Preparation of Lot Samples of Nut Meats for Mycotoxin Assay

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ABSTRACT

A procedure has been devised for preparing lot samples of mycotoxin-contaminated nut meats so that a representative analytical sample may be removed. The sample is rapidly reduced to coarse size. A relatively large portion (about 1/10 of total sample) of subsample is then split out and further comminuted to a fine particle size with the aid of a fat solvent (meat-solvent, w/v, 3:2). The analytical sample is removed from this mixture. The procedure was tested with shelled almonds and shelled walnuts using radioactive nuts to simulate the mycotoxin contamination and provide a simple, precise measure of the contaminated nut meat distribution. The pooled coefficient of variation was 18% for the subsamples and 4.4% for the analytical samples. Considering the dilution factors used (1.50 and 2.14 contaminated nuts/10^4 nuts) and the low degree of reliability of the lot sample, the sample preparation methods tested appear to be practical and reliable.

INTRODUCTION

The background and rationale of this study have been described previously (1) together with the experimental procedures and equipment. The basis of this study was the observation from the earlier work that a subsample (about 1/20 of the total sample) from the Dickens-Satterwhite subsampling mill (2) was representative of the total sample, but that further size reduction and mixing were needed to insure a representative analytical sample (ca. 50 g). It occurred to us that the system described in experiment 2 of the original study (1)—a two operation procedure consisting of a fine grind of peanuts with a disc mill (Baur) to form a paste, followed by further milling and mixing with a high speed agitator (Polytron) after formation of a liquid by addition of a high boiling point fat solvent (*n*-heptane)—could be simplified and readily applied to the 300 g subsample that is obtained from the usual 12 lb (5448 g) official sample of peanuts. It seemed likely that the two operations might be performed in one step.

EXPERIMENTAL PROCEDURES

The first attempt to accomplish simultaneous milling and mixing of peanuts and *n*-heptane employed a Waring Blendor jar equipped with a Polytron assembly (Will Scientific, Inc., cat. no. 25292) replacing the standard blades. The desired fluidity and size reduction were achieved (Table I) but the next experiment demonstrated that the standard Waring blades were equally effective. Subsequent experiments using the standard 1 qt Waring Blendor bowl were therefore aimed at determining the best means for bringing the components together, the optimum nut meat-solvent ratio, the effect of original particle size and the variety of nut meats for which the procedure could be applicable. Using the information on optimum mixing conditions derived from the foregoing experiments, two types of mixing units were examined for their effectiveness: Hobart vertical cutter-mixer and Polytron mixer in a 12 qt pail.

The effectiveness of each procedure was judged, first, on the ability to obtain a fluid system, and second, on the particle size distribution of the meal remaining after removal of the fat and solvent with a Soxhlet extractor.

Because the goal was to produce and maintain a fluid system, only addition of the solid to the liquid was examined. Various gross fractional additions of the solid to the liquid, with mixing after each addition, were employed. Because there was no observable change in the appearance of the slurry after addition of the solids to the liquid had been completed, a mixing time of 1 min after total solids addition was arbitrarily chosen. No increase in temperature of the mix over this time period was noted as determined by tactile sense.

In the first three experiments a nut meat-solvent ratio of 2:1 (w/v) was used (Table I). Since the slurry at this ratio was so thick that the blender speed constantly had to be readjusted, the slurry was thinned to a more easily handled consistency by using a 3:2 ratio. With this amount of solvent the slurry was still thick enough so that no separation occurred within the time adequate for removing a sample. The nut meat-solvent ratio of 3:2 (w/v) was used through the remainder of the experiments involving other mixing units and other nut meats. Chopped raw peanuts and whole raw peanuts were used in the first three experiments with the 2:1 ratio (w/v) of nut meat to solvent. Whole raw Brazil nuts, almonds, walnuts, pecans and cottonseed meats were tested to determine whether the procedure could be applied to these commodities using the 3:2 nut meat-solvent ratio (w/v).

To test the complete system from lot sample to analytical sample, radioactive nut meats were used as in experiments 6 to 15 of the original work (1). The use of radioactive nuts provides a known and predetermined dilution factor and a sensitive, precise measuring technique, although the radioactivity is probably evenly distributed through nuts in contrast to the very uneven distribution of aflatoxin through the contaminated nuts (3).

The first experiment of this new series with almonds was similar to the original experiment 8 with peanuts. Two radioactive shelled almonds (2.10 g) were ground with 14.0 kg of clean shelled almonds (dilution factor $1.5/10^4$) in a Dickens-Satterwhite mill. The radioactive meats were introduced individually at odd intervals during the run. The shunt sample (902 g) was ground with 600 ml of iso-octane in a 1 gal Waring Blendor. The ground meats were added in approximately three equal portions to the solvent in the Blendor bowl with thorough mixing after each addition. After the last addition, agitation at full speed was maintained for 1 min. No temperature rise of the blend was perceptible to the touch during this mixing regimen. Portions of this mix were transferred to 4½ oz plastic cups and weighed to the nearest gram. Radio activity was counted as described in the original paper (1). Because of the variable weights in the cups, each sample count was reduced to counts/min/g for the statistical interpretation. The variable geometry caused by the difference in fill was considered and was expected to contribute a negligible error.

The main body of ground almonds was divided with a

TABLE I

Particle Size Distribution of Various Nut and Seed Meats
Comminuted With Various Fat Solvents in Various Types of High Speed Mixing Devices

Nut or seed meats			Solvent		Mixer		Cumulative % through US standard mesh						
Type	Condition	Weight, g	Type	Volume ml	Type	Blade	14	20	40	60	80	100	200
Peanuts	raw, whole	---	---	---	D-S mill[a]	---	82	59	38	19	8	4	---
							(Chopped nuts used in subsequent experiments)						
Peanuts	chopped	300	*n*-heptane	150	Waring	Polytron	---	100	97	31[b]	1[b]	0.5	---
Peanuts	chopped	300	*n*-heptane	150	Waring	Regular	---	100	82	45	18	14	4
Peanuts	chopped	300	chloroform	200	Waring	Regular	---	100	74	61	56	51	41
Peanuts	chopped	2250	iso-octane	1500	12 qt pail with Polytron		---	100	55	34	2[b]	0	1
Peanuts	raw, whole	300	*n*-heptane	150	Waring	Regular	---	100	34[b]	25	0[b]	---	---
Peanuts	raw, whole	3500	*n*-heptane	1750	HVCM[c]	Scimitar	98	86	46	27	0[b]	---	---
Brazils	raw, whole	300	*n*-heptane	200	Waring	Regular	---	100	87	74	67	60	43
Almonds	raw, whole	300	*n*-heptane	200	Waring	Regular	---	100	34[b]	22	0[b]	---	---
Pecans	raw, whole	300	*n*-heptane	200	Waring	Regular	---	100	70	47	1[b]	---	---
Walnuts	raw, whole	300	*n*-heptane	200	Waring	Regular	---	100	83	62	51	48	32
Cottonseed	raw, whole	300	iso-octane	200	Waring	Regular	96	83	37	21	14	12	6

[a]Dickens-Satterwhite mill.

[b]Screen clogged by ultrafine particles.

[c]Hobart vertical cutter-mixer.

TABLE II

Degree of Homogeneity, Measured by Radioactivity, of Almond and Walnut Samples

Sample[a]	Dilution[b]	Mill	Grind	Sample cut	Sample weight, g	Number of samples	CV[c], %
Almonds	1.50	Dickens-Satterwhite	Coarse	D-S sub[d]	902	2	22.0
				Riffle	1000		
			Fine[e]	D-S sub[d]	71	7	4.4
				Riffle	58	15	4.7
Walnuts	2.14	Thomas	Coarse	Riffle	1200	2	13.0
				Riffle	1200		
			Fine[e]	Riffle	54	18	4.6
				Riffle	59	16	4.0

[a]Prepared for analysis by division of coarsely ground nut meats to ca. 1 kg units, further comminution of the 1 kg units in iso-octane (meat-solvent, 3:2, w/v) and division of the finely ground meats to ca. 50 g units.

[b]Radioactive nuts per 1×10^4 nuts by weight.

[c]Coefficient of variation of radioactivity counts.

[d]Dickens-Satterwhite mill subsample.

[e]Average sample weights.

TABLE III

Particle Size Distribution of Walnut Meats Ground Through Thomas Nut Mill and Almond Meats Ground Through Dickens-Satterwhite Mill

	Cumulative % through U.S. standard mesh					
Nut meats	8	14	20	40	60	80
Walnuts	40	24	10	3	----	---
Almonds	98	80	58	31	3	1

riffle sample splitter (A.H. Thomas cat. no. 8860) until a portion of ca. 1000 g was obtained. This portion was ground with iso-octane (w/v, 3:2) and handled in the same manner as the shunt sample.

When an attempt was made to duplicate this last experiment with walnuts, the screen of the Dickens-Satterwhite mill clogged almost immediately. On opening the mill it was seen that the nut meats had been smashed to a paste instead of the anticipated fragmentation. This result is sometimes encountered with shelled peanuts. The remainder of the sample was then comminuted with a Thomas Mills nut grinder (No. 3, Thomas Mills Mfg. Corp., Philadelphia, Pa.) with no difficulty. The particle size from this grinding operation is considerably larger than from the Dickens-Satterwhite mill when the latter mill works properly. Two radioactive shelled walnuts (2.25 g) were introduced individually at unequal intervals to the 10.5 kg batch of walnuts being ground (dilution factor $2.14/10^4$). The ground walnuts were then divided with a riffle sample splitter until two portions of approximately 1 kg (1200 g) each were obtained. Each portion was ground with iso-octane (w/v, 3:2) and analytical size samples (ca. 50 g) were prepared for radioactivity counting in the same manner as the almonds.

RESULTS AND DISCUSSION

From the size distribution data in Table I it can be concluded that very effective size reduction is achieved with peanuts, Brazil nuts, almonds, pecans and walnuts, starting with whole nut meats or partially comminuted nuts and mixing at a high shear rate with a suitable fat solvent. Cottonseed meats, however, were not as finely ground as the nut meats. The system worked approximately the same whether the mixer was a 1 qt Waring Blendor or a Polytron in a 12 qt pail, but for an unknown reason the Hobart vertical cutter-mixer was not as effective as the other pieces of equipment, even though the loading in relation to capacity was the same in all cases. A nut meat-solvent mixing ratio of 3:2 (w/v) provided an easily handled physical system with all the materials employed.

The rapid disintegration of the nut meats experienced in this sytem could not be caused by mechanical action alone. The change in structure on removal of the oil by the solvent may result in greater fragility of the meat. The effect was the same whether the solvent was *n*-heptane, iso-octane or chloroform. Theoretically, any good fat solvent should work, but from considerations of quantitation the solvent should have low volatility.

The data from the study with radioactive nuts (Table II) confirm the size reduction evidence. Both hard-meat nuts (almonds) and soft-meat nuts (walnuts) were prepared to provide analytical samples of nearly the same degree of uniformity within each of the subsamples; the pooled coefficient of variation is 4.4% (range, 4.0-4.7%). However, the subsamples were not as representative of the lot samples; the coefficients of variation were 22% for almonds and 13% for walnuts (pooled CV, 18%). These are admittedly rough figures because of the small number of samples (N = 2). Also, the more finely ground almonds (Table III) were not as uniform as the coarser walnuts beyond what would have been anticipated by the dilution factor. The samples were not mixed prior to splitting with the riffle, and this could be contributory to the lesser uniformity. However, because simplicity and speed were desirable elements in the sample preparation procedure, no attempt was made to mix the large lot samples. A practical test of three sample preparation procedures selected as effective was carried out in the previous study (1). The coefficients of variation for kilogram samples removed from lots of naturally contaminated peanuts (aflatoxin B, 25 μg/kg; total aflatoxins, 47 μg/kg) were 17, 14, and 14%, not much different from the values for the kilogram samples in this study.

Considering that the dilution factors used are smaller values than would normally be anticipated (3) and that the lot sample has a low degree of reliability of being representative of the lot (4,5), the sample preparation methods tested appear to be practical and reliable.

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