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Sample Preparation and Presampling of Pistachios

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A theory has been developed to quantify the reduction of subsample variance of aflatoxin contamination, which is observed when granular materials are wet slurried, rather than dry ground, during subsample homogenization. A coefficient of variation, based on particle size distribution, subsample size, and probability of contamination, is predicted. The theory is tested with dry ground and with wet slurried pistachios, and excellent quantitative agreement is obtained. A 32% increase in the mean aflatoxin level is observed as well when wet slurrying is applied. Although no statistical explanation for this effect can be found, it is suggested that it is related to physiochemical binding between the nut matrix, which is (partly) broken by wet slurrying, and aflatoxin, making the extraction of more toxin possible. Other parameters that may affect slurrying results have been investigated as well.

KEYWORDS: Aflatoxin; pistachios; subsampling; mean; variance

INTRODUCTION

Many granular materials, which contain contaminants, frequently have such contaminants distributed among the granules so that while the individual granules are uniform, the contaminant concentration varies widely among the granules. A small fraction of granules may contain the bulk of the contamination. Such distributions are common among nuts, pulses, spices, coffees, and some grains and seeds. When such lots are tested for contamination, it becomes important to take large samples to obtain a sample that is as representative as possible of the lot; 10 kg samples are not uncommon. The problem is that it is not possible to test a large sample as a whole; an aliquot must be taken. Extracting the entire sample, followed by taking a liquid aliquot, is not practical. The common solution is to grind the sample in some way, take a solid aliquot, and analyze the latter in the whole. This technique is referred to as subsampling.

Among the three required steps in the determination of contaminant concentration, the statistics of sampling and chemical analysis have been studied extensively, particularly by Whitaker and co-workers (*1*) and by ourselves (*2*). Analytic protocol in particular has been the subject of numerous AOAC studies (*3*). Very little has been done in the case of subsampling beyond some experimental observations on a number of commodities (*4*−*8*). It is generally understood that particle size plays a major role in the expected variance—the smaller the particles, the smaller the variance. Furthermore, the subsampling variance may be as much as 10 times that of analysis, but this will depend on the fineness of the grind. In an effort to reduce particle size, some laboratories, particularly in the European Union, have replaced the older method of dry grinding of solids by a wet slurrying technique. Quantitative relationships between particle size, subsample size, and possible other effects and variance have not been addressed. It is the purpose of the present paper to address these questions and others while testing various operating conditions, in particular with respect to pistachios.

THEORY

It seems intuitively clear that the finer the subsample is ground, the better it will represent the sample average, everything else being the same. We present some theory to quantify these ideas. The simplest case involves a lot of equal size particles (granules, nuts), a certain fraction p of which is contaminated at a fixed concentration c. The remaining fraction $1 - p$ is noncontaminated. It is assumed that the concentration of each contaminated particle is constant throughout. A sample of Q particles is taken. The sample size Q is too large to measure; instead, subsamples of N particles are taken, either by count or by weight. What is desired is the coefficient of variation (CV) of the amount of contaminant = standard deviation/mean among the subsamples. Note that only a single sample is under consideration; the sample variance, which would arise if many Q samples were considered, is not of interest here. If one does not comminute (grind) the sample, the subsamples will be distributed with a Poisson distribution (assuming N is large enough, typically $N > 30$ will suffice), with mean and variance each equal to Np and thus $\mathrm{CV} = (Np)^{-0.5}$. Now suppose the sample is comminuted so that each of the N particles is divided into s subparticles, each of the same size and having the same concentration as the particle from which it came. A set of subsamples is now chosen, each of the same weight as before, that is, corresponding to (but not necessarily from the same) N particles, which now contain Ns subparticles each. By the same argument, these comminuted subsamples will exhibit

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a CV = $(Nsp)^{-0.5}$. A factor of $s^{-0.5}$ has been gained by comminution. Strictly speaking, we have computed the CV of the number of subparticles, not their contaminant concentration, but for subparticles of equal size this is immaterial.

Of course, the assumption that all particles after comminution are of the same size is not valid. In general, particles will not break into exactly s subparticles in each case, but instead will break into a distribution of sizes that may be characterized by w_i, where w_i is the weight fraction of size i, or what is equivalent, the fraction of the N particles that broke into s_i size subparticles. The mean number of subparticles in fraction i becomes $w_i Nps_i$, as will the variance. What is needed is the mean and variance of the contaminant concentration. Let V be the volume in cubic centimeters and ρ the density of the noncomminuted particles. Then the amount of contaminant contained in a subparticle in fraction i is given by its volume V/s_i times its concentration $c\rho$. The mean total concentration becomes proportional to $\Sigma w_i Nps_i V/s_i\ c\rho = NV\rho pc$, the variance to $\Sigma w_i Nps_i (V/s_i\ c\rho)^2$, and CV = $[(\Sigma w_i/s_i)/Np]^{0.5}$, which reduces to $(Nsp)^{-0.5}$ when only a single size occurs.

The assumption that a kernel is contaminated, if at all, at a concentration c with probability p applies to subparticles which have the same c and p. However, this assumption in not valid in a real lot; it is well-known that contamination in tree nuts extends over as much as 8 decades of c, described by a probability density function $p(c)$ (*9*). The question then arises: what value should be used for p in the above expressions for CV? The answer lies in the effect of $p(c)$ on the mean and variance of contaminant concentration in such a lot. The mean is given by $\Sigma_i\ p_i c_i$, whereas the variance is proportional to $\Sigma_i\ p_i c_i^2 - (\Sigma_i\ p_i c_i)^2 = \sim\Sigma_i\ p_i c_i^2$, where binning of c is applied (*9*). This leads to a convoluted value of $\Sigma_i\ p_i c_i/\Sigma_i\ c_i$ for the mean factor in CV, with a corresponding expression for the variance. Thus, one would replace p in the above expression for CV by $(\Sigma_i\ p_i c_i/\Sigma_i\ c_i)^2/(\Sigma_i\ p_i c_i^2/\Sigma_i\ c_i^2)$.

The predicted CV (subsampling plus analysis) is computed from the sum of the variance due to the particle size distribution, described above, and an estimate of the analytic variance.

MATERIALS AND METHODS

Two types of experiments were carried out. Type II experiments used optimal methods and parameters, as far as possible, and were designed to explore the difference in measured aflatoxin contamination mean and variance obtained from dry grinding and from slurry grinding. Type I experiments, partly reported earlier (*6*), were designed to study the effect of a number of methods and parameters on slurry results. The method section gives the conditions for type II experiments in some detail; a paragraph follows indicating where type I experiments differed.

Both types of experiments used commercial eye-core (i.e., color sorter) pistachio rejects, kernels only (type II), or in-shell (type I). This material was chosen because it contains generally a high aflatoxin content, simplifying analysis. All chemicals were of reagent grade, and water was deionized. HPLC water consists of further cleaned deionized water through mineral removal and ultrafiltration. Dry grinding was carried out using a Hobart (Troy, OH) vertical cutter mixer (CM40), operating at 3500 rpm. In all cases, an approximately equal weight of dry ice was added to avoid "buttering" the grind (separating oil and solids and producing an emulsion). The Hobart operates by passing a heavy, sharpened, tilted blade through the nut/dry ice material, with limited vertical mixing. An integral mixing blade allows manual mixing during grinding. After a fixed amount of time (6 min) of grinding, the dry ice was allowed to evaporate by spreading the mixture to a depth of 5 cm and applying a heat lamp (100 W) with occasional stirring; 1.5 h sufficed to remove dry ice and reach ambient temperature. A free-flowing powder was the result. Next, the dry nut grind was mixed in a dry blend V-mixer (Patterson-Kelly, East Stroudsburgh, PA, capacity = 2 ft^3 = 57 L, 20 rpm,) for 1.5 min, and 12 subsamples (~50 g each) were taken. Samples were stored at −8 to 4 °C until used for analysis, low enough to avoid aflatoxin production. Cleanup required full disassembly of the Hobart with wash-down with 5% bleach. Cleanup of the V-mixer required solely wipe-down with minor water rinse, because all surfaces were visible. This will be referred to below as type II dry grinding and was designed primarily to use the best techniques available and to test the difference between dry grinding and wet slurry grinding.

Dry grinding for type I differed in the following way from type II grinding: (1) A different lot (of much lower aflatoxin content) was used as source, and all experiments were carried out on in-shell pistachios. (2) Grinding was tested at 3 and 6 min. (3) Dry ice was allowed to evaporate overnight on bulk stored material and without heating assistance. (4) No postgrind mixing was applied.

To avoid any sampling errors, the remainder of the dry-ground sample was slurried. For type II wet slurrying the liquid component was water. The liquid/solid ratio was 1.5 L/kg. The slurrying device was produced by Silverson Machines (Waterside, Chesham, Buckshire, U.K., model DX60), which incorporates a four-blade rotor with highly sharpened blades rotating within a stator with little (~0.02 cm) clearance. Depending on the stator used, it incorporates slits or holes in its sides, which allow exit of the shear-cut solid material. Silverson produces slurry shear cutters in a number of sizes. Many laboratories use the larger EX model, particularly for more difficult to cut solids or for slurries with less liquid. In the present case, most work was done with the slitted stator, using a DX unit. While cutting, a resulting countercurrent flow of cut-solids slurry is aided by an additional propeller mounted on the drive shaft. The entire assembly plus feed and the appropriate amount of water was placed in a cylindrical vessel, 35 cm diameter and 43 cm deep, ~8 cm above the bottom of the vessel, with the rotor operating at 1785 rpm for 30 min. By careful spacing of the upper down-draft propeller, good mixing could be accomplished throughout the entire vessel, except for the region immediately above the stator. Such material had to be hand stirred into the slurry, best part way through the experiment. Following slurry production up to 12 samples (~120 g each) were removed immediately from different parts of the slurry. Withdrawal needed to be done quickly as visual evidence of phase separation (shiny surface, but no layering) could be observed in a short time. Cleanup involved largely a hose-down, with stirring in a bleach solution, after removal and scrub-down of the stator.

Type I slurrying experiments differed in the following ways. (1) Experiments were carried out not only with the DX unit but also with a benchtop L4R unit (1 kg samples) for which preslurrying preparation was done using a Waring blender. This reduced shell fragment size below that achieved by the Hobart. For tests using MeOH/water blends as slurrying liquid, an AX model with an air-driven motor was used for safety. However, that unit could also not handle >1 kg and could not operate at over ~1100 rpm. (2) All tests were run at 2:1 liquid/solid ratios. (3) Slurrying experiments, carried out on the DX unit, were performed for 15, 30, 45, and 60 min. (4) A limited number of tests used a stator with holes. (5) Tests were run with 20% salt added to the slurry mix, rather than 2% at analysis.

In type II experiments, subsample bottles were transported to the analysis laboratory, warmed in a water bath to 25 °C, and stirred, and a 20 g dry ground subsample was immediately extracted. Analysis of dry ground samples followed standard AOAC HPLC procedure (*3*), except that extraction was carried out at 60 vol % MeOH, not 70 vol % MeOH [Whitaker et al. (*1*) noted little effect with water-slurried samples to 64 vol % MeOH]. Subsamples (with the addition of 2% NaCl) were extracted with 5 volumes (100 cm^3) of extraction fluid consisting of 60 vol % MeOH/water with 3 min blender mixing filtration, enough water was added to reduce MeOH to 30 vol %. Samples were diluted 25:1 using 30 vol % MeOH to match the HPLC detection capabilities. After further filtration, further sample preparation was carried out using a Zymark Benchmate2 (Hopkinton, MA) robot, which was used for sample cleanup. Passage through an immunoaffinity column (Vicam, Waltham, MA, type P), using pressure to aid flow, was followed by HPLC injection and postcolumn bromination, using a Kobra cell. The HPLC column was a reverse phase C_{18} column.

Detection was by fluorescence (360 nm/450 nm). The column was calibrated with spiked samples, which passed through the entire cleanup procedure. Quantitation was based on peak heights, not areas, as a relatively short column resulted in some peak overlaps. All results are reported as B_1 aflatoxin. Type I slurry subsamples differed in that the sample size was 50 g (20 g of solids, 30 g of water) and in that the added extraction solution consisted of 60 cm^3 MeOH plus 10 cm^3 water, the 30 cm^3 water in the slurry being considered as part of the extraction solution. The remainder of the analysis was identical to that of the dry ground sample.

In the section to follow, a significant increase in aflatoxin content was measured in the slurry ground compared to the dry ground material (see below). Following a suggestion made by a reviewer of the original submitted draft that this effect might simply be due to more efficient extraction of smaller particles, the effect of extraction parameters was explicitly tested in some follow-up type II experiments. The starting material was a fresh lot having low aflatoxin. Experiments were run in a triplicated 2 × 2 design, in which the extraction blending time was tested at 3 and 9 min and the extraction fluid (including slurry water as before) was tested at 60 and 70 vol % methanol. It might have been possible to test particle size as well by dry grinding for an extensive time, using repeated dry ice additions, but this seemed to be impractical.

Type I analysis differed in the following ways from type II. (1) Extraction was carried out using a wrist action shaker for 15 min, followed by centrifugation (4000*g*, 15 min). (2) All sample preparation was manual; no robot was used. (3) Passage through the VICAM column at the standard 1 cm^3/min was aided by controlled vacuum, not pressure. (4) Derivatization was precolumn, using trifluoroacetic acid. (5) The HPLC column used (Zorbax, ODS, 4.5 × 200 mm, Agilent Technologies, Wilmington, DE), was longer, avoiding overlap; hence, peak areas could be used. Otherwise, conditions and calibrations were the same as for type II experiments. (6) Tests were run not only at 60% MeOH extraction but also at 40−80% MeOH. It appeared that above 60% the fraction of MeOH did not affect results, but 60% may be marginal.

Particle size distributions were measured by sifting through a set of stainless steel screen wires at right angles (square openings) of known size. The particle size computed refers to the arithmetic average spacing between the finest screen passed and the next smaller screen. Dry ground sample was screened dry as well as by water wash to reduce electrostatic effects. Slurry samples were screened wet only. Attempts were made to characterize particle distributions by use of laser light scattering (LS250, Beckman Coulter Corp., Fullerton, CA). Although results were in general agreement with sifting, all particle distributions were mapped as bimodal, with a gap at 100 μm. This is presumed to be an instrumental artifact that could not be resolved, and such measurements were hence abandoned. In addition, an SMZ-10 stereomicroscope (Nikon) was used to identify individual particle shapes.

RESULTS AND DISCUSSION

Microscopic Observations. Measured observation on dried suspensions on glass slides indicated that the major axes of the larger particles (1 mm or more) were equal to ∼20%. Tilting the slides suggested that the particle depths were similar. On the basis of these observations, it was felt that a reasonable description of ground particle shape would be spheres.

Particle Size Distributions. The observed particle size distributions are listed in **Table 1**. Both sifts used water for wash-through. Dry sifting gave substantially the same results except for a somewhat lower amount of fines. The weights refer to the dry weight of material left on the sift screens. In view of the deduced spherical (see previous paragraph) particle shape, it was assumed that a particle would pass a screen if the diameter was smaller than the interwire spacing of the screen. s_i values were computed on the basis of the measured average weight of an unground pistachio kernel (0.5 g). The average number of particles per gram is computed as $2\langle s\rangle = 2/(\Sigma\, w_i/s_i)$. From this value weight-averaged diameters of 0.12 and 0.06 cm are derived form dry ground and wet slurried particles, respectively, in good agreement with sieving results. The gap between the cutters and the stator in the Hobart vertical cutter mixer amounts to ∼2 cm and the one in the Silverson slurry mixer to 0.030 cm. It appears that the Hobart grinds by impact with the blades, but the slurry mixer may actually cut.

Table 1. Experimental and Theoretical Particle Size, Mean, and CV of Ground Pistachio Subsamples

Sieving

			w_i, %	
screen size, μm	⟨diam⟩, cm	s_i	dry grind	wet slurry
2800	0.30000	35	0	0
2362	0.25810	56	1	0
1700	0.23100	114	6	0
1000	0.13500	388	26	6
850	0.09250	1207	15	10
710	0.07800	2013	9	11
600	0.06550	3400	8	9
425	0.05125	7098	10	12
250	0.03375	24853	18	12
180	0.02150	96134	3	6
150	0.01650	212686	1	4
125	0.01375	367522	1	4
105	0.01150	628200	0	5
75	0.00900	1310582	0	11
63	0.00690	2908334	0	7
45	0.00540	6067508	0	5

Statistics

	dry grind	water slurry
$\Sigma\,(w_i/s_i)$	0.00162	0.00034
no. of subparticles/g	1233	5882
wt av diameter, cm	0.12	0.06
p	0.00093	0.00122
N	40	40
CV, calcd (subsampling)	0.209	0.083
CV, anal.	0.05	0.05
CV, predicted (ss + anal)	0.215	0.097
CV, exptl	0.20	0.095
mean ± SE, exptl, ng/g	66 ± 4	87 ± 2

Probability of Contaminated Pistachios in the Sample (and Hence Subsample), *p*. To compute the weighted expressions for p in $(\Sigma_i\ p_i c_i/\Sigma_i\ c_i)^2/(\Sigma_i\ p_i c_i^2/\Sigma_i\ c_i^2)$, the single-kernel probability density function of the lot (strictly of the sample) is required. No direct measurements were made of the aflatoxin distributions in any of the samples received, nor was it likely that such information would be available in general in commercial use. However, previous work (*10*) had measured the aflatoxin distributions in a commercial pistachio process stream quite similar to the one considered here. The source was a stream of pistachio floater eye rejects for which the average concentration was 15.6 ng/g (kernels). This distribution was derived from 80 samples, 20 each at 1, 10, 100, and 1000 kernels. The number of samples is too small to obtain an adequate probability density function, but an estimate of p could be calculated from the above expression by using some smoothing of the experimental p. One obtains $p = 0.000219$, which compares favorably with the distribution shown in **Figure 1** at high c, as it should. To apply to the case at hand, a correction must be made to $p(c)$ for the lot mean. Previous work (*2*) had established that $p(c)$ was generally proportional to the lot mean for similar lots; that is, the shape did not change when the mean changed, the probabilities simply changed to account for the changed mean. In the present case the dry and slurry ground subsamples exhibited means of 66 and 87 ng/g (see below). Correcting for the mean, one obtains the p values needed for the present work, 0.00093

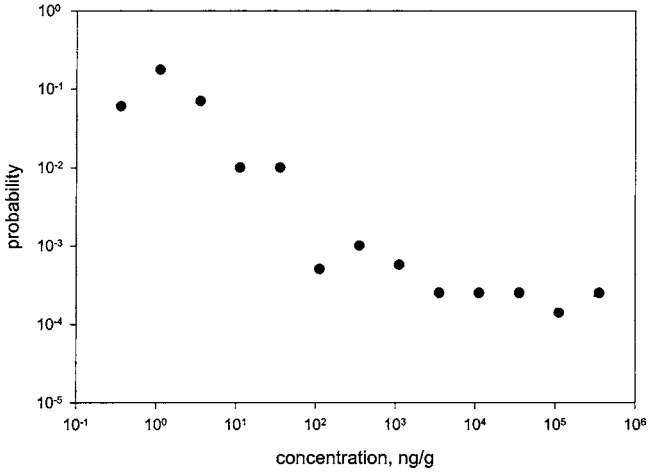

Figure 1. Probability of contamination of single kernels of eye core rejects (*10*) (total aflatoxin, smoothed).

Table 2. Experimental Aflatoxin Content of MeOH/Water Slurried Pistachios

grinding liquid[a]	aflatoxin content ± SD, ng/g
0/2/1/0	0.8 ± 0.1
0/2/1/0.2	0.9 ± 0.2
1.2/0.8/1/0	0.5 ± 0.1
2/0/1/0	0.9 ± 0.2

[a] Composition MeOH (cm^3)/water (cm^3)/solids (g)/NaCl (g).

for dry ground and 0.00122 for slurry ground eye core reject pistachios. These are listed in **Table 1**. It must be realized that this is an empirical approach, particularly as the two lots in question, although similar, are not identical (the older lot contained some aflatoxin G_1; the present did not). Thus, the source of assorted constants is the weakest part of the theoretical calculation of CV.

Computed Values of CV. The number of unground nuts of weight equal to the subsample is obtained directly from the measured weight of average kernels, 0.5 g. For type II experiments, $N = 40$. Combining values of the weight-averaged sum of $1/s_i$, N, and p, one computes for CV 0.209 (dry grind) and 0.083 (slurry grind). To this must be added the effect of analytic error, estimated in CV as 0.05 for type II and as 0.10−0.12 for type I (the increase is presumably due to passage of fluid through the immunoaffinity column, see below). Using the expression $CV = (CV_a^2 + CV_b^2)^{0.5}$, one obtains predicted CV (ss + anal) = 0.215 and 0.097, respectively, for dry grinding and water slurry grinding. No adjustable parameters are associated with this calculation. The experimental values, given in **Table 2**, amount to 0.20 and 0.095 for dry and slurry grinding, respectively. This excellent agreement with theory is probably somewhat fortuitous, given the uncertainty associated with the estimate of p.

The Effect of Slurrying on the Mean Aflatoxin. What was not expected, but is clearly noted, is the increase in the sample mean obtained by slurrying. We have no statistical theory to account for this increase, nor can we foresee any statistical property that could be responsible for this. Note that the two experiments which underlie the results of **Table 1** differ in only one respect, the sequence in which the extraction fluid is added. That is, in dry grinding the steps are (1) weigh 20 g of sample, (2) add 0.4 g of NaCl, (3) add 100 cm^3 of a blend of 40 cm^3 of water and 60 cm^3 of MeOH, (4) blend for 1 min in a Waring blender, and (5) filter. For wet grinding the sequence is (1) dry grind, (2) mix 10 kg of dry ground material and 20 L water, (3) slurry for 30 min, (4) weigh out 50 g of slurry, (5) add 1 g of NaCl (the amount NaCl is unimportant, see Table 2), (6) add blend of 10 cm^3 of water and 60 cm^3 of MeOH, (7) blend for 1 min in a Waring blender, and (8) filter. The remaining steps are the same. A 32% increase in m is noted. Velasco and Morris (*4*) saw a 20% increase in peanuts but a 7% decrease in copra, with little effect in corn and cottonseed. Whitaker et al. (*5*) obtained a 33% increase of m in peanuts but saw no change in CV. (However, his dry grind was very fine as well.) The matrix does seem to have an effect. It suggests that slurrying, aside from cutting, is not simply an addition of water, as had been assumed. The action may be one of swelling or possibly an exchange of H-bonding between aflatoxin and the matrix. At any rate, the effect is a nonequilibrium one.

Table 3. Effect of MeOH and Time on Extraction of Aflatoxin, ng/g

	sample:	dry grind		slurry grind
vol % MeOH		60%	70%	60%
3 min		1.8 ± 0.4	1.5 ± 0.1	2.4 ± 0.0
9 min		1.9 ± 0.4	1.8 ± 0.4	2.3 ± 0.1

As noted above, a reviewer suggested that the difference between dry grinding and slurrying might be due simply to efficiency of extraction of aflatoxin from smaller particles. Although the parameters used (3 min of blending and 60 vol % methanol) might be at the limit of adequacy, these parameters are used in a number of AOAC methods for aflatoxin. Nevertheless, the suggestion seemed to be worthwhile. An experimental design was run to test the effect of vol % MeOH and extraction time (Table 3) with the result that neither parameter showed significant effects.

One draws two conclusions. First, the standard wisdom, that extraction of aflatoxin from matrices by use of dry grinding and appropriate blends of MeOH/water can be relied upon to extract all of the aflatoxin from a matrix, appears not to be valid. It will be essential that for laboratories to agree all must follow the identical protocol (unless this effect can be quantified). Development of such a protocol by interlaboratory testing, such as is carried out by the AOAC, seems to be called for. Second, this nonequilibrium effect is interesting on its own account, and studies, such as the amount and composition, as well as the rate, of extraction should elucidate the underlying science. Moreover, such studies should be of direct application in toxicity as the water slurrying may bear some resemblance to what occurs in the gastrointestinal tract of mammals consuming aflatoxin-contaminated matrices.

ADDITIONAL EFFECTS

The remaining reported results refer to experiments of type I in which a number of method and parameter changes were tested to study their effect on slurry aflatoxin concentration results.

Analytic Dependence on VICAM Flow. VICAM Corp.'s written manuals recommend a flow rate of 1 drop (0.05 cm^3/s) through the immunoaffinity columns and further recommend that this flow rate be maintained by a pressure differential. On the other hand, technical staff at the company indicated verbally that vacuum differentials would work as well at the same flow rate. During the course of the work on type II methods we discovered that vacuum differential caused problems, presumably because the packing was too delicate to withstand a vacuum. Tests on a set of subsamples showed that pressure

differentials yielded an aflatoxin value of 381 ± 27 ng/g (five samples) or CV = 0.07, when pressure was used, but 245 ± 37 ng/g (eight samples) or CV = 0.15, with vacuum with another source. Calibration in both cases was carried out using vacuum. Unfortunately, all other type II experiments were carried out using vacuum. In addition, type II experiments used in-shell pistachios, which had little effect on aflatoxin values (the shells acted basically as a grinding agent), but made sieving experiments useless. It was concluded that most of these experiments were useful only in comparison with each other but could not be used for absolute values.

Grinding experiments were carried out for 3 and 6 min using the Hobart and for 15, 30, 45, and 60 min using the DX Silverson slurry grinder using the slotted stator almost exclusively. On the basis of the appearance of the grind, the shortest times were unsatisfactory, although within the accuracy of the measurements little effect was seen. The same was true when samples were taken from different parts of the sample container. We are told by colleagues that the more powerful EX grinders can operate in 15 min for most matrices. No tests were run on the efficiency of V-mixing.

The nature and amount of slurrying fluid were investigated. Various amounts of MeOH were substituted for water with results shown in **Table 2**. The effect of the solvent composition appears to be negligible, although the total aflatoxin content was low in this source. MeOH, however, had several disadvantages: (1) It is extremely hazardous and toxic; all experiments had to be carried out in a hood using an air-driven motor. (2) Disposal of the remaining slurry is costly. (3) Especially with pure MeOH, but also somewhat with 60 vol % MeOH, produced slurries separate extremely rapidly into layers, and it became very difficult to obtain representative samples of subsamples.

Use of Salt. NaCl (20% of nut weight) is generally added to dry ground nuts prior to extraction, presumably to increase ionic strength and so prevent H-bonding between proteins. Addition of NaCl prior to wet slurrying, as compared to addition after slurrying, had no substantial effect (see **Table 2**).

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