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«TRAINING ACTIVITIES ON FOOD CONTAMINATION CONTROL AND MONITORING WITH SPECIAL REFERENCE TO MYCOTOXINS»

J. Ya. SANDLER

GRAIN SAMPLING METHODS FOR MYCOTOXIN ANALYSIS

Centre of International Projects, GKNT

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Zh.Ya.Sandler

1. Basic notions and terms

Grain is transported to and from grain collecting centres and elevators in lorries, railroad waggons, marine or river vessels. Grain is stored in individual lots. The LOT usually means any amount of grain of uniform quality (judged on the basis of organoleptic properties) intended for simultaneous reception, unloading or storage in one silo bin or warehouse. It is the grain lot that usually represents the object of study. During its assessment, however, one has to take into account a major property of grain mass - its heterogeneity. Some grains are heterogeneous in form, size, moisture content and other signs of quality. As a result of self-sorting processes during reservoir loading, they can form zones differing considerably in these parameters from the basic grain mass. Various admixtures are also heterogeneous;their distribution in grain mass can be extremely uneven. Depending on density, they are concentrated in various grain layers, i.e. are present there in different amounts or are found in some layers while being absent in others. For example, mineral admixtures usually accumulate in areas of grain drop (at the bottom of a lorry body) while the light organic part - on top of the bulk or in the base of the cone of grain fallen from conveyer belts.

To eliminate or at least to possibly reduce the effect of grain mass heterogeneity, the sample for analysis is collected from a large number of smaller, the so called "single" samples, taken from various parts of the grain mass.

SINGLE SAMPLE or BATCH is a small amount of grain taken

from one place of a lot in a single intake. The reliability of judgement about a lot quality made on the basis of one single sample is very low. To have a representative sample for a given lot a series of single samples is drawn which are grouped later. The total mass of all single samples taken from the lot represents the INITIAL SAMPLE, BULK SAMPLE, LOT SAMPLE, or GENERAL SAMPLE. To determine some of its quality parameters, a small grain amount, a so called WEIGHED PORTION (or ANALYSIS SAMPLE, ANALYTICAL SAMPLE) is singled out. Its mass depends on the analysis kind and grain type.

The initial sample of bulky lots is too large. Out of it AVERAGE or SECONDARY SAMPLE (or SUBSPECIMEN)is taken, representing a part of the initial sample drawn out to determine the lot quality. For small lots the initial sample can at the same time be the average one.

2.Significance of adequate sampling for the assessment of grain quality

It is commonly assumed that the adequacy of the analysis results depends not only on the precision of the measuring device and the qualification of a technician performing the analysis. Indeed, the precision of the scales, proper weighing, purity of reagents, adequate measuring or titration, and many other things are of significance here. However, for such a complex matter as grain bulk, not lesser, if not greater, part is played by the technique of sampling for the analysis. No matter how carefully a technician performs the analysis, if the tested sample is not representative, the result would characterize only the quality of the grain sample brought to the laboratory and not that of the

whole lot. The lack of reliability of the figures of the contents of mycotoxins may lead to a real danger for human and animal health and the consumers of contaminated fodder will be subject to considerable economic losses caused by the death of animals or by the reduction of productivity. Thus, sampling represents one of major and significant elements in the whole grain analysis pattern in general and in the analysis of mycotoxins, in particular. Many authors claim that it is this stage that represents one of the basic sources of errors in the determination of mycotoxins content. For example, Whitaker (26) while studying cases of errors in the determination of aflatoxins in coarse-grained produce (maise, peanuts, pistachio nuts, etc.) presented the common determination error as a sum of lot sampling errors, taking the analysis sample, and the analysis itself (Fig.1). Comparison of variation coefficients presented in Fig.2 shows that the maximum spread of the results occurs at the first stage, i.e. initial sampling. This is due to uneven distribution of aflatoxins in the product mass. Thus, at the average aflatoxin content of 20 mg/kg the variation coefficient during initial sampling of 21.8 kg constituted 60%, during subspecimen taking (1.1 kg) - 18%, and in the process of the analysis of two weighed portions - 16%. The overall variation coefficient was approximately 80%. For cotton seeds the average variation coefficient for lots with a 20 mg/kg level of aflatoxin contamination was 8% during analysis, 18% in the subspecimen taking, and 100% in sampling (25). For maise (27), four error components were studied: the error of taking a 10 pound (4.54 kg) sample, the error of selection of a 1 kg coarsely ground (passing the 14 mesh sieve) subspecimen, the error of making a finely ground (passing the 20 mesh sieve) 50 g specimen, and the

I450

determination error itself. Variation coefficients for a lot with a 20 μg/kg aflatoxin contamination constituted 20, 7, 0, and 28%, respectively. Thus, maize differed considerably from peanuts in the error connected with sampling; however, this error turned out to be significant.

3. General requirements to sampling and to the preparation of specimens for the assessment of grain quality

There exist several general requirements to the methods of sampling and to the preparation of specimens for quality analysis. The basic requirement is the ensuring of sample representativeness, viz. the selected samples should characterize quality of the whole lot. This is attained by the observance of the following principles: the initial sample is collected from single samples; the average or secondary sample (subspecimen) for analysis is obtained through gradual reduction of the initial sample; weighed portions or analytical samples are also prepared by means of gradual reduction of the average or secondary sample (subspecimen).

4. General pattern of sampling and sample analysis for the assessment of grain quality accepted in the USSR

The general pattern of sampling is presented in Fig.3. The samples selected from every grain lot are inspected and compared. If they are homogeneous, they are united. The set of single samples represents the initial sample. In the case of apparent heterogeneity of samples, every homogeneous part is taken for a separate grain lot and an initial sample is formed from each of these lots.

While inspecting grain quality, it is extremely significant to organise thorough record keeping. For this purpose a sample

tag (label) with the crop name, strain, type and subtype, crop year, name of the enterprise to which it belongs, numbers of wharehouse, silo, waggon or ship name, lot mass, date of the initial sampling and sample mass, and signature of an official responsible for sampling, is put into the initial grain sample container. Later these data are recorded in a special book.

If the initial sample weighs up to 2 kg, it is at the same time considered as the average sample. If it exceeds 2 kg, a 2 kg amount is taken out of it by means of a separator or manually.

The average sample is collected somewhat differently if the initial sample is taken from a large homogeneous mass of grain, e.g. during vessel loading or unloading. In this case at the end of every loading or unloading day the taken single samples are mixed in the separator or manually, and approximately one eighth portion is drawn out of the mixture and placed into a separate container. At the end of the loading or unloading procedures, the grain is poured out of the container, mixed in the separator or manually and about 2 kg representing the average sample are taken.

An obligatory condition is the storage of a sample portion for a period of time during which there may be claims presented as to inadequate analysis performed. In such cases the sample is subject to a check (umpire) analysis.

The size of a weighed portion selected from the average sample for analysis is of significance for the assessment of the accuracy of the analysis. Naturally, an increase of the grain mass subject to analysis makes it more reliable and better reflecting the quality of the whole lot. The degree of influence of its heterogeneity diminishes. Besides, errors of the assessment

itself depending on the precision of a measuring device, technician's mistakes and occasional circumstances, are reduced considerably when the weighed portion mass is larger. Technically, however, it is impossible to analyse an extremely large weighed portion; its size has to be correlated with the type of the analysis and in some cases has to be differentiated depending on the crop and its targeted use, and also on the significance of the analysed parameter. The size of weighed portions is specified in standards for testing methods. Weighed portions are taken out of the average sample using a separator or manually. During high-volume supply periods, in order to ensure regular work of motor transport, grain quality assessment can be done on the basis of daily average samples.

Daily average samples are composed of grain lots homogeneous in quality and supplied during one day from one and the same farm. The lot homogeneity is established by type, subtype, colour, moisture content, grain pest contamination and content of impurities. To form the daily average sample from initial samples selected from every lorry, 200 cm^3 of grain from every 1.5 tons are taken. The grain is placed into a tightly sealed container which can be easily cleaned and washed. Every farm transporting grain by lots during one day, has its own containers. The average sample is formed out of the daily average sample using the technique described above.

5. Peculiarities of grain sampling for the analysis of mycotoxins

5.1. Factors influencing the volume of the sample for analysis

A. Discrete character of the distribution of mycotoxins.

The contamination of lots with mycotoxins bears a discrete

character. Micotoxins may be present only in a small part of the product, however, in a relatively high concentration. For example, the number of peanut kernels contaminated with aflatoxins can be less than 0.1% while the contamination level of individual kernels can reach 1 mln ppm. Fluctuations in aflatoxin content in individual kernels can reach 106 μg/kg (26).

In view of a heterogeneous nature of mycotoxin distribution, the taking of a homogeneous sample for analysis represents a rather complex problem. Thus, traditional methods of selection and sample preparation cannot be fully used in the analysis for mycotoxins. These methods should be subject to considerable changes. In particular, for the mycotoxin analysis a much greater sample mass is needed than that used in the assessment of other quality properties. At the same time it greatly depends on the tested product.

From the point of view of aflatoxin contamination, all products can be divided into two extreme types (15):

- with an extremely high degree of contamination heterogeneity (Type 1); and
- homogeneous type (Type 2).

Type 1 includes husked and raw peanuts and other oil seeds, intact or coarse-ground kernels, nuts and dried fruits. Type 2 represents such liquids as vegetable oil, milk and dairy products. There exists also Type 3 characterized by an intermediate degree of contamination; to this type belong: fine-grist flour, peanut oil, fermented products, oilseed cake, dried cereal grain. The size of a representative lot sample depends on the product

1458

type. For Type 1 the lot size should be as large as possible, while for Types 2 and 3 - considerably smaller.

B.Kernel size.

Another factor influencing the sample volume is the size of grains. For Brazil nuts weighing 8-10 g each a large sample is needed, compared to that for peanuts having a weight of less than 0.5 g.

The sample size also depends on the volume of the tested lot. The greater is the lot, the larger should be the general sample. However, if a lot is very large, the volume of the sample does not play a decisive role: lots of husked peanuts weighing 40,000 pounds or 100,000 pounds can be represented by samples of identical volumes.

It should be noted that recently the peanut sample weight in the analysis for aflatoxins has been raised in the USA from 12 pounds first to 24, then to 48 and, finally, to three samples 48 pounds each. This increase of the sample size testifies to the producer's need to have a more reliable assessment. On the other hand, some investigators (26) claim that in the analysis of peanuts for aflatoxins the major source of errors lies in the inadequate (less than 22 kg) sample weight.

5.2. Principal pattern of sampling in the analysis for mycotoxins

The general (initial) sample is drawn either by continuously operating automatic sampler or by combining single samples taken from a maximally large number of container portions. Since the whole general (initial) sample cannot be extracted prior to analysis for mycotoxins, a secondary sample (subspecimen) should be prepared by gradual sample reduction. Before this procedure,

however, the initial sample should be thoroughly ground and mixed. The dispersion degree does not play a decisive role; however, the subspecimen mass depends on the particle size. For coarsely-ground products its size should be larger than for finely ground ones. In view of the fact that the grinding diminishes the size and increases the number of particles, an error in taking the secondary sample is usually smaller than that during the primary (initial) sampling. A subspecimen with the mass of 1-5 kg is preferable.

Before taking the analytical sample (weighed portions for analysis) the subspecimen should be also ground, thoroughly mixed and gradually reduced because only a small portion of the subspecimen is subject to the analysis. The size of the weighed portion varies in different methods from 20 to 100 g. As far as sample representativeness and solvent economy are concerned, most suitable is the weighed portion of 50 g.

Thus, the general pattern of grain analysis for mycotoxins is as follows: taking a lot (general, initial) sample, its thorough mixing, coarse grinding to reduce particles size, mixing to obtain a homogeneous product, taking a secondary sample (subspecimen), further grinding and taking a sample for analysis.

5.3. Technique of sampling in the analysis for mycotoxins

A. General information

Samples for the analysis of vegetative raw materials can be taken at various instants: in the field, during harvest, storage, processing, commercial operations, reprocessing and final processing, product supply to the food industry because mycotoxin

contamination can occur at any of these stages. The technique of sampling in each case will be different (15) since these stages have their specific features related to the distribution of contaminated grain within a lot, accessibility of inspection and sampling. However, as far as it is possible, samples should be taken from a product containing a large number of small particles. Thus, it is more expedient to perform sampling of maize grain rather than of corn-cobs; at the same time ground maize is preferable to maize in grain (10). Evidently, sampling from a lot mixed during harvest, loading-unloading works, transportation, grinding, crushing gives more representative samples in view of a discrete character of mycotoxins distribution. This statement is correct both for contamination in field and in other stages of the processing of vegetative raw materials. For example, moisture condensation or water permeation in storehouses can cause partial mildewing of a portion a lot during storage. That is why it is impossible to predict in what storage place sampling should be performed in order to have a proper idea about aflatoxin concentration in the lot. We shall discuss only a technique of sampling raw materials without dealing with intermediate and final products of their processing.

B. Field sampling(examination of grain for mycotoxins)

It is extremely difficult to select a representative maize sample before harvest because the distribution of contaminated corn-cobs bears a random character. Besides, one corn-cob can contain no contaminated grain at all, while another one has only one contaminated grain or all of them. Thus, to obtain a representative sample, one should select a large number of corn-cobs to

husk them later. Approximate variation coefficients at the average aflatoxin content in husked maize grain 100 ppm and at randomized corn-cob sampling in the field were 133 for 33 cobs, 83 for 100, 62 for 200, 48 for 400, 39 for 800, and 29 for the whole field (10). Thus, it is more expedient to conduct sampling from husked maize in the period of harvesting these samples reflect the quality of a greater number of corn-cobs. The same considerations are also justified for other crops growing in groups.

C. Grain bulk sampling

While analysing a grain lot in a bin (metallic store-house), the sampling of grain flow at the moment of grain mixing during bin loading-unloading can be recommended as the only reliable method because configuration of the bin and limited access to it make other methods of obtaining representative samples complicated (10). Various samplers automatically crossing the grain flow at certain time intervals (14) are used. This sampling technique is considered to be most precise because representative transverse grain flow cross sections are systematically isolated and the initial (general) sample is made out of them. In such cases the error made by an operator during sampling is eliminated. Usually a mechanical separator located between the sampler and sampling place and intended for the reduction of the sample size to the volume needed, is connected with the sample selecting device. In the absence of this device or when it is impossible to use it for any reason, a special operator can be appointed to make samples with the help of a shovel placed into grain flow at certain time intervals. In this case the grain flow should be subject to a rather frequent sampling. Single samples, however, should not be too large for

the general sample not to exceed the permissible level. If the continuously operating automatic sampler cannot be applied (when the bulk lot is in the bin, lorry, railroad waggon, or similar container), sampling can be performed by means of probes which should reach the container bottom. Both manual and mechanical probes are used.

D. Sampling from the lot in sacks

The best way of taking samples from the lot in sacks is to perform this procedure during filling or emptying the sacks. In this case samples are taken with a shovel or in handfuls and are placed into a collecting container. After the sealing of the sacks and their placing on trays, sampling becomes complicated. For sampling purposes sack probes are used. In view of the fact that the number of sacks in one lot is large, it is recommended to select samples from one quarter of the number of the sacks.

5.4. Devices used for sampling and for the preparation of specimens

Various devices (18) for sampling and for the preparation of specimens during mycotoxins analysis are in use which are similar to those mentioned above in discussing the sampling for general grain quality assessment. Most common, however, are continuously operating samplers, the Hobbart vertical self-cutting separator-mixer (HVSM) and the Dickens-Satterwhite grinder. The use of these devices makes sampling and sample selection cheaper, quicker, and more efficient. Using the Hobbart mixer, it is possible to grind and at the same time mix a 20 pound sample of Brazil nuts in hulls within 2-3 minutes; the variation coeffi-

cient in this case is about 3%. The Dickens-Satterwhite grinder (12) is simple and compact; it enables the sample reduction simultaneously with subspecimen taking. Its productivity rate is 3 kg/min. Besides, it permits continuous withdrawing of a 5% product portion, as a result of which a sample of the product passing the grinder becomes representative; however, its size is too large. Therefore, when this grinder is used, the whole subspecimen should be extracted with later selection of a corresponding portion of the extract for further analysis (11). This technique is broadly used in the USA to inspect all peanuts supplied to the food industry.

5.5. Measures to prevent progressive mildewing in the period between sampling and assessment of mycotoxins content

While selecting samples for any analysis, one should strictly observe the cleanliness of devices and containers used which should be dry and possess no foreign smell. Sampling should be performed in a way that samples themselves and also the sampling equipment and containers for their storage are protected from harmful external effects. In view of the fact that samples, depending on concrete conditions, are transported or stored for various periods of time from the instant of sampling to the time of study, one should observe measures preventing mold growth within this period. It is preferable to reduce the time of sample transportation and to keep samples dry and cool ($0^{\circ}C$ and below). It is also necessary to do everything possible to eliminate sample moistening during transportation and storage. For this purpose it is not re-

commended to keep raw and insufficiently dried seeds in plastic conteiners if the latter are not stored in cool atmosphere. It is better to use fabric or paper sacks. Mold growth and toxin production can take place within several hours; thus, samples after selection should be dried as soon as possible at a temperature of about 80-90°C for three or more hours, to reduce moisture content to 12-13%. When it is necessary to study the grain microflora, the grain should be dried to the same moisture content at a temperature of 60°C in the course of a longer period of time. In case a sample was cooled before drying, it should be immediately dried and stored dry before the analysis.

It should be also taken into account that sealed samples cannot be cooled sufficiently rapidly to 0°C. For this reason the package should be kept open till the grain is cooled. Sample sacks should be spacy enough permitting loose grain distribution within a sack to speed up the cooling process.

In view of the fact that moisture may rapidly condense on grain cooled to 0°C with dry ice, liquid nitrogen, or in a refrigerator, which leads to the increase of moisture content to the undesired level, it is recommended to analyse the samples immediately after cooling or to keep them in water-proof containers after refrigerator storage till ambient temperature is reached. The problem concerning the use of preservatives, such as acetic acid, propionic acid, etc. has not been solved yet. For such recommendations it is necessary to study possible danger for the health of the working personnel caused by these substances in view of their corrosion activity and toxicity and also because of the effect of such treatment on the analysis results due to possible destruction of all representatives of the microflora.

Grinding and further sampling should be performed immediately, as far as circumstances permit, after initial sampling. If it is impossible to analyse secondary samples immediately, they should be cooled or kept dry.

6. Review of existing methods of sampling and of the preparation of specimens of vegetative raw materials for mycotoxins analysis

Unfortunately, there exist no methods of sampling of the preparation of specimens for the mycotoxins analysis. Various international organizations (Association of Official Analytical Chemists (AOAC), UN Food and Agricultural Organization (FAO), World Health Organization (WHO), Un Environmental Programme (UNEP), American Association of Cereal Chemists (AACC), International Standardization Organization (ISO), and others) and individual investigators have elaborated a number of methods in this respect (4, 10, 13, 15-18, 24-27). Exporters of produce which can be contaminated by mycotoxins usually deal directly with consumers and jointly elaborate agreements related to the methods of sampling. Most common methods of sampling and of the preparation of specimens in respect to different products are discussed below. It should be noted that for grain crops sampling is easier than, for example, for peanuts, in view of grain fineness and the aflatoxin contamination of a lesser number of grains.

For maize analysis for mycotoxins it is recommended to collect the whole field crop and select the representative sample in the flow of threshed maize grain (10). It is preferable to make the selection directly after harvest by combine prior to storage. Selection can be also made from a randomly chosen 1 acre field plot using a table of randomized field plot numbers, into which

the field is divided (8). It is noted, however, that in the later case the sampling can turn out to be baissed. The sample shoud be not lighter that 10 pounds (4.54 kg). Flow sampling is preferred to a single sample taking which,in its turn,is better than corncob sampling in the field. If, however, field sampling has to be made, it should be randomized but not chaotic. One should draw a sketch of the field, divide the field into N approximately equal sections, select one cob from each section, and unite the cobs into the general sample which later on has to be husked before passing through a sieve No. 14. After thorough mixing, a secondary 1 kg sample should be taken out of the general one by gradual reduction. The whole sample should be ground to pass the sieve No. 20 and after thorough mixing 50 g analytical specimens are isolated.

The following pattern of maize sampling (raw material) from a lot is recommended (15, 18):

Maize lots in collection centres and those sent to processing (reprocessing)

↓

Select samples from each sack or container with a general mass of about 70 kg

↓

Mix

↓

Obtain 5 kg samples (cross-type separator using a separator or some other suitable technique)

↓

Grind (every grain should be split into 4-5 particles)

↓

↓

Place in thin layer and inspect in the light of an ultraviolet lamp with high radiation intensity

The lot admitted to storage

Presumably contaminated lot

↓

Grind the whole lot to be passed through a 20 mesh sieve

↓

Mix

↓

Take a sample for analysis

↓

Analyse for aflatoxins presence by column chromatography (screening method)

The lot with the negative result of the analysis for the presence of aflatoxins

↓

The lot with the positive result of the analysis for the presence of aflatoxins

↓

Extract a sample for TLC and quantitative estimation of aflatoxins

↓

Confirm the presence of aflatoxins by means of obtaining chemical derivatives

↓

Direct the lot to the

end use depending on the level of aflatoxins content

For medium size and fine grains (maize, rice, peanut kernels, cotton seeds, etc.) ISO recommends to spread the sample selected for the analysis in a thin layer on a clean surface, reduce the sample to about 1 kg by cross-type separation, and to grind the sample in a blade grinder (coffee grain grinder can be used) to pass a 14 mesh sieve (0.8 mm). The sample should be thoroughly mixed and by means of further cross-type separation reduced to the amount needed for the aflatoxins analysis.

It should be said that the International Organization on oilseeds considers the existing methods as insufficiently strict in respect to peanuts, claiming that both hulled and raw peanuts used for human consumption always demand extremely thorough sampling. Taking into account, however, that strict methods of sampling are much more time-consuming, more simple techniques described above can be used if the consumer has no objections in this respect.

One of most strict sampling patterns which can be recommended for application is the one adopted in Canada and the USA (26). It is applicable for seeds of all oil crops and also cereals, e.g. maize. Following this pattern, the samples, in order to make them representative, are taken from every fourth sack out of lots containing more than 400 sacks or approximately 50,000 pounds (22.5 tons) till a maximum amount of 100 subspecimens is collected. If the lot exceeds 400 sacks, it can be divided into smaller lots (sublots), the number of which should not exceed 4. Each of these should consist of no more than 400 sacks. Samples from every lot

are taken evenly in regular time intervals to obtain a maximum of 100 subspecimens, e.g. one from every eighth sack if there are 800 sacks. No samples are drawn from mouldy sacks. The total mass of all subspecimens should not be inferior to 36 pounds (16.2 kg). Then the selected samples are transported to a laboratory for analysis, thoroughly mixed, and divided into three portions of equal size; each of these should have a mass not less than 12 pounds (5.4 kg). They are coded, e.g. A, B, and C. Every portion is thoroughly ground in a grinder and a necessary amount of the product is withdrawn for the analysis. If portion A contains no more than 4 µg/kg aflatoxins, the portion is considered as satisfactory, while the level of 20 µg/kg and higher makes the portion unsuitable. If portion A is satisfactory, no analysis of portions B and C is needed. When the analysis of portion A reveales the level higher than 4 µg/kg aflatoxins, it is necessary to analyse portions B and C. In the case when the concentration of aflatoxins in both portions does not exceed 4 µg/kg, the result obtained for portion A can be discarded and the whole lot can be considered satisfactory. When the aflatoxins content exceeds 20 µg/kg, the lot is unsuitable for use. However, if one or both results for portions B and C exceed the 4 µg/kg level, the average figure is taken for all three portions. If the level does not exceed 20 µg/kg, the lot is considered satisfactory, otherwise - unsatisfactory.

It should be noted that the level of 20 µg/kg is the maximum permissible content in Canada and the USA; other countries can establish their own standards.

According to one of recent patterns established in the USA to control aflatoxin contamination of peanuts supplied to food

enterprises (26), it is recommended to take a lot sample with a mass of 144 pounds (65.4 kg) from peanut lots weighing 88-220 tons and then to divide it into three samples 48 pounds (21.8 kg) each (Fig.4) using continuously operating automatic sampler or other suitable techniques (one sample from every fourth sack in a sack-contained lot). One of the samples is passed through the Dickens grinder to obtain a secondary 1.1 kg sample which is completely extracted with 3l of a methanol water (55:45) solution and 1 l of hexane. A portion for analysis is taken from the extract. The analysis of the second and third samples is performed according to the same technique depending on the aflatoxin content observed in the process of the analysis of the first portion. A similar pattern is proposed (13) for the analysis of maize and cotton seeds.

The technique elaborated in FRG for roasted peanuts requires a 5 kg sample (23).

Every supply of imported Brazil nuts to the USA is subject to an obligatory analysis for aflatoxins and only after such an analysis the import certificate is given. Brazil nuts are supplied in sacks containing 500-2,000 pieces. The sampling is performed in docks or storehouses; the lot sample contains form 30 to 60 pounds. It is ground in a HVSM to give a homogeneous sample (19). In such cases it is sometimes necessary to pass the sample two and more times through a HVSM. In view of the fact that importers have the right to analyse the sample only in grain, th whole sample is shelled, kernel pulp is mixed before grinding with equal amounts of ground shells. The importer pays for the cost of shelling. It has been found that it is advisable to grind the samples without shelling because hard shells help to obtain the needed degree of

homogeneity (9). The addition of such liquids as heptane followed by mixing and grinding is also recommended (9). This technique provides for the highest degree of homogeneity with a 1% variation coefficient and can be applied to samples weighing from 500 g to 20 kg and more. For samples of hulled kernel pulp, dry grinding of a lot sample is proposed. The ground sample should be mixed and a 300 g specimen is to be taken out of it, mixed with heptane, and then finally ground in a Warring mixer (9). This method, with the substitution of heptane with water, was used for cotton seeds, peanuts, peanut meal and peanut oil, cotton meal, copra, and maize (21, 22).

Pistachio nuts are also imported into the USA after analysis for aflatoxins. The supply lot usually consists of 300-500 sacks 70 kg each. The lot sample collected by a probe from sacks weighs 30-60 pounds, depending on the lot size. The whole lot sample is then ground in a HVSM and tested in a manner similar to that used for analysis of Brazil nuts (9).

7. Economic aspect of sampling and of the preparation of specimens for the mycotoxins analysis

Sampling and the preparation of specimens is not only the most complex but, frequently, most costly step of analysis for mycotoxins. This is explained by the fact that the cost of analysis includes the cost of the product constituting the sample, the costs of sampling and analysis itself, and the cost of sample transportation from the sampling area to the place of analysis (11). Indeed, on the one hand, the larger the initial (lot) sample, the more representative it is. On the other hand, however, food produce, and in particular, nuts are rather expensive, while the

changing of the form of the product (ground grain, halves, fines) lowers the quality of the former. Therefore, one of the Dickens grinder advantages is the fact that only 5% of ground product are withdrawn for analysis while the remaining part still possesses a certain commercial value although lower than that of whole grain.The cost of packing the lot sample is also of significance together with the cost of transportation of the sample to the laboratory. Naturally, these costs increase for larger samples. At the same time, the distance to the place of analysis is too long for some products. For example, a sample of Brazil nuts has to be transported to a distance of 2-3 thousand miles. Thus, the laboratories should be located as close as possible to the producing areas. Mobile laboratories are also expedient.

The equipment and techniques used are quite costly, not always convenient, and require qualified personnel.

The above discussed considerations force many investigators (10) not to recommend any common method of sampling and specimen preparation which could be convenient in all situations, and only some general directions reflecting our present-day knowledge of mycotoxins can be specified for concrete cases. At the same time, there is no doubt that only harmonised methods of sampling can yield comparable results. Further studies in this field should be aimed at elaborating a sufficiently reliable and inexpensive technique.

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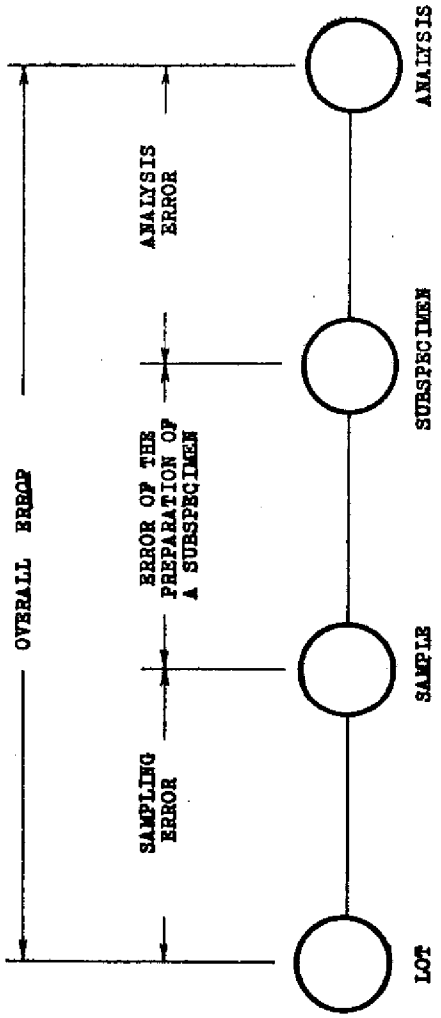

Fig.1. Typical sequence of operations used in the evaluation of the concentration of aflatoxins, X, and corresponding contributions to the overall error of determination.

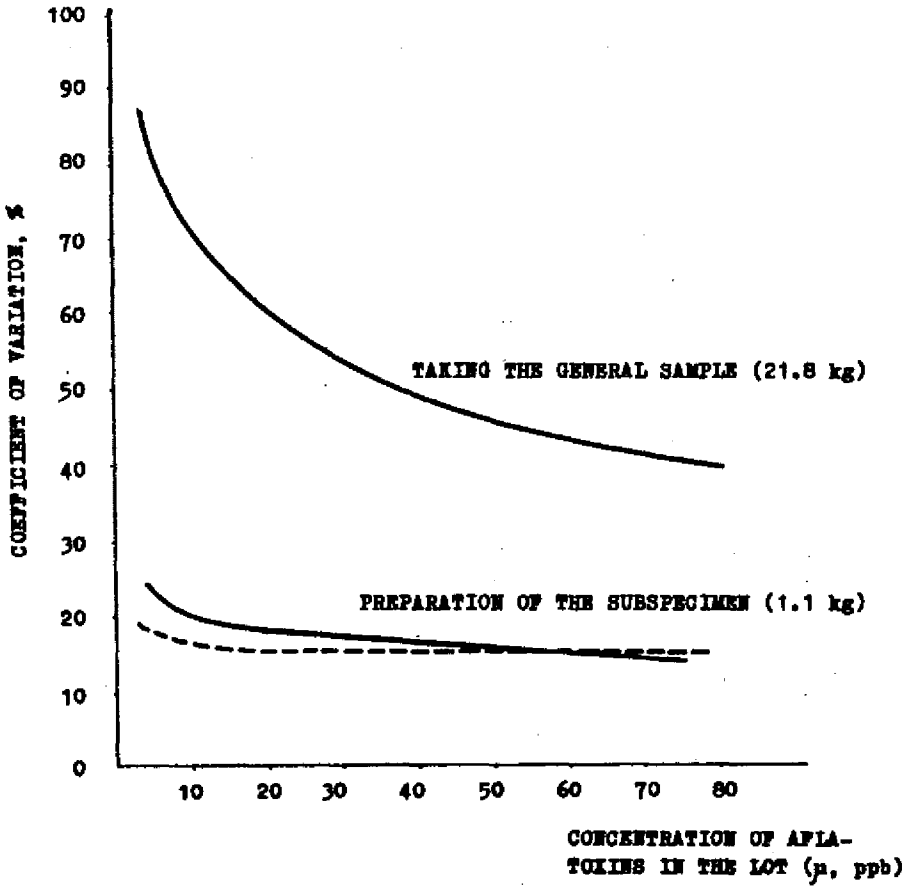

Fig.2. Coefficients of variation related to the preparation of subspecimens and analytical procedures in testing peanuts for aflatoxins.

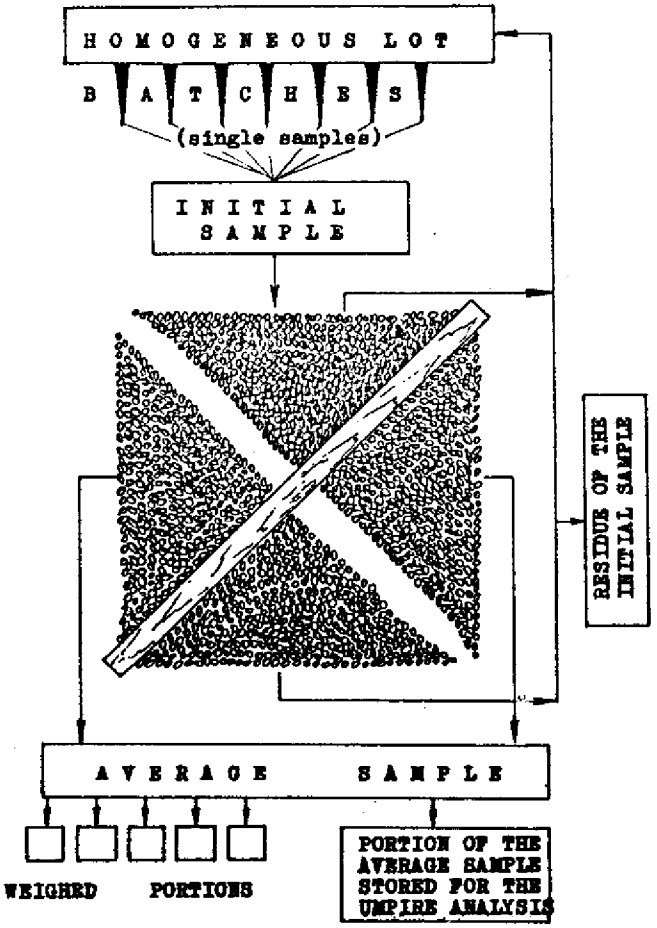

Fig.3. Sampling and specimen preparation scheme.

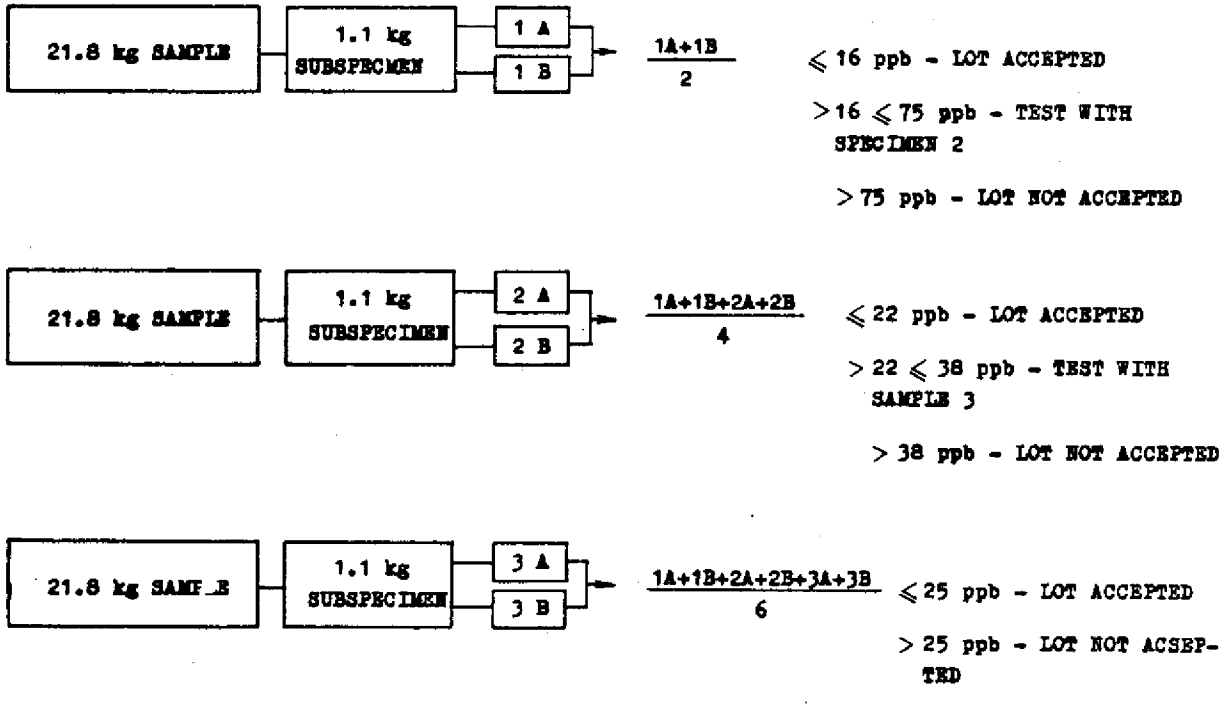

Fig.4. Pattern of the analysis of peanuts for aflatoxins accepted in the USA.