# Subsampling Theory and Comparison of US and European Preparation Methods for Aflatoxin Determination in Pistachios

**Thomas F. Schatzki** *and* **N. Toyofuku,** *USDA-ARS, Western Regional Research Center, Albany*

## INTRODUCTION

The effect of experimental parameters on the mean and variance of measurements of contaminants in subsampled granular commodities was investigated. Subsampling involves the homogenization of a generally large (10 kg) granular sample, followed by taking an aliquot thereof for actual analysis. Currently two methods of homogenization are in use, dry grinding and wet slurry grinding. This makes studies of homogenization particularly pertinent, since dry grinding is in common use in the US (a producing region), while labs in the European Union (EU – a consuming region) are using wet grinding. The problem is approached here both theoretically and experimentally, using aflatoxin determination in pistachios as an example.

## RESULTS

The theoretical approach to variance follows to clarify the large sampling variances commonly observed in tree nuts and similar commodities. These variances arise from the broad distribution of aflatoxin among individual granules (kernels). Homogenization reduces the particle size, thus greatly increasing the number of particles per unit weight, allowing one in turn to obtain aliquots with variances no larger than (or even smaller than) those obtained in the sampling step. The resulting variance is, accordingly, related to the particle size distribution as well as to the size of the aliquot.

Experimental tests are run by first dry grinding a large sample, testing a portion thereof, followed by wet grinding and retesting. A number of grinding parameters were investigated, among which were time of grinding, solvent used for wet grinding (water:methanol), grinding head used in the slurry grinder, amount of solvent and extraction fluid composition. The theory of subsampling was tested by grinding under assorted conditions and by use of the measured particle size distribution, to good effect. It was noted that the variance obtained by wet grinding was substantially totally accounted for by the variance of the final chemical analysis, while dry grinding introduced an additional variance that could not be reduced to below roughly twice the analytic variance. The variance due to sampling (the initial step in the analysis) was, as expected, much larger than either of the other two contributions.

Experiments further showed that the mean value obtained by wet grinding exceeded by 30% that obtained by dry grinding when pistachios were ground in water. Results in other commodities may differ, in some cases even reversing the trend (Spanjer et. al.). A theoretical basis for this effect could not be found, but may be associated with either of two effects that could not be controlled. One involved the difficulty of maintaining a homogeneous slurry while withdrawing a subsample, due to rapid settling of the slurry. Such settling was routinely observed, but probably was not enough to account for the 30% difference in means observed. The other might be an increased extraction of aflatoxin from a ground pistachio matrix by the presence of water in the slurry (prior to the actual aflatoxin extraction step).

## CONCLUSIONS AND PRACTICAL APPLICATION

In light of the much larger variance introduced by sampling, the subsampling variance is virtually negligible. The change in mean can be removed by calibration, or better by inter-laboratory agreement to use the same subsampling protocol, with adjustment of acceptance level, if needed. The simpler and faster equipment cleanup between samples needed in wet grinding argues for standardization on the latter method.

*Funded by the California Pistachio Commission.*