurs when a good lot (a lot with a mycotoxin level truly below a defined maximum level) is considered to be unacceptable because the sample test result

is greater than the ML. This type of misclassification is called the seller's risk. The second type of misclassification occurs when a bad lot (a lot with a mycotoxin concentration truly above a defined ML) is considered to be acceptable because the sample test result is below the ML [6]. The frequency with which these two misclassifications occur depends upon the design of the sampling plan and can be evaluated with help of an operating characteristics (OC) curve. However, no method had been developed to evaluate and design sampling plans to detect aflatoxin in dried figs.

2. EXPERIMENTAL

A balanced nested design was used to measure the variability and distributional among sample test results selected from each contaminated lot. Description of sample selection, sample preparation, and aflatoxin quantification is given below.

2.1 Sample selection

Twenty commercial lots of dried figs, sixteen of them were rejected lots because of suspicion of being contaminated with aflatoxin, were collected from producer warehouses in Turkey. Lots chosen for the study were standard commercial size of about 10 tonnes. One hundred incremental portions of about 1600 g each were randomly removed from different locations in the lot to achieve a representative aggregate sample and minimize spatial heterogeneity of aflatoxin contaminated figs within the lot. The 100 increments were collected and pooled together as a composite or aggregate sample of about 160 kg. The aggregate sample was thoroughly mixed for about 15 minutes. After mixing of the 160 kg aggregate sample for 15 minutes, it was divided into sixteen 10 kg laboratory samples.

Each 10 kg laboratory sample was achieved by collecting 200-300 g portions of dried figs randomly from the 160 kg aggregate sample. Each 10 kg laboratory samples were transferred into the plastic bags for the water-slurry comminution. By minimizing the spatial heterogeneity among contaminated figs in the lot, the aflatoxin concentration among the sixteen 10 kg laboratory sample was assumed to be an unbiased estimate of the aflatoxin distribution and variability among 10 kg laboratory sample test results taken from the lot.

2.2 Sample preparation

Each 10 kg sample was blended for 3 minutes with water on a 1:1 mass basis using Robot Coupe model R45 vertical cutter mixer. Two 110 g water-slurry portions (55 ml water:55 g dried figs) were removed from each water-sample blend. The size of the test portion used in thestudy is defined by the dry fig mass of 55 g in the water-slurry portion.

2.3 Analytical method

The analytical method chosen for this study was the same as that typically used by the cooperating laboratory to quantify aflatoxin in commercial lots of dried figs [3]. The appropriate extraction solvent was added to the 110 g water-slurry portion and blended for 1 minute. Aflatoxins were extracted from each 110 g water-slurry portion using 330 ml methanol:water in a 4:1 ratio (the methanol:water solvent ratio was adjusted for the water in the water-slurry portion). Both AFB_1 and AFT were quantified by liquid chromatography (LC) with fluorescence detector in each of the two aliquots taken from the extract. Since two 110 g water slurry portions were removed from each 10 kg water-sample slurry blend, a total of four AFB1 and four AFT aflatoxin measurements were made on each of the 16x10 kg laboratory samples.

3. RESULTS AND DISCUSSION

Of the 20 lots identified for the sampling study, 4 lots had an average aflatoxin concentrations below 1.0 µg/kg AFT because 14 to 16 of the 16 sample test results were below 1 µg/kg AFT. With so few sample test results above 1 µg/kg AFT, the goodness of fit test may be questionable and as a result these four lots were not used in the distribution study. The 16-aflatoxin sample test results for each of the remaining 16 lots are shown in Table 1. For ease of viewing, the 16-aflatoxin sample test results were ranked from low to high to show the range among sample test results for each

lot. The mean, median, and variance among the 16-Aflatoxin sample test results are also shown in Table 1.

Table 1. Aflatoxin test results (µg/kg total aflatoxins) among 16-ten kg laboratory samples taken from each of 16 lots of dried figs.

Lot	Sample Number																Mean	Median	Variance
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	(µg/kg)	(µg/kg)	
12	2.8	2.9	3.0	5.0	6.1	6.1	6.2	7.0	7.2	7.9	8.0	9.0	10.9	11.2	11.2	14.4	7.4	7.1	11.1
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.6	0.9	1.0	1.3	2.8	160.7	10.5	0.0	1606.1
20	0.0	0.3	0.4	0.6	0.9	1.0	6.8	6.9	7.8	8.8	10.1	11.1	22.7	32.6	53.4	60.1	14.0	7.3	358.8
15	13.9	14.9	16.7	17.2	18.7	18.9	19.8	19.8	22.2	23.0	24.6	26.1	27.4	28.2	32.4	45.2	23.1	21.0	61.4
11	2.2	3.2	5.0	8.4	8.9	9.2	10.0	10.8	12.6	14.7	19.1	39.6	53.8	57.0	65.6	118.4	27.4	11.7	1016.5
9	15.3	16.2	16.7	22.0	27.3	27.9	29.8	31.1	36.9	38.2	40.8	44.4	48.9	54.2	57.4	70.2	36.1	34.0	254.7
18	22.3	27.4	27.9	28.8	30.4	32.3	34.6	36.0	37.8	38.6	40.8	41.6	43.0	43.2	45.8	57.1	36.7	36.9	74.8
14	26.9	27.7	28.2	29.6	31.1	33.3	34.8	38.7	39.6	42.1	42.6	43.4	44.2	45.3	46.1	59.7	38.3	39.1	78.2
17	1.2	4.3	4.7	5.0	5.4	17.8	20.0	25.2	29.8	44.1	47.3	47.6	63.2	75.4	108.2	115.4	38.4	27.5	1326.0
16	17.3	20.3	21.0	64.4	65.4	72.8	73.2	73.8	74.9	77.7	79.2	80.8	80.9	86.2	86.4	92.3	66.7	74.3	598.9
7	17.8	18.0	20.6	65.2	70.0	71.7	75.4	75.6	76.3	76.8	86.1	87.0	93.8	99.3	107.7	108.4	71.9	75.9	857.6
13	10.9	13.3	20.7	36.3	41.0	47.3	49.4	51.0	53.0	99.1	110.0	110.3	156.1	218.1	242.8	259.4	94.9	52.0	6793.9
8	18.2	19.2	42.6	50.2	65.9	66.3	72.1	90.6	105.8	123.2	129.1	129.2	166.8	192.4	213.6	253.1	108.6	98.2	4838.4
6	55.3	66.0	76.2	78.3	82.9	73.7	112.9	113.8	114.4	119.1	120.7	126.6	128.1	139.0	154.9	277.0	116.2	114.1	2603.0
5	67.8	76.4	85.9	91.6	93.3	100.7	104.4	105.0	125.7	128.1	128.4	135.2	156.2	158.4	201.4	285.4	127.8	115.3	2958.8
10	202.9	213.9	216.8	227.9	232.4	244.0	249.3	252.2	252.8	254.8	266.7	269.6	280.3	326.7	334.0	374.1	262.4	252.5	2197.0

3.1 Operating characteristics curve

The OC curve associated with the aflatoxin test procedure used in this study (10 kg laboratory sample of 584 figs, water-slurry comminution of the laboratory sample, aflatoxin extraction from a test portion consisting of 55 g of fig mass, quantification of aflatoxin in one aliquot by LC) was computed for an accept/reject limit of 10 µg/kg AFT (Fig. 1).

For the aflatoxin sampling plan described above, the OC curve shown in Figure 1 predicts that about 87.0, 60.4, 22.8, and 2.5% of the lots at 5, 10, 20, and 40 µg/kg AFT, respectively, would be accepted by the sampling plan. The sampling plan accepts a very small percentage of lots above 40 µg/kg AFT. The percent lots rejected by the above sampling plan can be calculated by subtracting the percent lots accepted from 100 (% rejected = 100 - % accepted).

For example, 13.0, 39.6, 77.2, and 97.5% of the lots at 5, 10, 20, and 40 total µg/kg, respectively, would be rejected by the sampling plan. Based on a ML of 10 µg/kg AFT, the 13.0% of lots at 5 µg/kg that are rejected by the sampling plan is a point estimate of the good lots rejected or the seller's risk for a lot at 5 µg/kg. The 22.8% of lots accepted by the sampling plan at 20 µg/kg is a point estimate of the bad lots accepted or buyer's risk at 20 µg/kg. A perfect sampling plan would accept 100% of all lots with aflatoxin concentrations below the ML of 10 µg/kg and reject 100% of all lots with aflatoxin concentrations above the ML of 10 µg/kg.

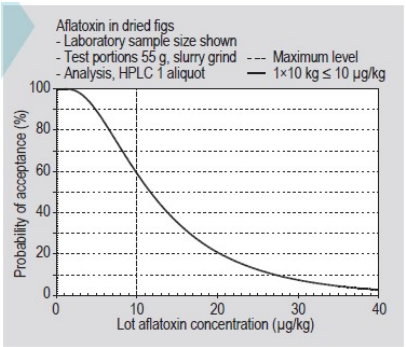

Figure 1. Operating characteristic curve showing the performance of an aflatoxin sampling plan for dried figs that uses a single 10 kg laboratory sample of 584 figs, water-slurry comminution, 55 g test portion, quantify aflatoxin in 1 aliquot liquid chromatography methods, and an accept/reject limit of 10 µg/kg total aflatoxins.

4. CONCLUSION

The negative binomial distribution was found to give acceptable fits to the 16 observed distributions over a wide range of lot concentrations. It is important to be able to predict the buyer's and seller's risks associated with a sampling plan used to detect aflatoxin in dried figs so that sampling plans can be designed to reduce the risk of misclassifying lots. Once the magnitude of the buyer's and seller's risks are known, sampling plan design elements, such as laboratory sample size and/or accept/reject limits, can be changed to make the risks of misclassifying lots more acceptable to the buyer and seller of the product being inspected. By changing these sampling plan design elements (such as laboratory sample size), it is possible to adjust the performance of the sampling plan according to risks levels agreed upon by the buyer and seller.

AKNOWLEDGMENT

We wish to thank the International Nut&Dried Fruit Council (INC) for its financial support, and Republic of Turkey Ministry of Food, Agriculture and Livestock, Aydın Directorate of Provincial Food, Agriculture and Livestock, and Aegean Exporters Associations for their financial and logistic supports for implementation of the present study. PRO-METROFOOD project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 739568. This presentation is supported by PRO-METROFOOD project.

REFERENCES

[1] Food and Agriculture Organization (FAO), 2003. Worldwide Regulations for Mycotoxins in Food and Feeds in 2003. FAO Food and Nutrition Paper 81. FAO, Viale della Terme di Caracalla, 00100, Rome, Italy. 165 pp.

[2] International Commission on Microbiological Specifications for Foods (ICMSF), 1996. Microorganisms in Food 5, Blackie Academic and Professional, Champman and Hall, New York, NY.

[3] Official Methods of Analysis, 2005. 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method 999.07.

[4] Samson, R.A. and Van Reenen Hoekstra, E.S., 1988. Introduction to Food Borne Fungi, 3rd Ed., Grafisch bedrijf Ponsen&Looijen b.v., Wageningen, 299 pp.

[5] Vazgecer, B., 2014. Aflatoxin in dried figs. WHO/FAO pre-CCEURO Workshop 29 September 2014, Den Haag. Available at: https://www.codexalimentarius.nl/sites/codexalimentarius.nl/files/bijlagen/EN%20Betul.pdf.

[6] Whitaker, T.B., Giesbrecht, F.G., Wu, J., Hagler, W.M., Jr. and Dowell, F.E., 1994. Predicting the distribution of aflatoxin test results from farmers' stock peanuts. Journal of AOAC International 77