Distribution of Aflatoxin in Pistachios. 2. Distribution in Freshly Harvested Pistachios

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The aflatoxin sample probability distribution $\{P_i(n)\}$ (fraction of samples exhibiting aflatoxin concentrations in ranges of log C) for field-run and for finished pistachios was calculated for several crop years since 1980, using orchard, survey, and certification data from assorted sources. Sample size n was 100 nuts for one year, 3200 nuts for the remainder. The [single-nut] crop probability distributions $\{p_i, c_i\}$ were derived using the methods described in Schatzki (*J. Agric. Food Chem.* **1995**, *43*, 1561), where p_i is the probability of a single nut having concentration c_i. The $\{p_i, c_i\}$ distributions of field-run material were found to be consistent, but with some evidence of a decrease at the highest aflatoxin concentrations during 1981–1991. As a result, the average aflatoxin concentration in U.S. crops appears to have decreased from about 10 to 1.5 ng/g in that period. In finished pistachios the predicted $\{p_i\}$ distribution of contaminated nuts was found to be 2–4-fold lower in the crop years for which both field-run and finished data were available, suggesting that sorting for quality removes a large part of the aflatoxin present at harvest.

Keywords: *Aflatoxin; pistachios; U.S. crops; field-run; sorted; 1981–1991*

INTRODUCTION

The fungi *Aspergillus flavus* and *Aspergillus parasiticus* occur on a number of tree and ground nuts and, when present, may produce the deleterious mycotoxin aflatoxin. Aflatoxin is known to be a potent carcinogen in animals (Palmgren and Hayes, 1987). As a consequence, a number of countries have set limits on the concentration of aflatoxin that may be present in a lot offered for human consumption. Limits vary from 2 ng/g (ppb) (Germany) to 20 ng/g (United States). It is thus of interest to the producer as well as to the processor to know whether or not nuts are contaminated at harvest time and how such contamination, if any, varies from year to year. Previous work (Schatzki, 1995) had shown that aflatoxin contamination of tree nuts can be characterized in terms of a lot probability distribution $\{p_i, c_i\}$, which describes the fraction p_i of contaminated nuts that contain c_i ng of aflatoxin/g of nuts ({} stands for "the set of all ...). This fraction, in turn, may be derived from the sample probability distribution $\{P_i(n)\}$. The latter is estimated from the fraction of samples, all of n nuts each, which fall into each range [i] of the sample aflatoxin concentration C. By use of the relations given there, several sample probability distributions, each run at a different n, may be combined to yield a single lot distribution $\{p_i\}$.

A large number of samples must be measured to obtain a reasonable measure of $\{P_i(n)\}$ and $\{p_i\}$. Since pistachios are expensive and the analysis is costly, such large sets of measurements are rare. Only four studies covering more than 100 samples could be found. Wood and co-workers (Wood, 1989; Wood and Pohland, 1992) reported on surveys for aflatoxins in foods and feeds. Since these data refer, however, to single samples from lots which might not be comparable, these results were not used here. Sommer et al. (1986) and P. Bolin (DFA of California, Fresno, unpublished results, 1992) made aflatoxin measurements on samples taken from a large number of lots which could, under reasonable assumptions, be considered comparable and would be representative of an entire crop year. Bolin (1992) also made available the results of an unpublished study carried out during the mid 1980s. All of this work referred to field-run pistachios, i.e. pistachios just before harvest or after receipt at the plant but before any additional sorting. In addition, data were available for finished pistachios for two crop years, 1990–1991.

MATERIALS AND METHODS

Sommer 1981. Sommer et al. (1986) sampled pistachios in the tree at or immediately preceding harvest. "Early split" nuts were selected on the basis of a visible hull split along the suture line through which the shell and kernel could be viewed (Sommer, University of California, Davis, personal communication, 1992). Nuts with a split exposing only shell [either because the shell had not opened or because the split occurred at a nonsuture position ("growth split")] were not selected, nor were (presumably insect or bird attacked) nuts with blackened hulls, as it was thought any process sorting would remove such nuts from the final product. Selected nuts as well as unsplit green (control) nuts were cooled to about 4 °C, shelled, and sorted according to whether they were infested with navel orange worm. Nuts were combined into 50 nut samples (1980 harvest) or 100 nut samples (1981 harvest), and aflatoxin content was measured at a detection level of about 2 ng/g on the kernel material only.

To construct probability distributions $\{P_i(n)\}$ only 1981 early split data were used [Table 2 of Sommer et al. (1986)] as the 1980 data with $n = 50$ consisted of only 33 samples. Data from Kettleman City and Madera, CA, were compared, separately for insect-infested and noninfested nuts, by means of a Kolmogorov–Smirnov test (Siegel, 1956). The distributions were found to differ only at the $P = 0.20$ level ($D = 0.205$, $N_1 = 61$, $N_2 = 59$). Accordingly, Kettleman City and Madera data were combined to yield a data set consisting of 120 insect-infested and 240 insect-free samples. Control nuts (non early splits) showed substantially no aflatoxin in either year. Sample-by-sample, rather than binned, kernel-based data were obtained from Sommer (1992). These data were converted to kernel plus shell on the observation that shell and kernel weights are approximately equal and little aflatoxin is present

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Table 1. Experimental Sample Probability Distributions,[a] $\{P_i(n)\}$

C, ng/g	Sommer 1981 infested	Sommer 1981 noninfested	Sommer 1981 total	DFA 1983–1986	DFA 1990–1991 raw	DFA 1990–1991 finished
<1.0	0.342	0.788	0.636	0.914	0.918	0.955
1.0–3.2	0.217	0.158	0.178	0.012	0.014	0.017
3.2–9.9	0.175	0.017	0.069	0.018	0.026	0.013
10–31	0.125	0.013	0.050	0.022	0.030	0.007
32–99	0.008	0.004	0.006	0.012	0.011	0.006
100–316	0.042	0.000	0.017	0.016	0.001	0.002
317–999	0.008	0.017	0.017	0.004		
1000–3160	0.058	0.004	0.022	0.002		
3170–9999	0.017	0.004	0.008			
10000–31600	0.008		0.003			
N, no. of samples	120	240	360	504	703	537
n, nuts/sample	100	100	100	3200	3200	3200
$\langle C\rangle \pm$ SE			152 ± 59	9.8 ± 4.0	1.5 ± 0.3	0.92 ± 0.40
$E^2(C)/V(C)$			0.018	0.012	0.030	0.010

[a] Fraction of samples falling in C range indicated.

Table 2. Single Nut Probability Distributions,[a] $p_i \times 10^6$

c_i, ng/g	Sommer 1981 total	Sommer 1981 orchard	DFA 1983–1986	DFA 1990–1991 raw	DFA 1990–1991 finished
$<10^2$	996290	999779			
$<3.2 \times 10^3$			999973	999974	999986
1.8×10^2	1800	107			
5.6×10^2	680	41			
1.8×10^3	500	30			
5.6×10^3	60	3.6	3.8	4.4	5.3
1.8×10^4	170	10	5.6	8.1	4.1
5.6×10^4	170	10	6.9	9.4	2.2
1.8×10^5	220	13	3.8	3.4	1.9
5.6×10^5	80	4.8	5.0	0.3	0.6
1.8×10^6	30	1.8	1.2		
5.6×10^6			0.6		
$\langle c\rangle \pm$ SE	152 ± 60	9.1 ± 14.6	9.5 ± 3.9	1.5 ± 0.3	0.9 ± 0.4

[a] Probability of a nut having aflatoxin concentration c_i.

in the shells; thus, sample concentrations, C, were halved. Logarithmic bins of half-decade size were chosen on the basis of the precision with which aflatoxin was measured in this study [see Schatzki (1995) for discussion]. The fractions of samples values falling into each bin are shown in Table 1 separately for infested, insect-free, and total sample. Total sample data were converted [using the fractions as estimates of $\{P_i(n)\}$] to the single nut $\{p_i\}$ distribution, as described in Schatzki (1995), and are shown in column 2 of Table 2. (An estimated slope of $\gamma = -\mathrm{d} \log p_i/\mathrm{d} \log c_i = 0.35$ was used. Use of $\gamma = 0.8$, representative of the limited region $c_i < 10^4$ ng/g, would have increased the p_i values about 2-fold at $c_1 = 180$ ng/g and less than 30% in the rest of that range.)

Sommer et al. (1986) estimated that the nuts which were collected amounted to 2% of the hanging nuts. To test this estimate, during the 1992 harvest 5400 kg of pistachios was collected following orchard shake-down and sorted for early split, growth split, other hull damage, and blackened nuts. Early split nuts indeed accounted for 2% of the total, with the additional sorted-out nuts accounting for an additional 4%. Unpublished work from this laboratory suggests that the aflatoxin distribution in other damaged nuts is similar to that in early splits. If this is so, the Sommer distribution should be multiplied by 0.06 to yield a distribution comparable to that of field-run pistachios. The result of this multiplication is shown in the column labeled Sommer "orchard" in Table 2.

DFA of California 1983–1986. During 1983–1986 DFA, sponsored by the California Pistachio Commission, conducted a survey of aflatoxin in California pistachios. The protocol and results have been reported in memorandum form only and were obtained from P. Bolin (1992). Six or eight growers and processors, during each of the four mentioned harvest years, supplied 4.5 kg samples from up to six or eight lots at three points in the process: field green (presumably just before harvest), green delivered (start of processing), and dry (start of storage). In 1983 samples were taken after 5 months of

Table 3. Aflatoxin Positive Samples, DFA 1983–1986

harvest year	green, field	green, plant	dry, unsorted	overall
1983	6/35 = 17%	5/36 = 14%	3/28 = 11%	12%
1984	3/64 = 5%	3/59 = 5%	5/59 = 8%	6%
1985	1/35 = 3%	1/28 = 4%	1/28 = 4%	3%
1986	1/46 = 2%	3/39 = 8%	6/38 = 16%	8%
overall	6%	7%	10%	8%

storage (before sorting) as well, but these were not used in the present work. The samples were homogenized, and aflatoxin was determined on 50 g aliquots using HPLC with TFA (trifluoroacetic acid) derivatization. Resulting sensitivity was 1 ng/g. Nut size was not reported, assuming 20 nuts (shell plus kernel) per ounce (28 g) yields $n = 3200$.

The protocol was designed to track individual lots through the process to detect the effect of processing. Since the three processing steps differed little in time (samples were drawn within a day or so of each other), reasonable agreement between the aflatoxin levels for a given lot at each step might be expected. Such was not found to be the case. Of the 149 lots for which data were available at all three steps, 116 showed three negative results, 30 showed one positive result only, and 3 showed two positive results (but of widely different values). Furthermore, positives were equally distributed among the three steps. One concludes that the three measurements for each lot corresponded to a 3-fold sampling for which $P_i(n) \le 0.33$ in almost all cases. Results had been recorded in such a way that relation to actual processor could not be derived. Hence, results from all processors and all lots were lumped and the fraction of samples having detectable levels of aflatoxin recorded. These results are shown in Table 3, grouped by year and processing stage. A year-to-year variation is observed which is not monotonic, possibly due to the short time span, nor does it alternate as does the pistachio harvest size. Accordingly, data for all four years were lumped as well. Binning, as described for the Sommer data above, was then applied. The estimated $\{P_i(n)\}$ result is shown in Table 1. These data were converted to the single-nut distribution function, as above, and shown in Table 2.

DFA of California 1990–1991. Another set of results based on a quality control (certification) program DFA runs on pistachio nut samples submitted by the industry is available. Single 4.5 kg samples are used for each lot. Lots are identified as raw product (dry, unsorted) and finished product (sorted), but no other information is available on each lot. Submission is voluntary. The protocol and sensitivity are that described above for the other DFA data. Aflatoxin is reported on the basis of dry whole nut (shell and kernel). Data for the 1990 and 1991 harvests were obtained from P. Bolin. For the 1990 harvest, aflatoxin in 694 samples was determined, of which 483 were raw product and 211 were finished product. For the 1991 harvest, 220 raw and 326 finished products were tested. Since only a single sample was available from each lot and no statistics relating lots were available, it was assumed that the samples submitted (to DFA) were representative of the 1990 and 1991 production. Hence, lots were treated as random selections from the entire crop year, the raw product being comparable to field-run product. The finished product derived from lots that had been dry stored for up to 1 year or more and then sorted to remove low-quality (discolored, overly small, shell-free, etc.) nuts. Although occasional problems do occur in storage, such as moisture damage, these are relatively rare. As far as is known, none of the finished lots tested represented problem product. While sorting protocol varies somewhat from processor to processor, these differences are small. It was therefore supposed that the finished lots again represented random samples resulting from a generalized "sorting process" applied to a single crop year and could be treated to yield estimated probabilities $\{P_i(n)\}$.

Fractions of samples with detectable levels amounted to 44/483 = 9% and 14/220 = 6% for raw product and 11/326 = 3% and 13/211 = 6% for finished product in 1990 and 1991, respectively. As in the DFA 1983–1986 data, a significant ($P < 0.0001$) variation is noted between years but, again, the time period is short. Accordingly, 1990 and 1991 data were lumped to yield the $\{P_i(n)\}$ distributions given in Table 1. Calculated p_i results are shown in Table 2.

Expected Values. The expected values of the sample concentration C may be estimated as $\langle C\rangle = E(C) = \sum_i P_i(n) * C_i$, $E(C^2) = \sum_i P_i * C_i^2$, $V(C) = E(C^2) - E^2(C)$, and standard error of $\langle C\rangle = (V(C)/N)^{0.5}$, where $P_i(n)$ is the fraction of samples in bin i, C_i is the midpoint thereof, i.e. $C_i = (C_i^+ * C_i^-)^{0.5}$, with C_0 taken as zero, and N is the number of samples. $\langle C\rangle$ and $E^2(C)/V(C)$, used below, are listed as the last rows of Table 1. Sommer "orchard" data are not included in Table 1. Corresponding values can be obtained from the "total" data by using $0.06 * P_{i\neq 0}(n)$ to yield $\langle C\rangle = 9.1 \pm 14.5$ ng/g.

In Schatzki (1995) it was shown that the corresponding values for the lot concentration c are given by $\langle c\rangle = E(c) = \sum_i p_i c_i$, $V(c) = \sum_i p_i c_i^2/n$. Lot average $\langle c\rangle$ values are listed in Table 2. The $\langle c\rangle$ values corresponding to nonprocessed pistachios (1981 orchard, DFA 1983–1986, and DFA 1990–1991, raw) were fitted to the average crop year by weighted linear least squares, yielding a slope of -1.254 ng g^{-1} year^{-1}, $P = 0.097$.

RESULTS AND CONCLUSIONS

The lot averaged $\langle c\rangle$ values, given in Table 2, closely match the sample averages $\langle C\rangle$, given in Table 1 or computed above, as they should. The large standard error of the orchard result arises from smallness of sample. Only a total of 360 * 100 = 36000 nuts were run, while p_i amounts to but a few times 10^{-5}, even when all of the values at high c_i, which dominate $\sum_i p_i * c_i^2$, were combined. Thus, $N * np_i < 1$ and there is not enough material to get a representative lot average. In Schatzki (1995) it was pointed out that if the fraction of uncontaminated samples, $P_0(n)$, is less than $\exp(-E^2(C)/V(C)) \approx 1 - E^2(C)/V(C)$, a distributive contamination (i.e. at least two p_i) is called for. An estimate of $P_0(n)$ is given on the first line of Table 1. A comparison of this line with the last line of Table 1 indicates the inequality to be true for all four distributions and particularly for the Sommer one.

The derived $\{p_i\}$ distributions are plotted to a common scale in Figure 1. (Points for which $p_i = 0$ are not shown.) Note that no adjustable parameter was used to derive these plots, yet comparable results are obtained despite the different provenances, the drastically diverse sample sizes, $n = 100$ and $n = 3200$, and the use of the 6% estimate for damaged nuts (the former resulting in a log 32 shift in the ordinate, the latter a log 0.06 shift in the abscissa). No such agreement is obtained between columns of sample fractions listed in Table 1. In light of the agreement shown in Figure 1,

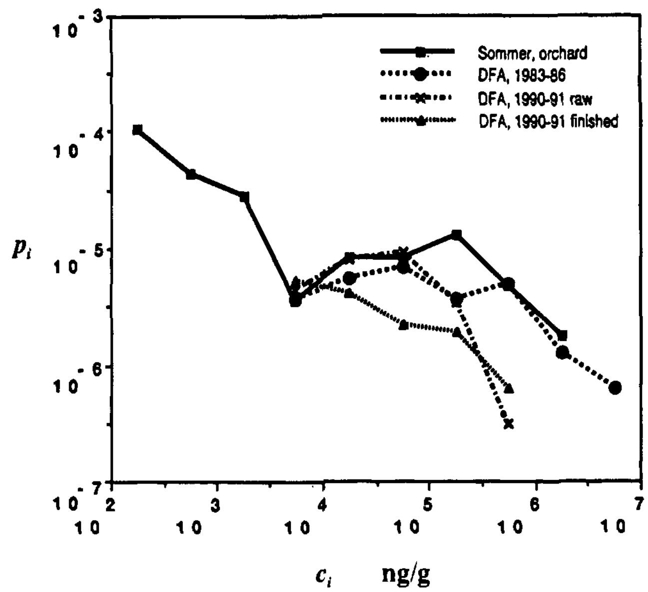

Figure 1. Probability of a single nut having aflatoxin concentration c_i.

it is concluded that these curves truly represent the U.S. pistachio raw product during the 1980s and early 1990s.

All of the p_i drop to zero within a decade of 10^6 ng/g. This is in agreement with extensive published data on selected single ($n = 1$) tree and ground nuts, many of which report aflatoxin in the range of a few times 10^5 but rarely above 10^6 ng/g. The highest value reported in a single nut appears to be 4×10^6 ng/g in parts of a peanut kernel (Cucullu et al., 1966). The lower reported limit of the determined distributions depends on the sample size used, as all of the studies had detection limits of about 1 ng/g. Whether the distributions truly have a lower limit or whether they extend to infinitesimal concentrations cannot be established from the data at hand. Whether the drop off in the distribution around 10^4 ng/g is real remains to be seen. Such curves as are shown in Figure 1 should eventually lead to a model of aflatoxin production in the field.

The roughly 2–4-fold dropoff between the 1990–1991 raw and finished data must be assigned to the sorting process. In light of the fact that only about 25% of total product is removed to convert field-run pistachios to high-quality finished product, the removed material (most of which is sold at a lower price, but for human consumption) must have considerable aflatoxin content. These results also indicate the danger of blending out-sorted product back into high-quality streams in terms of the final aflatoxin content. Studies of the distribution in these process streams are now in progress in our laboratory.

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