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Sampling, sample preparation, and sampling plans for foodstuffs for mycotoxin analysis

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Abstract - Traditional means of sampling and sample preparation of agricultural crops and foodstuffs are generally not adequate for mycotoxin analyses. The main reason for this is that mycotoxin contamination is usually of a heterogeneous nature and this presents problems in sampling and in the preparation of a homogeneous sample for analysis. Sampling plans, sampling equipment, and sample preparation are discussed.

INTRODUCTION AND BACKGROUND

The obvious reason for sampling any given lot of material is to obtain a portion for the estimation or observation of attributes of the particular lot. It is also obvious that the sample must be representative of the lot if meaningful results are to be obtained. Traditional methods of sampling and sample preparation of agricultural crops and foodstuffs are usualy not adequate for mycotoxin analyses since mycotoxin contamination is usually heterogeneous, which creates problems in obtaining a representative sample for analysis. It has been shown that a small percentage of the particles in a lot may be contaminated and these contaminated particles may have extremely high mycotoxin levels. Therefore, in order to obtain a representative sample for analysis, it is necessary to take a relatively large number of particles cumulated from a number of sites in the lot and then properly prepare the sample in order to obtain a precise measurement of the mycotoxin contamination in the sample.

The exceptions are for foods that are either flowable liquids, e.g., milk or beer, or foods which have been made into pastes or powders by a grinding process, e.g., almond paste or smooth peanut butter, or flour. The first class of foods requires only a stirring whereas the latter classes require some mixing and blending to assure homogeneity. In analyzing foodstuffs for mycotoxins we are interested in determining the true mycotoxin concentration (average or mean) of the contaminant in a given lot. We will see later that an accurate and precise estimate of the true concentration of a lot is dependent upon at least three distinct but interdependent parts; namely, (1) sampling, (2) sample preparation, and (3) analysis. The analytical aspects of this problem will usually have the least error; the sampling part will have the largest, and sample preparation will usually have an error lying somewhere between these two. Methods of analysis will be discussed only as they interrelate with sampling and sample preparation.

Although we speak about sampling plans for mycotoxin analysis, the plans developed to date have been devised for aflatoxin contamination. There is little if any evidence to indicate that the nature of contamination by other mycotoxins will be different from that of aflatoxin. Therefore, the information presented here for aflatoxin can be considered to apply to other mycotoxins until evidence is generated to show that a specific mycotoxin should be treated differently.

HETEROGENEOUS CONTAMINATION

Shortly after it was recognized that the aflatoxin problem (the causative factor in Turkey X disease) was associated with peanut meal (Ref. 1), the heterogeneous nature of contamination of individual peanut kernels was observed, thus alerting investigators to the sampling and sample preparation problem in estimating the aflatoxin concentration of a particular lot of peanuts. The initial work was done by Cucullu et al. (Ref. 2) by visually selecting suspected nuts from two lots of peanuts. Aflatoxin was found in about half the selected nuts from these lots and the concentration ranged from a detectable trace to a high of 1,100,000 µg of aflatoxin per kg (ppb) of peanuts with an average concentration of 112,000 µg/kg. From these findings it was estimated that one highly contaminated kernel in 10,000

*Deceased

could result in a concentration of 50 µg of aflatoxin per kg of peanuts (Ref. 3). Analysis of two separate 2 kg samples from one of the lots of peanuts revealed 30 and 400 µg aflatoxin B1/kg, respectively. Of 100 individual Brazil nut kernels analyzed, five were contaminated at levels of 50 to 25,000 µg/kg. In an investigation of 771 cottonseed kernels, Cucullu et al. (Ref. 4) found that 18% were contaminated and that the concentration among individual kernels ranged from 150 to 5,750,000 µg/kg. Fuller et al. (Ref. 5) made estimations from analyses of walnuts indicating aflatoxin contamination of 1 in 28,500 walnuts; in another study, 1 in 29,000 hazelnuts were found to be contaminated (Ref. 6).

Analyses of 256 randomly selected kernels from a known contaminated lot of corn revealed no aflatoxin; however, 5 of 10 selected defective kernels from the same lot were found to contain aflatoxin (Ref. 3). In another study, Shotwell et al. (Ref. 7) analyzed corn kernels that showed bright fluorescence under ultraviolet light. Most of the fluorescing kernels contained aflatoxin and some at extremely high concentrations, e.g., more than 400,000 µg/kg. These studies clearly illustrate that only a few individual kernels or seeds from a lot may be contaminated and the concentrations may be extremely high. Table 1 summarizes data for contamination of individual kernels.

TABLE 1. Assay of single kernels for aflatoxin[1].

	Commodity and Selection				
	Peanuts	Cottonseed	Corn		Brazil Nuts
Selection	Defects	Random	Defects	Random	Defects
Number of Kernels Examined	40	150	10	256	100
Number Positive for Alfatoxin	22	28	5	0	5
Range of Aflatoxin Concentration[2]					
Low	Trace	0.057	Trace	0	0.05
High	1000	600	8	0	25

[1]After Stoloff et al., 1969.
[2]Micrograms of total aflatoxins per gram kernel.

The analysis of multiple samples from contaminated lots also illustrates the effect of the heterogeneous nature of the contamination. Analyses of 72 samples each from two bins of corn ranged from 0 to 376 µg/kg with an average concentration of 21 µg/kg from one bin, and 0 to 332 µg/kg with a mean of 15 for the second bin (Ref. 8). In the case of peanuts, Whitaker et al. (Ref. 9) show the results of analysis of ten 4.5 kg samples each from 29 lots of contaminated peanuts. A typical result gave values of 0, 0, 3, 4, 4, 5, 15, 60, 160, and 165 µg/kg, with an average concentration of 36 µg/kg. Dickens (Ref. 10) further demonstrated the extent of the problem by displaying a clear plastic bag of rice in which 0.1% of the rice kernels were colored red. He removed approximately 250 kernels by scooping a small beaker full and pouring the kernels out on a surface where the red kernels could be readily counted. By removing several beakers full and observing them he did not get any red kernels. He pointed out that, in taking 250 kernel samples from a Poisson distribution one would expect on the average that 78 of 100 samples would have zero red kernels. This clearly illustrates the futility of analyzing small samples drawn from lots in which a small percentage of particles are contaminated.

"Pockets" of contamination may be found in bulk storage of grains, oilseeds, oilseed cakes, flours, or ground mixed feeds. Dickens (Ref. 11,12) has shown that peanuts become contaminated in storage because of inadequate ventilation of warehouses. When outside temperatures are below the temperature of the stored product, convection currents take place which cause upward moisture movement and condensation occurs either directly on the surface layer of the product or underneath the roof of the warehouse. Condensation on the surface layer and condensation which drips from the roof of the warehouse is conducive to mold growth on the product. Wet spots caused by condensate drip may create pockets of highly contaminated material. Wet spots can also occur from rains if there are leaks in the roof or side walls. Sampling lots with these isolated pockets of contamination requires that the total sample be a cumulation of small samples taken from many different locations in the lot in order to reduce the possibility of erroneous results.

"Pockets" of contamination may be caused by other factors such as contamination from certain areas of a field at the time of harvest (Ref. 13), the mixture of small loads of contaminated material with good loads in bins or silos, or wet spots in storage containers of cereal flours or mixed feeds.

These investigations with individual kernels, multiple samples from lots, the demonstration with rice, and the possibilities for the development of pockets of contamination all show the necessity for correct methods of taking a sample and a sound statistical basis for the development of acceptable sampling plans.

ASSOCIATED ERRORS

Most of the published statistically designed sampling plans have been developed through the efforts of Whitaker and Dickens and their colleagues at North Carolina State University (Ref. 14,15, 9, 16, 17). They chose to use the negative binomial distribution to design and evaluate aflatoxin sampling plans because the distribution closely resembled the observed distribution of contaminated particles according to their aflatoxin concentration (Ref. 14). Also, the distribution has been used to describe the incidence of contagious diseases (Ref. 9). Whitaker et al. (Ref. 18) use the Monte Carlo solution techniques to predict the acceptance probabilities associated with aflatoxin testing programs because Monte Carlo can account for interrelated factors such as multiple samples, subsamples, and analyses. Their approach in attacking this problem is described in a publication which breaks down the total error involved with analysis of peanuts into its component parts and expresses these specific errors as coefficients of variations (C.V.) (Ref. 19). With peanuts, the total error is the sum of the errors associated with sampling, subsampling and analysis. For a lot concentration of 25 µg/kg, the C.V.'s for a 4.54 kg sample, a 280 g subsample, and one analysis, are approximately 110, 35, and 23%, respectively, with a total CV of 118%.

In another study (Fig. 1) at a 20 µg/kg aflatoxin concentration in peanuts when using a 48 lb. (21.8 kg) sample, a 1100 g subsample and the analysis of 2 aliquots of methanol-water extract from the subsample, the coefficients of variation were 55, 18, and 16%, respectively, with a total CV of 80% (Ref. 20). A similar study was conducted by Whitaker et al. (Ref. 21) for cottonseed. The approximate C.V.'s for a lot with a 20 µg/kg concentration was 100% for a 4.54 kg sample, 18% for a 100 g subsample, and 8% for one analysis by the Velasco method (Fig. 2).

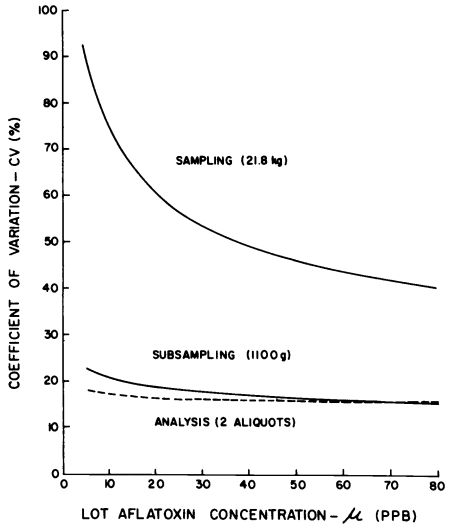

Fig. 1. Coefficient of variation associated with the sampling, subsampling, and analytical steps of the peanut aflatoxin testing program (Ref. 20).

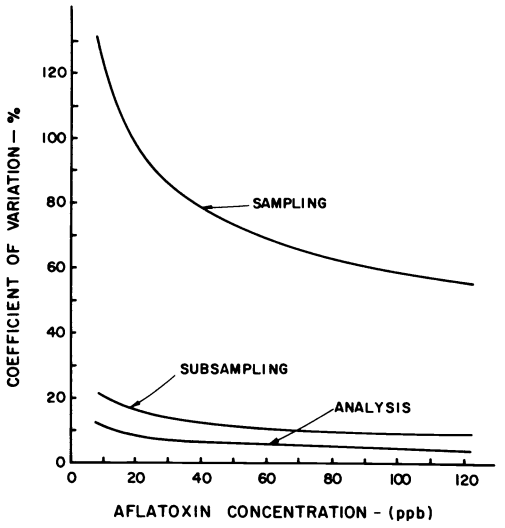

Fig. 2 Coefficient of variation characterizing sampling, subsampling, and anlysis is shown as a function of aflatoxin concentration for cottonseed (Ref. 21).

In a study with corn (Ref. 17), the total error was broken down into four components: sampling error, coarse subsampling, fine subsampling, and analytical error (Fig. 3). The coefficient of variation associated with the 4.54 kg sample, 1 kg coarse subsample (passes a #14 mesh screen), a 50 g fine subsample (passes a #20 mesh screen), and one analysis by the CB method were found to be 20, 7, 0 and 28%, respectively, for a lot with a concentration of

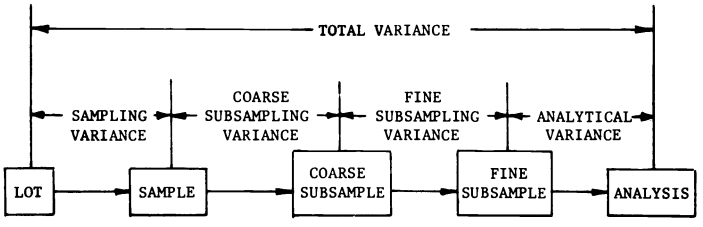

Fig. 3. Typical steps to estimate the aflatoxin concentration in shelled corn and the associated variance components (Ref. 17).

20 µg/kg total aflatoxin. For this size sample (10 lbs.), the study shows that corn is quite different from peanuts and cottonseed in that the analytical error was the greatest and the error associated with sampling was much less than the others and even less than the analytical error.

These studies draw attention to the fact that the sampling error is usually the largest contributor to the total error; so improved sampling can make the greatest contribution toward the accuracy of analytical results from which acceptance or rejection decisions are made. To date, this segment of the problem has received little attention compared to the analytical errors; this is exemplified by the fact that there are nearly 500 references for aflatoxin methods in the literature compared to about 25 for sampling for aflatoxins.

SAMPLING PROCEDURE

A general discussion of sampling and sample preparation equipment can be found in Chapter 26, Official Methods of Analysis of AOAC (Ref. 22). A representative sample can best be obtained by the use of automatic continuous samplers in situations where such equipment can be used, such as manufacturing process streams of materials. When this is not possible, e.g., when a bulk lot is in a bin, truck, box car or similar container, probe samples should be taken by means of probes which can reach to the bottom of the container. Both hand-operated and mechanical probes are available for this purpose. When the lot is bagged, samples are best taken from the bags while they are being filled or emptied into containers. These samples may consist of portions taken by scoop or by hand, "grabs", and composited in a collection container. After the bags are closed the job becomes more difficult, but samples can be removed by means of small triers (probes). For lots comprising a relatively small number of bags it is best to sample each bag. As the number of bags in a lot becomes large, a good practice is to remove material from one-fourth of the bags.

Usually the amount of sample material removed from the lot is more than is required, so it is necessary to thoroughly mix this material before removing the required amount of sample. After mixing, the sample can be subdivided to the required size by use of mechanical dividers or by applying the "quartering" technique. A procedure has been worked out for subdividing pistachio nuts employing simple shop-built equipment (Ref. 23).

Since the recognition of the aflatoxin problem, it has generally been the practice to require at least 1 kg samples; and the United States Food and Drug Administration has advocated a minimum of a 15 lb (6.8 kg) sample. The size of the lot under investigation usually does not affect the random variability associated with sampling if the sample size is small compared to the size of the lot. A properly drawn 48 lb. sample is as representative of a 100,000 lb. lot of raw shelled peanuts as it is for a 40,000 lb. lot. Over the years, the size of the sample for the control of aflatoxin in peanuts in the United States has risen from 12 lbs. (5.4 kg), to 24 lbs. (10.9 kg), to 48 lbs. (21.8 kg), to the current 144 lb. sample (three 48 lb. samples). This increase in size evolved as more reliable test results were required by the manufacturer. Increasing sample size has the advantage of simultaneously reducing the number of good lots rejected and the number of bad lots accepted by a testing program. The disadvantage of a large sample is the increase in cost of the samples and sample preparation (to be discussed below). A West German plan described by Waibel (Ref. 24) requires a 5 kg sample for roasted peanuts. Another factor in obtaining a represenatative lot sample, in addition to consideration for the nature of the contamination, is the size of the individual kernels or grains. In general, a larger sample will be required for something like Brazil nuts, which weight 8-10 g each, than for peanuts, which may weight less than 0.5 g each.

Table 2 presents data for samples taken by the United States Food and Drug Administration for regulatory control of aflatoxin in food and feedstuffs.

Initially a single sample was generally used for aflatoxin control work. However, as the sample size increased, so did costs of sampling. Recently in an effort to reduce the cost of the sample, sampling plans requiring multiple samples have been developed (e.g, the Swiss plan of Kuntti and Schlatter (Ref. 25) calls for ten-250 g samples of almonds, and the current United States peanut program requires three 48 lb (21.8 kg) samples, (Ref. 26,27). The advantages of using multiple samples, particularly when they are used in sequential plans such as the United States peanut plan (discussed below), is that a sequential plan may be designed that would give the same protection as a single sample plan but would use less sample on the average.

SAMPLE PREPARATION AND EQUIPMENT

Assuming that a representative lot sample can be obtained, the next step in the process is to prepare the sample for analysis. Photographs of typical sample preparation equipment are shown in Figure 4. In general, this will involve mixing and blending of the material,

TABLE 2. Product sample sizes used by the United States Food and Drug Administration for mycotoxin analysis.

Product	Package Type	Lot Size	Number of Sample Units	Unit Size	Total Sample Size
			(minimum)	(minimum)	(minimum)
	consumer	NA	24	8 oz.	12 lbs.
Peanut butter-smooth	bulk	NA	12	1 lb.	12 lbs.
Peanut butter-crunchy					
Peanuts-shelled, roasted or unroasted	consumer or				
Peanuts-ground for topping	bulk	NA	48	1 lb.	48 lbs.
Tree nuts (except in-shell				initial sample	
Brazil and all pistachio	consumer or	NA	10	1 lb.	10 lbs.
nuts in import status)-	bulk		as followup to positive analysis		
in-shell, shelled, slices or flour			50	1 lb.	50 lbs.
Tree nuts-paste			12	1 lb.	12 lbs.
			(minimum)	(minimum)	(minimum)
Brazil nuts-in-shell	bulk	<200 bags	20	1 lb.	20 lbs.
(in import status)		201-800 bags	40	1 lb.	40 lbs.
		801-2,000 bags	60	1 lb.	60 lbs.
Pistachio nuts-in-shell	bulk	multiples of			50 lbs. for each
(in import status)		75,000 lbs.	20% of units		multiple of 75,000 lbs. or less
Pistachio nuts-shelled	bulk	multiples of			25 lbs. for each
(in import status)		75,000 lbs.	20% of units		multiple of 75,000 lbs.
Corn-shelled, meal, flour	consumer				
or grits	or bulk	NA	10	1 lb.	10 lbs.
Cottonseed	bulk	NA	15	4 lbs.	60 lbs.
			(minimum)	(minimum)	(minimum)
Oilseed meals-peanut meal cottonseed meal	bulk	NA	20	1 lb.	20 lbs.
Edible seeds-pumpkin melon, sesame, etc.	bulk	NA	50	1 lb.	50 lbs.
Ginger root-dried, whole	bulk	"n" units	$\sqrt{n}$		15 lbs.
-ground	consumer	NA	10	10 x 1 oz.	10 lbs.
	bulk	NA			10 lbs.
Milk-whole, low fat, skim	consumer	NA	10	1 lb.	10 lbs.
Small grains-e.g. sorghum, wheat, barley	bulk	NA	10	1 lb.	10 lbs.
	consumer or				
Dried Fruit-e.g. figs	bulk	NA	50	1 lb.	50 lbs.
			(minimum)	(minimum)	(minimum)
Mixtures containing commodities susceptible to mycotoxin contamination -					
Commodity particles relatively large	consumer or		50	1 lb.	50 lbs.
Commodity particles finely ground	bulk	NA	10	1 lb.	10 lbs.

[1] NA denotes - does not apply.
[2] To be collected from random sites in the lot.
[3] One-half lb. is adequate for most purposes.
[4] Optional sampling program for seeds or dried fruits with a low incidence of contamination: Take initial 10 x 1 lb. sample. If any aflatoxin is detected, resample 50 x 1 lb. for a determination of contamination on which to base a regulatory judgement.

coarse grinding to reduce the particle size so the material will pass a standard #14 mesh screen, mixing to obtain uniformity, and subdividing to obtain a portion for further grinding to produce a flowable material which can be subdivided to the specified size of the analytical sample (25-100 g).

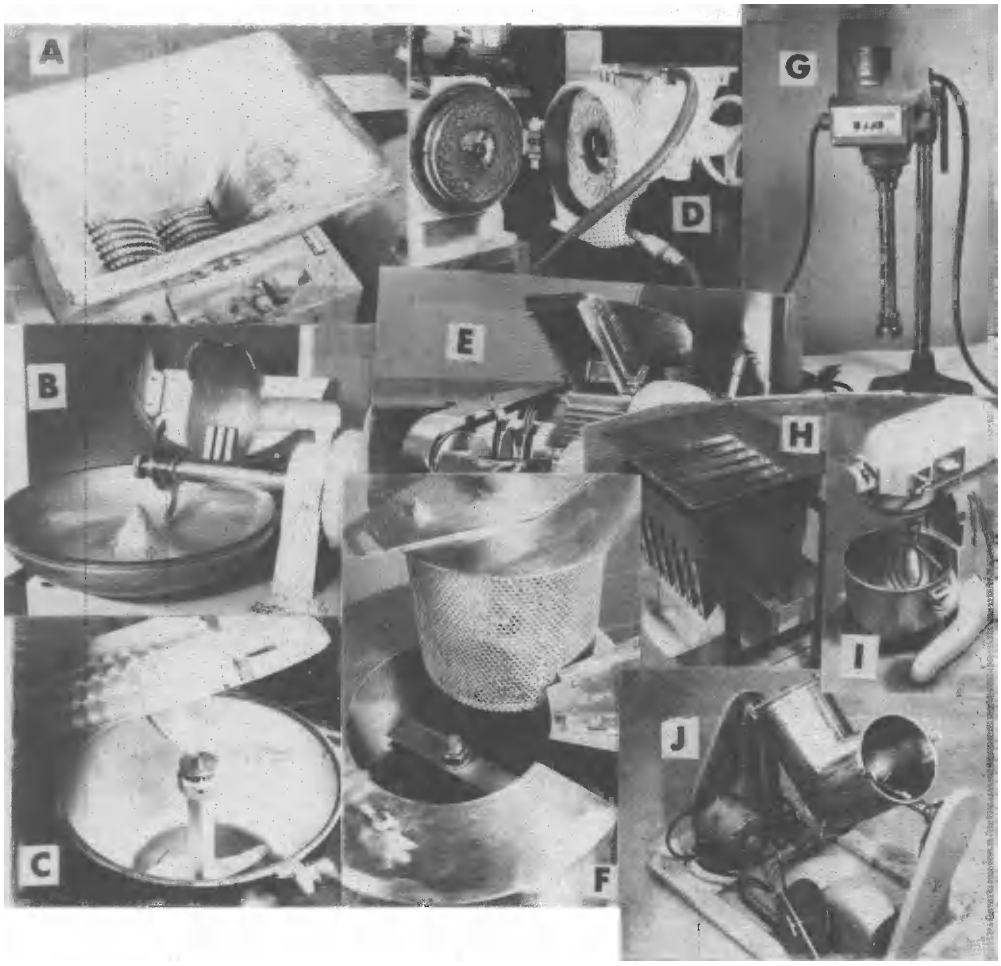

Fig. 4 (A) Thomas Mill nut grinder; (B) food cutter; (C) Hobart vertical cutter-mixer; (D) Bauer disc mill; (E) Fitzpatrick hammer mill; (F) Dickens subsampling mill; (G) Willems Polytron; (H) Jones riffle sample splitter; (I) Hobart planetary mixer; (J) Patterson-Kelly twin shell intensifier-blender (Ref. 3).

An important factor in investigating sample preparation procedures is the problem of evaluation so that valid comparisons between different procedures can be made. The reason for this is the inherent error in the TLC analytical methods where coefficients of variation range from 10 to 35% even when run under ideal conditions. One laboratory overcame this obstacle by employing radioactive kernels of the foodstuff being investigated (Ref. 3,28). One or several kernels were removed from the lot sample and were made radioactive by placing them in a neutron activator. These radioactive kernels were then added back to the lot sample being prepared. After preparation, a number of analytical size samples were removed and the radioactivity of the samples measured and compared for uniformity. Two practical procedures which are now extensively used evolved from these investigations. One employing a commercial piece of equipment known as a HVCM (Hobart Vertical Cutter Mixer; Fig 4) is capable of simultaneously grinding and blending a 25 pound sample of in-the-shell Brazil nuts to produce analytical size samples with a coefficient of variation of 3% in only 2-3 minutes. It was found that the hard shell was necessary as a grinding aid to produce this degree of homogeneity. In grinding nut meats an equal portion (weight) of crushed oyster shell was added to the nut meats as a substitute for Brazil nut shells and found to be equally effective as a grinding and blending aid. This procedure is used in the United States to prepare samples of each lot of imported Brazil nuts and pistachio nuts. When it is necessary to grind two or more charges from a lot sample, it is important that a weighed portion from each charge be composited to make up the sample for analyses, because no practical means has been found to adequately mix the ground material from two or more charges to produce a homogeneous mass before removing the analytical sample.

The other procedure to come out of these investigations employs the principle of producing a readily flowable slurry by adding a liquid such as heptane to ground kernels; the mixture is then blended and finely ground by use of a Polytron (Fig. 4). This procedure was found to produce the highest degree of homogeneity, 1% C.V., and can be used to prepare samples from

500 g to 20 or more kg. It has not been employed to any extent for practical control work because other less cumbersome and less costly procedures are available. The principle was employed by Stoloff and Dantzman (Ref. 28) to develop a procedure using dry grinding of the lot sample, mixing, and subdividing to produce a 300 g sample which is mixed with heptane to produce a slurry which is then finely ground in a Waring Blendor. This is a method recommended for small samples of nut meats. This slurry grinding technique using water in place of heptane was later employed by Velasco and Morris (Ref. 29) for the development of a procedure for the preparation of cottonseed, peanuts, peanut butter, peanut meal, cottonseed meal, copra, and corn for analysis. A water slurry method for peanuts, developed by Whitaker and Dickens, has been approved as a modification of AOAC Method II and is used in aflatoxin analysis for all peanuts produced in the United States (Ref. 30,31). With this method, a 1100 g subsample of comminuted peanuts from the Dickens mill is blended with 1600 ml of water. Aflatoxin is extracted from a 196 g portion of the water slurry by blending the 196 g portion with 218 ml of methanol, 65 ml of water, and 160 ml of hexane. A 50 ml portion of the methanol-water extract is then analyzed according to AOAC Method II.

Another significant investigation led to the development of the Dickens subsampling mill for peanut kernels (Fig. 4) (Ref. 32). This is a simple compact mill developed to simultaneously comminute and subsample peanut kernels at a rate of about 3 kg per minute. As the peanuts are ground through the mill, a 5% portion is continuously removed to give a representative subsample of the material passing through the mill. This procedure is extensively used in the United States for the control of all peanuts going into food manufacture (Ref. 26). It is estimated that nearly 100,000 lots of peanuts are analyzed in this manner each year. Since the Dickens mill has been in use it has been applied to a number of agricultural commodities in addition to raw peanut kernels. In some instances it is necessary to modify the screen size in order to produce the desired subsample.

Lot samples of cottonseed can be dehulled by passing them through a Bauer disc mill (Fig. 4) with the discs set wide enough to just crack the hulls of the seed; the seed is then passed over a small beater to separate the hulls from the kernels (Ref. 33). The kernels are then ground by passing them through a Dickens subsampling mill.

Other pieces of equipment such as hammer mills, grinders, food choppers, twin shell blenders, planetary mixers, etc., have been used in sample preparation, but the HVCM and the Dickens mill are the most extensively used, particularly for preparing in-the-shell nuts or when the preparation of large samples is required. Both of these machines, when properly used, do the job cheaply, rapidly and efficiently.

SAMPLING PLANS

Based on the sample assay results obtained through the use of a sampling plan, a lot of foodstuffs is judged to be acceptable or unacceptable. There is no practical way of determining the actual amount of aflatoxin in a given lot since the aflatoxin would have to be extracted from the entire lot and analyzed. Therefore in practice, a sampling plan has a risk (probability) to the consumer that the accepted lot has more than the acceptable level of aflatoxin and a risk (probability) to the processor that a rejected lot has less than the acceptable level of aflatoxin. A plot of these probabilities vs. the aflatoxin concentration in a lot, as determined by the sampling plan, is called an "operating characteristics" (OC) curve. The shape of the curve is dependent upon a particular sampling plan. Figure 5 is a typical OC curve for raw shelled peanut plans used in the United States.

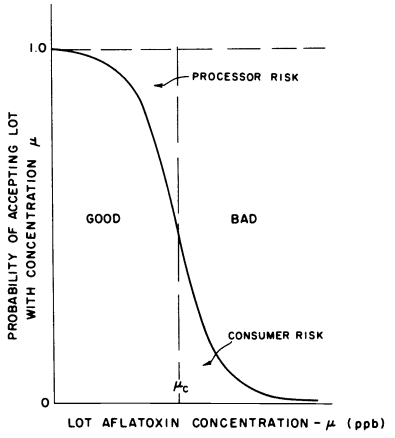

Fig. 5. Typical operating characteristic curve for evaluating sampling plans (Ref. 20).

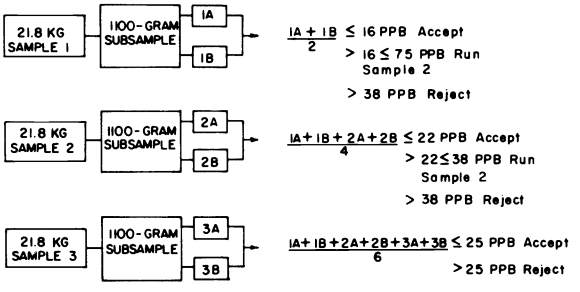

Fig. 6. Schematic of the United States peanut aflatoxin testing program (Ref. 20).

Raw shelled peanuts

The aflatoxin testing program currently in use in the United States to test all lots of raw shelled peanuts before they go to food manufacturers for processing is a multi-sample sequential type of testing plan as described by Whitaker (Ref. 27) and illustrated in Figure 6. A 144 lb. (65.4 kg) sample is randomly taken by continuous automatic samplers, or collections are made from every fourth bag, or collections are made by other approved methods from bulk containers. This lot sample is divided into three 48 lb (21.8 kg) samples. One sample is passed through the Dickens subsampling mill and the entire (1100 g) subsample is extracted in 3 liters of methanol-water (55:45) and 1 liter of hexane. Duplicate 50 ml aliquots of the extract are analyzed by the AOAC Official First Action Method II (Ref 22). The results are averaged, and if the mean is less than 16 µg/kg total aflatoxin, the lot is accepted. If the mean is greater than 75 µg/kg, the lot is rejected. If the mean is greater than 16 and less than or equal to 75, the second 48 lb. (21.8 kg) sample is analyzed in the same manner as the first sample. The four results are averaged and if the mean is 22 µg/kg or less, the lot is accepted. If the mean is greater than 38 µg/kg, the lot is rejected. If the mean is greater than 22 and less than or equal to 38, the third sample is processed and analyzed like the first two. This time the six results are averaged. If the mean is 25 µg/kg or less, the lot is accepted and if it is more than 25, the lot is rejected.

Coker (Ref. 34) developed a low-cost testing plan for raw shelled peanuts that employs a combination of: (1) sequential samples, (2) water slurry sample preparation, (3) multiple assays, and (4) a column detection analytical technique. The plan is designed to test a 20 ton lot of raw shelled peanuts. Three discrete 3 1/2 kg samples are drawn by removing approximately 180 g from 20 sacks for each sample (a total of 60 sacks are sampled from each lot). Each 3 1/2 kg sample is passed through a Dickens mill and the subsample is homogenized by comminuting in a Waring Blendor with tap water (Ref. 29). The water slurry is analyzed in duplicate by the Romer (Ref. 35) column detection or TLC method. The lot is accepted if one or more samples contain less than 10 µg/kg.

The Federal Health Office Berlin (Fed. Rep. of Germany) has developed sampling plans for the control of aflatoxin at a level of 5 µg/kg for peanuts and peanut products (Ref. 36). These authors point out that it is not possible to determine whether or not a batch complies with the limit of 5 µg/kg with samples of 10 kg or less. Recognizing the fact that it is more economical not to process raw materials which are too highly contaminated than to reject products after they have undergone processing, they emphasize sampling at the point of importation. They elect to call this "preventive testing". In this plan a 100 kg sample is drawn from a batch by taking random samples of 0.5 kg each. The entire 100 kg sample is ground and homogenized, and two samples are analyzed. Both tests must produce nearly identical results (as a check for homogeneity). If the averaged results are less than 4 µg/kg, the lot is accepted, if more than 4 µg/kg, it is rejected and must be reprocessed.

An alternative plan may be used where the 100 kg sample is randomly divided into 10 kg units. Two units are randomly chosen and analyzed. If the averaged results are less than 1 µg/kg, the lot is accepted; if the average is more than 20 µg/kg, the entire lot is rejected or subjected to a sorting process. If the results lie between the 1 µg/kg and 20 µg/kg limits, another 10 kg unit is selected at random and analyzed as above, and the results are averaged with those from the first two units. The lot is then accepted, rejected, or further tested depending upon the values given in Table 3. Reprocessed lots are treated in the same manner as described above.

TABLE 3. Lot acceptance and rejection limits on sample concentration of aflatoxin B1 when the sequential testing plan of the Federal Health Office is used (Ref. 34).

Acceptance Limit (µg/kg)	Total Weight of Sample Units Tested	Rejection Limit (µg/kg)
1	20 kg	20
2	30 kg	14
2.5	40 kg	10
3	50 kg	8
3	60 kg	7
3.5	70 kg	6
3.5	80 kg	5
3.5	90 kg	4.5
4	100 kg	4

Brazil nuts

In the United States each import shipment (lot) of in-the-shell Brazil nuts is sampled and analyzed before entry is allowed. The shipments are all in bags, and lots range from 500 to 2,000 bags. The bags are randomly sampled on the docks or warehouses by means of a trier (probe) to obtain lot samples of 20 to 60 pounds (9.1 to 27.2 kg). The entire lot sample is

ground in an HVCM (Ref. 3) to give a homogeneous sample for analysis. Two or more charges to the HVCM are required for the larger samples; when this is necessary, appropriate weighed portions are removed from each charge to make up the required analytical sample. The importer has the option of analyzing a "kernel only" sample. In these instances the entire sample is shelled, and the nut meats are mixed with an equal weight of crushed oyster shell before grinding (the importer pays the cost of shelling).

Pistachio nuts

In the United States each import shipment of pistachio nuts is sampled and analyzed before entry is allowed into the country. The shipments (lots) usually range in size from 300 to 500 bags (70 kg each). The bags are randomly sampled by means of a trier to give the required lot sample, 20 to 60 pounds (9.1 to 27.2 kg), depending on the size of the lot. The entire lot sample is then ground in an HVCM with the same precautions as taken with Brazil nuts.

Milk and milk products

As mentioned earlier in this discussion, liquids such as beer and milk require no special sample preparation because the bulk lot and the lot sample can be readily made homogeneous by simple stirring before the sample is removed for analysis. Because some milk products such as yogurt, ice cream, butter or cottage (fresh) cheese are homogeneous, a sample taken from any portion of the lot will give a representative sample for analysis. This principle is not valid for cheese, however. Kiermeier and Behringer (Ref. 37) investigated fermented and aged cheeses and found B and G aflatoxins in addition to the M1 which is usually the only aflatoxin found in milk as a metabolite of B1 ingested by the cow. The B and G aflatoxins apparently are formed by fungi growing on the cheese. He found that the amounts were quite different in portions taken from different parts of the cheese. He also points out that one cheese is not necessarily representative of cheeses from a given lot. Thus these facts should be taken into consideration in sampling cheese for aflatoxin analysis.

ECONOMIC ASPECTS

The main economic factors involved with the overall cost of an aflatoxin testing program are the cost of the material making up the sample, the cost of sample preparation, the cost for the actual analysis, and in some instances the cost of shipment of samples from the point of sampling to the place that the analysis is performed. Each of these can amount to a sizeable sum and options are available to the decision-maker.

It is readily apparent that large samples are necessary to obtain reliable analytical results. Since the foodstuffs of concern are generally rather expensive, particularly the tree nuts, at today's prices the cost of a sample can easily amount to $100 to $200. In addition, in these times of limited food and feed supplies in some countries, it is not prudent or desirable to use these materials unwisely.

In fact, there is a great potential for considerable savings in determining the aflatoxin content of a lot by applying some of the techniques presented here; e.g., use of sequential samples, use of subsampling mills to remove representative samples from large lot samples, use of the water slurry technique to homogenize the subsample, and the adoption of sensitive analytical methods for analyses. The latter point can bring about considerable savings in reagents if the methods are modified to use homogenized representative samples only large enough to give detection at the level of contamination being controlled and to use amounts of reagents according to the smaller sample.

Procedures for sample preparation all change the form of the lot sample and in some instances the procedure is destructive, e.g., grinding shell and kernel in the case of hard shell nuts and the addition of oyster shell to nut meats in the use of the HVCM. The changing of form (kernels or seeds to a ground material) reduces the value of nuts in at least most instances, e.g., pecan halves are more valuable than chopped pecans and pecan flour is of considerably less value. As pointed out earlier, the Dickens mill is used extensively in the control of peanuts in the United States. The currently used sequential plan uses only as many of the three 48 lb. (21.8) samples as necessary to reach a decision. As a result, less than 65.4 kg of sample per lot is used on the average.

Ayres (Ref. 38) suggests a plan to minimize the sample costs for pecan processing. The market place requires some small pieces of nuts and during the chopping process to produce these some comparatively very low value pecan meal is formed. By selecting the halves and the large pieces on a statistical basis for chopping, a small sample of the pecan meal can be used as a representative sample for analysis. Similar studies of manufacturing processes for foodstuffs with the view of minimizing sample costs are needed.

The cost of analysis must be considered in multiple sample plans in which up to ten samples are analyzed to evaluate a lot. The costs may be justified for expensive foodstuffs when chemical reagents are cheap and the analyst's time is not too costly.

In some instances Brazil nut samples are shipped 2,000 to 3,000 miles from the point of sampling to the analyzing laboratory. Some find it economical to shell the nuts before shipping, thus saving the shipping costs of the shells.

CONCLUSION

It can be seen from the information presented that complex procedures are requird to produce an analytical sample which is representative of any given lot. In several instances statistically sound sampling plans have been devised for some foodstuffs but such plans are still needed for others. The costs of sampling for mycotoxins are relatively large compared to costs of sampling for more conventional food analyses. It is highly desirable that investigations be conducted to devise economically feasible sampling plans so that suspect foodstuffs can be readily analyzed with a minimal of loss of foods for this purpose.

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