Machine Vision System for Automated Detection of Aflatoxin-Contaminated Pistachios

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The previously reported [Pearson, T. *Lebensm. Wiss. -Technol.* **1996**, *28* (6), 203−209] channel image sorter has been used to re-sort color-sort and hand-sort rejects and to sort mainstream (pre-hand-sort) U.S. pistachios. Sorting was carried out at commercial speeds of up to 163 kg/channel/h. Recoveries of good nuts of 39−67% on re-sorted product and 97.8% on mainstream nuts were achieved. Aflatoxin levels were reduced from 8.6−4.8 to 0.04−2.5 ng/g on color-sort rejects and to 15 from 22 ng/g on hand-sort rejects. For mainstream product, aflatoxin levels were reduced from 0.12 to 0 ng/g by image sorting, compared to reduction to 0.04 ng/g by hand sorting. Quality for mainstream sorting improved significantly, particularly for other damage, serious insect damage, gross defects, and loose kernels. Re-sort quality improved as well, but recovered product will still require dyeing. The sorter should find application in product recovery, in preparation of product for very stringent markets [Schatzki, T. F. *J. Agric. Food Chem.* **1998**, *46* (1), 2−4], and in presorting the very large samples required for testing lots with high reliability, that is, low standard error of the mean [Schatzki, T. F. *J. Agric. Food Chem.* **1998**, *46* (1), 2−4].

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INTRODUCTION

In previous publications (Pearson, 1996, 1997) one of the authors described a visible image based sorting machine that was designed to sort pistachios. The basis of the sort was the machine recognition of a dark stain present at the lips of the shell split of a dehulled nut (Figure 1). The classification rule differed from the usual color sorter in that a particular image pattern was sought rather than an overall color, which would be influenced by the exposed pistachio kernel visible through the shell split. The motivation for designing this particular recognition algorithm had been that it had previously been noted that the dark lip stain mentioned above had been found to be present on pistachios which had been subject to early hull splitting in the orchard (Pearson et al., 1994). Sommer et al. (1986) had noted earlier that aflatoxin in pistachios occurred only in early-split nuts. It thus followed that Pearson's sorting algorithm should be able to selectively remove nuts infected with preharvest aflatoxin from any U.S. pistachio process stream. This point remained to be proven, however. It was the purpose of the present work to test this question.

MATERIALS AND METHODS

Input to Sorter. Input nuts for all sorting tests consisted of bins drawn from storage of commercial processors, with no special selection for these tests. Except as indicated otherwise, all input streams consisted of sinkers. Five sorting tests were run, which are hereafter indicated by 1−5. Test 1 was carried out at processor A, using 1995 crop product, tests 2−5 were performed at processor B with 1996 crop. The processors correspond to similarly named processors in Schatzki and Pan (1996), which should be consulted for details of the commercial sorting process. Nuts from processor A were unsized; those at processor B consisted in all cases of 21/25s, that is, 21−25 nuts/ounce or 740−881 nuts/kg. In what follows, "accepts" refer to the good product and "rejects" to the bad product, although the sorter may have been operating in reverse mode, redirecting the good nuts (as indicated below). In all cases the smaller fraction was rejected by the sorter. The material flow at processor B is given in Figure 2. Flow at processor A was similar, except that sizing followed hand sorting. The input to the five sorts was as follows (sort 1 at processor A, sorts 2−5 at processor B):

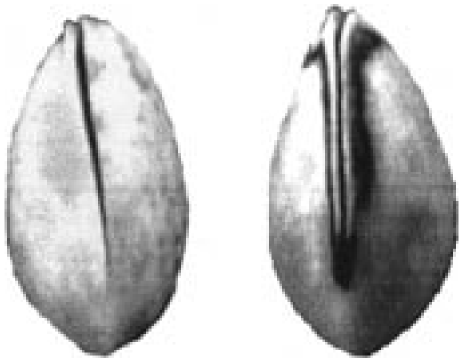

Figure 1. Normal pistachio (left) and lip-stained pistachio (right) after hulling split staining.

(1) Mixed Sinkers and Floaters. These nuts had been rejected by automated color sorters during routine processing. A total of 761 kg, contained in two bins, was used. Bin 1, containing 426 kg, had been rejected on the basis of a single color sorter pass; bin 2 resulted from rejection in each of three sequential color passes. Nuts in bin 1 had a slightly better appearance by visual inspection.

(2) Color Sort Rejects (Second Pass).

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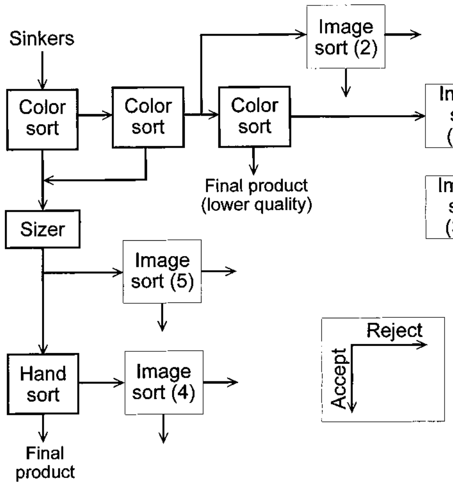

Figure 2. Image sorter tests in a commercial sorter stream, processor B.

(3) Color Sort Rejects (Third Pass). The accepts from the image sort were re-sorted by the image sorter on a second pass.

(4) Hand Pick Out (Hand Sorting Rejects).

(5) Output of the Sizer. The nuts in this sort consist largely of first color sort accepts, plus some accepts of the second color sort. This comprises the main product stream and, except for the hand-sorting operation, consists of the prime product being offered for human consumption.

Sorter. The image sorter used has been described previously (Pearson, 1996, 1997). Sorting discrimination was developed using the SAS procedure DISCRIM (SAS, 1988), following Pearson (1996). A training set consisting of 360 stained (by vision) and 380 unstained nuts (color sorter rejects from processor B) was found to have substantially similar covariances of the two populations in terms of the three image parameters MS, LFR, and HSR. These parameters are described in detail by Pearson (1996). Briefly, MS is the number of pixels with a moderate slope across the nut, LFR is the number of pixels with a low slope and low intensity, and HSR is the number of regions that have high slope and high intensity, where low, moderate, and high are specified in Pearson. Assuming normal distribution, pooled covariances resulted thus in a linear discriminant function. The centers of the two populations were found to be

$$C_{\mathrm{stained}} = -6.05894 + 0.02143 \times \mathrm{MS} + 0.00857 \times \mathrm{LFR} + 0.27073 \times \mathrm{HSR}$$

$$C_{\mathrm{unstained}} = -7.96656 + 0.01369 \times \mathrm{MS} + 0.02102 \times \mathrm{LFR} + 0.06399 \times \mathrm{HSR}$$

The centers were used for all five sorting discriminants as attempts to use other discriminants were found not to be successful. To adjust the fraction accepted, the "threshold" parameter was adjusted for different sorts (SAS, 1988). This adjustment was based on the visual appearance of the sorted nuts, essentially by ensuring that the bad nuts contained all or most of the nuts with adhering hull and very dark nuts. Presumably, adjustment of the threshold would result in different good/bad nut ratios, although the point was not explicitely tested. For sort 5 of the main process stream, it was found that too many false rejects were triggered by dust (skin, shell particles, etc.). An airstream was installed above the rollers to remove most of this dust, but this did not solve the problem completely. Accordingly, the discriminant function was modified by adding the requirement that nuts would be rejected only if $\mathrm{HSR} > 0$, $\mathrm{MS} > 20$, $\mathrm{LFR} < 400$, and $\mathrm{MS} > 0.09 \times \mathrm{LFR}$, leading to a piecewise linear discriminant function. Later versions of the software, not used here, allow discriminant functions to be established automatically by feeding the sorter a set of training nuts. This will allow for changes dictated by nut size and other parameters that might change in commercial operation.

A constant feed tray was used to feed the sorter, which was supplied by bucket and shovel loading. All other material flow was handled in the same way. Large debris (twigs, etc.) was removed manually. An overall feed rate of 95−163 kg/channel/h was achieved, and it is believed that a feed rate of 180 kg/channel/h or better could be achieved with automatic feeding devices provided only that the reject rate does not exceed 45 kg/channel/h, as beyond this the pneumatic ejector valve becomes limiting. In the list below, the amounts sorted are indicated according to recovered material. Up to 5% of input was lost due to material handling.

Aflatoxin Analysis. In all cases both the accept stream and the reject stream were analyzed separately except for the case of multiple passes (double-sort), for which the aflatoxin concentration of the material used in re-sorting was computed from a material balance. Samples were analyzed following the protocol described previously (Schatzki and Pan, 1996). For sort 1 500 g samples were used, which were ground until kernels passed a No. 10 screen (10 wires/2.54 cm). (Shells were included, but were not required to pass the screen.) One hundred samples were analyzed from the accept stream and 160 from the reject stream (in both cases material sorted from bins 1 and 2 was blended before any analysis was performed). The purpose of the large number of samples was to develop an aflatoxin distribution function [see Schatzki (1998)]. For sorts 2−5 eight 16 kg samples were taken (unless sample shortage forced smaller and/or fewer samples, see next section) from each stream, 8 kg subsamples were ground with 50% dry ice in a Hobart VCM40 vertical cutter mixer (duration 3 min), and the two subsamples were blended, using a V-mixer (duration 1 min). Three solid aliquots were then taken from each sample and analyzed separately. Further analysis followed the Schatzki and Pan (1996) protocol, which includes affinity column cleanup and solvent change and reduction, precolumn derivatization with trifluoroacetic acid, and reverse column HPLC with fluorescence detection. Results are reported on the basis of total extraction fluid, as was done there. The more usual method of reporting aflatoxin on the basis of recovered fluid yields values ≈75% of those reported here but does not take into account fluid left in the cake after centrifugation. Aflatoxin is reported as $B_1 + G_1$, of which B_1 accounted for ≈90%. The means and standard errors of the mean reported below are based on sample mean averages.

Quality Check. One bin (862 kg) run through sorting process 5 was tested for quality following the USDA procedure (Code of Federal Regulations) by manual inspection of three sets of 500 nuts each sampled from the input and the accept and reject streams. The reject stream was re-sorted, and the accept and reject streams from this sort were inspected as well. This inspection was carried out by processor B staff. The presence of insect and feeding damage was verified by X-raying 500 nut samples of these streams. No aflatoxin analysis was done on any of this material.

RESULTS AND DISCUSSION

Aflatoxin Levels. The aflatoxin levels resulting from the image sorting are given in Table 1, which includes the pertinent sorting parameters, the variables of the analysis, and the aflatoxin results. The standard deviation (SD) shown is the experimental one, based on the sample results.

In sample preparation for analysis, the ground samples were not sifted and reground as needed, as should have been done, except for those of test 1 and some of test 5. As a result, the variability between solid aliquots within a sample (not shown) and the variability between samples (indicated by the SD) are, in some cases, larger than they ought to be. [A derivation in Schatzki (1998) shows that the SD should be given approximately by

Table 1. Aflatoxin Results following Image Sorting

	test 1	test 2	test 3, pass 1	test 3, pass 2	test 4	test 5
input stream						
provenance (processor, stream)	A, color rejects	B, color rejects of 1 pass	B, color rejects of 2 passes	test 3, pass 1 accepts	B, HPO rejects	B, sizer accepts
ng/g, calcd	8.6	3.4	4.8	2.45	21.7	0.12
sorting mode	normal	normal	reverse	normal	normal	normal
threshold, %	20, 30		10	33		40, 45[a]
accept stream[b]						
wt, kg	404	68	326	202	445	4540
ng/g	0.04 ± 0.01	1.05 ± 1.8	2.45[c]	2.5 ± 1.5	15.5 ± 20.4	0
no. of samples	100	8		9	8	8
total no. of nuts tested	50000	106000		119250	106000	106000
reject stream[d]						
wt, kg	333	28	507	127	243	103
ng/g	19.0 ± 5.2	9.0 ± 20.0	6.3 ± 7.6	2.4 ± 2.3	33.0 ± 53.1	5.2 ± 8.7
no. of samples	160	3	4	8	8	14
total no. of nuts tested	80000	39750	53000	106000	106000	90200

[a] Piecewise linear discriminant. [b] Good nuts. [c] Calculated. [d] Bad nuts.

sqrt(250 000 × sample mean/total number of nuts tested).] In effect, in those cases when large kernel particles remained in the grind the total number of nuts tested is smaller than that shown. Nevertheless, the means are not affected.

Turning now to the individual results, test 1, which tested the backsorting of color rejects at processor A, shows unequivocally that the image sorter sorts for aflatoxin. The aflatoxin means of the accept and reject streams differ by 3.65 times the larger SD, indicating that the hypothesis that they are the same is rejected with $p = 0.0001$. Note that the sort generates two almost equal size streams, so that 55% of the product was recovered. The recovery rate of the singly rejected input bin was 58% and that of the triply rejected bin was 51%, suggesting that little is gained with respect to aflatoxin levels from multiple color sorting. Visual inspection of the good product indicates no visible defects, but the shells exhibit a yellowish or “golden” appearance, characteristic of “light stain” and thus are a lower value product than the white shelled nuts discussed below in test 5. Discussions with the processor indicated that the good sorted product would need to be dyed before sale. Of course, this product is fully wholesome, as compared to the reject stream, which would just pass the FDA action level of 20 ng/g total aflatoxin (20/75% or 26.7 ng/g on the basis used here). The calculated aflatoxin level of the input stream amounts to 8.6 ng/g, which is comparable to the 1 (sinkers), 7.8 (floaters) ng/g, from the same processor in the 1992 crop year (Schatzki and Pan, 1996).

Test 2 used an input stream rather similar to that of test 1, double rejects from a color sorter, albeit from another processor. The difference in input aflatoxin level from that of test 1 presumably arose from different color-sorting parameters used by the two processors or possibly different farm lots or crop years. Here, again, the accept and reject streams differ by 4.4 SD, accepting the lower SD as more representative [the upper, 20 ng/g, is more than twice the theoretical value of 7.5 ng/g], showing clear aflatoxin separation. Again, comparable amounts of accept and reject are obtained (68 and 28 kg).

Test 3 took as input nuts that had been thrice rejected by a color sort. The rather similar aflatoxin level to that of a double rejection, 4.8 versus 3.4, again suggests that little is gained in this respect by multiple color sorting. The subsequent image sort does generate lower aflatoxin levels for the accepts (the accept aflatoxin is calculated from the subsequent sort), although now accept and reject streams do not differ even at the $p = 0.05$ level. The experimental SD is close to the theoretical one of 5.5 ng/g. At roughly 1 SD the hypothesis that aflatoxin difference is obtained is only rejected at $p = 0.16$.

When the accepts of this sort are re-sorted, as shown in column 3, pass 2, nothing is accomplished as far as aflatoxin elimination is concerned. Thus, multiple image sorting appears to act like multiple color sorting: whatever is accomplished is done on the first pass.

The last backsort attempt is illustrated by test 4, which sorts rejects generated by manual pick outs of product that has already been accepted by the color sorter. Schatzki and Pan noted an extreme range of aflatoxin levels in such pick outs (1−24 ng/g), depending which process stream was considered. The calculated 21.7 ng/g seen here certainly falls within that range. (Since this material was destined for human consumption as kernels, a direct aflatoxin determination is certainly called for.) The levels resulting from image resorting (15.5 and 33.3 ng/g) do show higher levels in the rejects, but not significantly so. Both are, of course, marginal or worse for human consumption. Interestingly enough, the rejects at 33.3 ng/g look better than the rejects from 3, pass 2, at 6.3 ng/g, which simply illustrates the fact that aflatoxin levels are set by a very few, highly infected nuts, which cannot be identified visually on the basis of present knowledge.

Finally, consider test 5. This test is very different from the other four in that it is not a re-sort but the image sort of the main process stream (only the hand-sorting operation, plus possible roasting and salting, remains before release for final sale). This product is expected to be quite clean and, indeed, a material balance calculation showed that the input stream contained but 0.12 ng/g aflatoxin, less than the 0.59 ng/g found for this processor for the fully sorted 1993 crop. Accordingly, a very large amount of material needed to be sorted to obtain an appreciable reject stream. Six 1 ton bins were sorted, and aflatoxin was measured on samples taken from the first two bins jointly and the next three separately. The sixth ton was used for quality checks only (see below). Table 1 shows only the weighted average of the five tons. For the discriminant function as shown in Table 1 and discussed above, and an overall sorting rate of 163 kg/channel/h, the reject rate was quite constant at 2.25 ± 0.19%. Not 1 of the 24 subsamples (8 samples, 3 aliquots each) of the accepts showed any aflatoxin (the detection limits in these analyses is ≈0.03 ng/g), whereas the rejects tested

Table 2. Quality Results following Image Sorting, Percent Defective

	1	2	3	4	5
provenance (processor B)	sizer accepts	accepts from sorting col 1	rejects from sorting col 1	accepts from sorting col 3	rejects from sorting col 3
wt, kg	909	841		14	6
light stain	11.8 ± 1.8	9.6 ± 2.3	13.9	13.6	14.8
dark stain	0.7 ± 0.6	0.7 ± 0.5	13.5	6.5	30.0
clean split[c]	82.8 ± 2.4	85.1 ± 2.0	55.2	70.0	20.0
nonsplits	1.6 ± 0.8	2.6 ± 0.7	0.9	1.0	0.7
split, not on suture	0.2 ± 0.2	0.2 ± 0.3	0.3	0.3	0.4
adhering hull	0.3 ± 0.3	0.3 ± 0.4	2.9	1.2	8.9
other damage	0.5 ± 0.4	0.1 ± 0.2	0.9	1.1	0.4
serious insect	1.1 ± 0.2	0.4 ± 0.4	2.8	2.8	3.0
gross defect	0.4 ± 0.2	0	1.6	1.2	2.5
loose meats	0.4 ± 0.0	0.1 ± 0.2	6.4	1.6	17.7
foreign matter	0	0	0	0	0
loose shell	0.3 ± 0.3	0.3 ± 0.3	0.6	0.6	0.6
DBOM[d]	0	0	0	0	1.1
insect and feeding damage (X-ray)		0.3		0.9	5.8

[a] Computed. [b] Missing weight lost to spillage. [c] Nondefect. [d] Damaged by other means.

at 5.2 ± 8.7 ng/g. The SD is more than twice the theoretical 3.8 ng/g, which is surprising since three of the five tons had been sifted through a No. 10 sieve. However, the 2 ton sublot tested at 1.5 (five samples) and the 1 ton sublots at 0.0, 9.6, and 12.0 ng/g (three samples each), which suggests that the bins might not have been comparable. In any event, the 2.2% reject product showed significant aflatoxin, while the clean product showed none. Whether the absence of aflatoxin in a 106 000 nut (131 kg) test is adequate to prove the absence of such infected nuts in the entire accept stream is open to debate; clearly a test of 10 times such sample size would be more preferable. The point is of some importance for using the sorter for testing and will be discussed further below.

We were informed by processor B that hand-sorting removes 0.3−0.4% of product. At the 0.12 ng/g of input to hand-sorting, computed here, and 21.7 ng/g of pick outs, one computes that hand-sorting reduces aflatoxin levels only to 0.04 ng/g. Although the exact value is very much subject to the extensive material balance calculations used, and may be higher, it is clear from the present test that the image sorter is capable of reducing aflatoxin by at least 0.1 ng/g in streams which are already quite clean. Furthermore, it is noted that eight samples were taken from the image sorter accept stream, each of which showed no measurable aflatoxin. Using a detection level of 0.03 ng/g (Schatzki and Pan, 1996), one would be 95% confident (binomial, $n = 8$) that the accept stream contains $<0.31 \times 0.03$ ng/g ≈ 0.01 ng/g. It would thus appear that image sorting alone can reduce the aflatoxin level to a value lower than that achieved by hand-sorting.

Quality Improvement. Quality inspection for 500 nut lots of an image sort of color sorter accepts is shown in Table 2. The list of defects includes those commonly checked for manually before sale. Column “1” shows the percentage of nuts having the defect in the input stream, which is the same as that of test 5 above. The SD shown arises from repeating the test on three samples. Column “2” shows this percentage for the accepts, column “3” for the rejects, which were re-sorted once again to see whether re-sorting by the image sorter might improve quality. There exists no theory about the SD here. Comparison of columns 2 and 3 shows that defects in the reject stream are higher in virtually all cases except for nonsplit nuts (the image sorter is looking for the darkened region of the visible kernel, which is missing in these nuts). However, the amount sorted out is so small that only for serious insect damage, gross defects (generally evidence of insect feeding), possibly other damage, and the amount of loose kernels is a significant difference noted between input and accepts. It is rather interesting that a second image sort does make some difference in the quality of the product, as it does for color sorting. This is in distinction to aflatoxin reduction, noted above.

One further test of quality was made in case of the processor A color sort rejects (test 1) but not shown in Table 2. In this case the image reject population contained smaller nuts than the accept population. On the basis of two randomly selected samples consisting of 74 nuts from the reject and accept group, respectively, it was found that the nut weight of rejects extended (with one exception) from 0.7 to 1.8 g/nut, with an average of 1.18 ± 0.26 g/nut, whereas accepts ran from 0.9 to 1.9 g/nut, with an average of 1.42 ± 0.22 g/nut.

CONCLUSIONS

Image sorting, using the sorter described previously (Pearson, 1996, 1997) can be used to reduce aflatoxin levels in U.S. pistachios (the situation in pistachios that may have postharvest aflatoxin, not subject to the staining patterns used here, is not addressed). Much like with color sorters, multiple sorting by the same sorter does not improve aflatoxin levels significantly, but the use of both sorters in tandem does do so. Multiple sorting does improve quality in image sorting, much as it does in color sorting. Backsorting reject product does produce significantly lower aflatoxin and even achieves zero levels in favorable cases, but the clean product still suffers from appearance problems that prevent achievement of top quality. Sorting a mainstream product following color sorting appears to reduce aflatoxin to nondetectable levels, or at least reduces aflatoxin by 0.1 ng/g. Such sorting also improves quality levels significantly, which should simplify hand-sorting following this step.

In another publication (Schatzki, 1998) it was suggested that the image sorter might be used to reduce a large sample to manageable size. Such large samples are required to reduce the standard error of the sample mean to the point where a test is representative of the lot mean when testing pistachios. If a sample of k kg is taken and reduced to a dirty (reject) portion of $0.022k$ kg by image sorting, and if the clean portion is truly at 0 ng/g, then the aflatoxin level of the k kg input sample is $0.022t$ ng/g, where t is the test result of the $0.022k$ reject portion. It was seen above that the $0.978k$ (accept) portion might indeed be aflatoxin-free. However, suppose it is not, but actually contains a ng/g aflatoxin. Then, from a material balance, one has that the input contained $0.978a + 0.022t$ ng/g aflatoxin, whereas $0.022t$ was reported. Thus, as long as $0.978a \approx a$ is of order $0.022t$ or less, the reported value will not be much smaller than the true one. In the case the test is carried out to ensure that the lot is below, say, 4 ng/g, all that is required is that the accept portion is well below 4 ng/g. From the results shown here, that is very likely. One concludes that the use of this sorter to reduce test sample size is well justified.

Finally, it should be pointed out that the aflatoxin separation achieved here may be a function of the distribution of aflatoxin in the unsorted lot and the sorting parameter used, particularly the threshold value. An excellent separation was achieved in test 1 while achieving 55% recovery. Adjustment of the threshold will affect the level of aflatoxin in the accept product and the amount recovered as well. The excellent separation in test 1 must be a function of the distribution in that input stream. Tests of this concept were not carried out here. The observations noted here are fully consistent with the hypothesis that image sorting selects all of the early splits but that these are diluted (perhaps to 50%) with other nuts also rejected.

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