Effect of manual sorting on Aflatoxins content in peanuts (*Arachis Hypogaea*, L.) from a Ghanaian market

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ABSTRACT

Aflatoxins have been of major public health concern ever since they were discovered.
A simple physical manual sorting procedure and blanching to facilitate the elimination of aflatoxins in raw peanuts was designed, conducted and verified using workshop participants. Two processors were then trained on the technology. Six streams of kernels namely, raw unsorted kernels, pre-sorted kernels (immature and shrivelled kernels), three levels of bad discoloured kernels (≤10% discoloured kernels, ≤50% discoloured kernels and >50% discoloured kernels) and good kernels were obtained during the verification exercise. Analyses carried out on these samples using High Performance Liquid Chromatography (HPLC) gave total aflatoxin levels ranging from none detected to 60.42 µg/kg for good kernels and very, very bad kernels, respectively. Total aflatoxin content of the testa recorded 5.34 µg/kg. During the training session for the two processors, shrivelled and immature kernels were found to be the most susceptible to aflatoxin contamination. Thorough manual sorting of blanched kernels, offers a practical possibility in reducing significantly, aflatoxin levels to below regulatory limits.

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Introduction

Peanut (*Arachis hypogaea, L.*) is grown and consumed worldwide. It is composed of 50% fat, 30% protein and gives 585 Kcal/100g of energy on consumption (USDA, 1999). Peanuts have high satiety effect which is enhanced by their rich source of fibre and protein (Burton-Freeman 2000; Holt *et al* 1995). In recent years, nuts have received considerable attention as one of the foods that have beneficial effects for cardiovascular health. Studies have confirmed that consuming nuts as a snack food at least five times per week may lower the risk of cardiovascular disease, type 2 diabetes and gall bladder disease (Tsai *et al.,* 2004; Jiang *et al* 2002; Hu *et al*., 1998). Peanuts are among the nuts to which epidemiological data have linked such benefits (Kris-Etherton *et al.,* 1999; Hu *et al.,* 1998; Prineas *et al.,* 1993). The high satiety values of peanuts evoke a strong compensatory dietary response in the form of reduced energy intake that offsets two-thirds of the energy by nuts (Mattes *et al.,* 2005; Alper & Mattes, 2002).

In Ghana, peanuts are cultivated nationwide and was ranked 8th in 2007 in the country's commodities production with a volume of 440,000 metric tonnes (FAOSTATS, 2007). Such high production goes into many end uses including ingredient for various snacks as well as main dishes. However, the consumption of peanut products can be unsafe to the consumer because of the issue of mycotoxins particularly aflatoxins.

Aflatoxins are secondary metabolites produced by *Aspergillus flavus* Link: Fries, and *A. parasiticus* Speare that occur in many

commodities used for human food and animal feed. These compounds have a high acute toxicity, as well as immunosuppressive, mutagenic, teratogenic, estrogenic and carcinogenic activities (Klich *et al.,* 2009) and are classified as group 1 carcinogens by the International Agency for Research on Cancer (IARC) (Peraica *et al.,* 1999). Aflatoxins occur more during postharvest than during pre-harvest conditions (Wild & Hall, 2000). These toxins are known to increase in food during storage (Kaaya & Kyamuhangire, 2006). High temperatures, high humidity, as well as insect and rodent damage result in accumulation of these toxins (Hell & Mutegi, 2011). Nearly 80% of aflatoxin contamination can be attributed to small, shriveled seeds (Davidson *et al.,* 1982), mouldy and stained seeds (Fandohan *et al*., 2005; Tuner *et al*., 2005; Awuah & Kpodo, 1996; Park, 2002). Sorting can be done using physical characteristics like colour, size, and density (De Mello & Scussel, 2009). In addition, kernels that float in water have been found to contain up to 95% aflatoxin (Philips *et al.,* 1994; Kirskey *et al.,* 1989).

Food safety in Africa and indeed the world over in relation to mycotoxins is a broad and diversified subject matter to deal with. Conditions in countries vary widely, in their geographical location, climate, soil and cultural habits. As a result, different tolerance levels have been set by individual countries to regulate foods contaminated with mycotoxins. The objective of this study was to investigate the influence of manual sorting on aflatoxin levels in peanuts sold on the Ghanaian markets.

This is important in view of reservations about cost and safety concerns for current chemical detoxification methods (alkaline treatment, acid treatment, ozone treatment and ammonia treatment) as well as irradiation methods. In ionizing radiation (eg. X'rays, gamma rays, and ultraviolet rays) potential changes may occur in molecules of the irradiated food. These changes may be quite harmful to living organisms exposed to large doses of the ionizing radiation (Needhidasan & Melvin Samuel, 2013). Thermal processing has been reported to reduce aflatoxins in peanut meal (Coomes *et al.,* 1966). However, this seems to be insufficient because aflatoxins are heat resistant within the temperature range of food processing (80-121^{0}C).

Materials and methods

Peanut kernels used in this study were purchased from the Nima market in the Greater Accra Region of Ghana. The kernels were purchased in 50 kg batches with each batch made up of 5.0 kg samples from 10 sellers bulked together.

A systematic sorting process that could lead to a total elimination of aflatoxins in samples of peanuts was developed, and the aflatoxin content of each sorted fraction determined using 4.0 kg of kernels sampled from a batch of 50 kg of the raw peanuts. The process involved a pre-sorting exercise involving sieving of the kernels to sort out immature and shrivelled kernels and damaged kernels from the raw unsorted kernels (RUP). This was followed by blanching and dehulling of the pre-sorted kernels, and further sorting of the dehulled kernels to obtain fractions of good clean kernels and discoloured kernels (Fig. 1). The discoloured kernels were further categorized into kernels with less than 50% discolouration and those with more than 50% discolouration (Figs. 1, 2, 3, and 4).

Blanching involved preheating an oven (Wagnet Oven, Model: GP/50/55/250/DIG, Leader Engineering, St. Helens Merseyside, WA9 5GZ, England) for 15 min to a temperature of 140^{0}C. The peanut kernels were then spread in two aluminium pans and placed in the oven for 30 min. The kernels were allowed to cool under a ceiling fan after which the reddish seed coats (testa) were manually removed by rubbing and winnowing. Good sorting primarily targets good kernels, and this process involves the removal of discoloured and damaged kernels as well as shrivelled and immature kernels. The sorting exercise resulted in six main streams of fractions that were subjected to aflatoxins analysis. These

include raw unsorted kernels, shrivelled and immature kernels, testa, discoloured kernels and good or unstained kernels (Fig. 1).

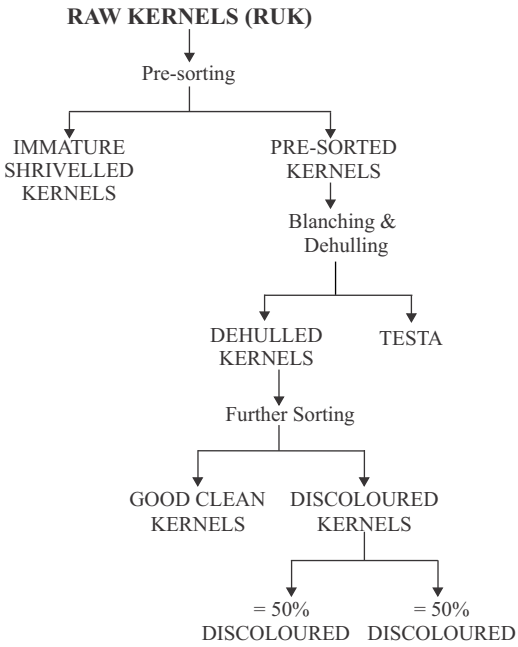

Fig. 1. The Sorting Process Chart

Fig. 2. Raw unsorted peanuts

Fig. 3. Removal of testa

Fig. 4. Sorted clean kernels

Training of selected stakeholders

Training sessions were held at the CSIR-Food Research Institute, Accra, Ghana, to train participants on the sorting procedure and consequent reduction of aflatoxins in market samples of peanuts. Two groups, made up of 10 participants each at the CSIR-Food Research Institute participated in the first training. The sorting procedure was carried out and the separate fractions obtained from each group analysed for aflatoxins and the effectiveness of the process evaluated. The training was similarly carried out for two other processors, one from Accra and the other from Tema, both in the Greater Accra Region of Ghana. The processor from Accra (Processor 1) was a student of the University of Ghana involved in a research to produce canned aflatoxin-free peanut soup base for local consumption and export. The processor from Tema (Processor 2) on the other hand was a local entrepreneur working with researchers at the CSIR-Food Research Institute on the preparation and production of ready-to-eat aflatoxin-free peanut chocho spread. The sorted fractions from the processors were also analysed for their aflatoxins content to assess the effectiveness of their operation.

Aflatoxin Analysis

The method described by Stroka and Anklam (1997) which is based on the standard method (JAOAC, 1991) was used in the determination of Aflatoxins in each of the sorted out peanut kernel fraction. Test portions were extracted with a solvent solution (methanol/ water) plus hexane. The sample extract was filtered, 10 ml of filtrate diluted with Phosphate Buffered Saline Solution (PBS) to a specified solvent concentration, and applied to immu-noaffinity column (R-Biopharm RHONE LTD EASI-EXTRACT AFLATOXIN) containing antibo-dies specific to aflatoxins B_1, B_2, G_1, and G_2. Aflatoxins were removed from the immun-oaffinity columns with neat methanol. The aflatoxins were then quantified by reverse-phase

high performance liquid chromatography (RP-HPLC) with post column derivatization (PCD) involving bromination. The PCD was achieved with pyrimidinum hydrobromide perbromide (PBPB) followed by fluorescence detection.

In order to achieve reasonable homogeneity, a slurry of the nuts was prepared before proceeding to the extraction process. Approximately 50 g of the test portion was weighed into a blender jar, 5 g of Sodium chloride, 200 ml of methanol/water solvent and 100 ml N-Hexane was then added. The mixture was then blended for 3 min with a high speed blender (Waring commercial blender) and subsequently filtered through Whatman No.4 filter paper. Aliquots of 10 ml of the filtrate was pipetted into a beaker containing 60 ml of phosphate buffered saline (PBS), mixed with a plastic spatula/stirrer and applied onto an immunoaffinity column. The filtrate was passed through the column at a flow rate of approximately 3 ml/min by gravity. Distilled water (15ml) was applied in little portions of approximately 5ml - at a maximum flow rate of 5ml/min and dried by passing air through the immunoaffinity column by means of a syringe for 10 seconds. Aflatoxins were eluted and quantified as described by Stroka & Anklam (1997) (JAOAC, 1991).

Statistical Analysis

Data obtained from the aflatoxins assay were subjected to analysis of variance (ANOVA) using GenStat (12th edn). To determine the significance of observed differences between two treatment means, the least significant difference (LSD) was used to separate the means at 5% probability level.

Results and discussion

Sorting process development and aflatoxins verification exercise

A total of nine fractions of kernels emanating from the thorough sorting process developed were obtained and analysed for their aflatoxins content during the verification exercise. This sorting process, is expected to be a pre-requisite for further processing of peanut into the myriad of products on the market as it has a direct bearing on the safety of the product. The sorted out kernel portions comprised of the control raw unsorted peanuts (RUP), immature and shrivelled kernels, pre-sorted deshelled kernels, total discoloured kernels, less than 50% discoloured kernels, more than 50% discoloured kernels, dehulled shrivelled and immature kernels, good (clean) kernels, and the testa. The aflatoxins content of these different categories of kernels are given in Table 1.

Total aflatoxins content of the control which is the unsorted raw kernels was 19.75 µg /kg (Table 1). The individual aflatoxins levels tested ranged from "none detected" to 18.36 µg/kg representing aflatoxin G2 and aflatoxin B1 respectively. Sorted out immature shrivelled kernels recorded total aflatoxins content of 60.45 µg /kg with individual aflatoxins showing values that ranged from 0.25 µg/kg for aflatoxin G2 to 42.37 µg/kg for aflatoxin B1. As expected these kernels had the highest level of total aflatoxin and the highest in aflatoxin B1 (Table 1). The bulk of kernels remaining after pre-sorting, recorded a highly reduced aflatoxin levels. Interestingly, Ya Xu *et al*., (2017) recorded a 96.7% reduction in aflatoxin B1 through manual sorting. Cole *et al.*, (1995) stated that sizing and electronic sorting reduced aflatoxin concentration of peanut lots by 29% and 70% respectively. Blanched kernels, followed by electronic colour sorting further reduced aflatoxin level by 91%. In our present study, the discoloured kernels sorted out after dehulling were further categorized into various fractions based on the degree of discolouration and tested for their aflatoxin content. The discoloured kernels were separated into $\geq$ 50% discoloured, $\leq$ 50 discoloured and $\leq$ 10% discoloured. The results showed an increase in total aflatoxins with increasing discolouration. No aflatoxins were detected in the final clean kernels.

TABLE 1

Aflatoxin levels in sorted peanut fractions

Sample	*Fraction*	*Aflatoxins Levels (µg /kg)*[1]				
	(%)	*B1*	*B2*	*G1*	*G2*	*Total*
Raw unsorted peanuts (RUP)	100.00	18.36±0.04	1.35±0.15	0.04±0.002	ND	19.75±0.75
Immature and shrivelled kernels	15.00	42.37±0.35	17.68±0.80	0.50±0.05	0.25±0.03	60.45±1.61
Dehulled shrivelled, immature kernels	1.20	42.17±0.65	17.66±0.80	0.34±0.02	0.25±0.03	60.42±1.08
Presorted dehulled kernels	84.00	2.93±0.43	0.670±0.08	1.13±0.19	0.60±0.05	5.33±0.93
≤10% discoloured kernels	4.20	0.12±0.01	0.02±0.002	ND	ND	0.14±0.02
≤50% discoloured kernels	3.09	0.08±0.02	ND	0.16±0.003	ND	0.24±0.01
≥50% discoloured kernels	1.10	0.17±0.01	0.06±0.002	0.18±0.002	ND	0.41±0.03
Good (clean) kernels	74.48	ND	ND	ND	ND	ND
Testa	1.28	2.26±0.18	1.08±0.02	1.65 ± 013	0.35±0.01	5.34±0.33

[1]Values are means ± SD of duplicate determinations

ND = None detected

Sorting performance by Trainee Groups

The categories of kernels sorted out during the training exercise were raw unsorted kernels (RUK), immature and shrivelled kernels, presorted kernels for blanching, ≥ 50% discoloured, < 50% discoloured, and good clean kernels (no discolouration) just as was the case with the verification exercise. Aflatoxin analyses showed that the raw unsorted kernels (RUK) used by the two groups had a total aflatoxin content of 23.25 µg/kg (Table 2). The amounts for Aflatoxin B1, B2, G1 and G2 were 19.13 µg/kg; 2.98 µg/kg; 1.14 µg/kg, and "not detected", respectively. From an initial weight of 5 kg of RUK, the amount of presorted material was as follows: Group 1 sorted out 0.3 kg of immature and shrivelled kernels representing 6% while Group 2 sorted out 0.9 kg of immature and shrivelled kernels representing 18% (Table 2). The loss of 6 - 18% in the presorted immature and shrivelled kernels represented either extremely poor quality starting material, and/or "over"-sorting by untrained workers. The total aflatoxin content for the presorted kernels was 152.29 µg/kg for Group 1 and 148.03 µg/kg for Group 2. Additionally, high levels of Aflatoxins in the presorted material were over 10 times the regulatory limits for raw peanuts to be used as ingredients. This could however, find their way to the peanut products if not sorted out.

Following blanching and subsequent removal of the skin, peanut kernels with ≥ 50% discoloration were removed (Fraction 1). The

remaining material (Fraction 2) consisted of kernels with some discoloration (< 50%) and those free from any discoloration. For Fraction (1) kernels with ≥ 50% discoloration for Group 1 was 0.25 kg or 5% of RUK and that for Group 2 was 0.20 kg or only 4% of RUK (Table 3). Aflatoxins were not detected in these samples for the two groups. However, these samples must be discarded, and considered as losses from the raw material due to the discoloration they had. This is in order to produce high quality peanut products. Previous experiments indicated that removal of kernels after blanching, with ≥ 50% discoloured kernels will result in peanuts with aflatoxin contents below the regulatory limit (EU requirements).

TABLE 2

Aflatoxins content of presorted fractions of peanut during group training

Sample	*Fraction*	*Aflatoxins Levels (µg /kg)*[1]				
	(%)	*B1*	*B2*	*G1*	*G2*	*Total*
Group 1						
Raw unsorted peanuts (RUP)	100.00	19.13±1.00	2.98±0.10	1.14±0.06	ND	23.25±0.39
Immature and shrivelled kernels	6.00	103.66±7.21	8.97±1.03	35.23±6.03	4.43±0.47	152.29±3.69
Presorted kernels for blanching	94.00	4.25±0.41	1.02±0.14	ND	ND	5.27±0.32
Group 2						
Raw unsorted peanuts (RUP)	100.00	19.13±1.0	2.98±0.1	1.14±0.06	ND	23.25±0.39
Immature and shriveled kernels	18.00	101.20±4.19	8.71±0.21	34.02±3.82	4.10±0.28	148.03±2.13
Presorted kernels for blanching	82.00	3.14±0.22	1.11±0.24	ND	ND	4.25±0.26

[1]Values are means ± SD of duplicate determinations
ND = None detected

To verify that removal of Fraction 1 kernels would result in peanuts below the regulatory limit of 15 µg/kg for ingredients, another fraction (Fraction 2 with < 50% discoloration) was removed from the kernels. Fraction 2 recorded no aflatoxins for both Group 1 and Group 2. Kernels with ≥ 50% discoloration which was 4% (0.20kg) of RUK for Group 2, and kernels with < 50% was 24% of RUK for Group 1 and Group 2. Aflatoxins were again not detected in these samples (Table 3).

In the remaining clean unstained kernels, Group 1 had 3.45 kg or 69% of RUK and Group 2 had 3.0 kg or 60% of RUK. As expected, kernels from both groups were free from aflatoxins, as analysed by HPLC (Table 3). The

percentages of the sorted blanched kernels appeared to be low at 69% and 60%, respectively, for Groups 1 and 2. Several factors contributed to this loss. Among these are losses due to low quality peanuts which could be avoided by accepting only good quality material, losses due to blanching resulting in 6.5% loss. This is an expected loss and is unavoidable in the processing of the product, including those products from unsorted deskinned peanuts.

TABLE 3

Material balance and total aflatoxins content at post blanching sorting during group training

Sample	*Fraction*		*Total Aflatoxins (µg /kg)*
	kg	%	
Group 1			
Raw Unsorted Kernels (RUK)	5.00	100.00	23.25±0.39
Discoloured kernels (≥ 50% surface discoloration)	0.25	5.00	ND
Discoloured kernels (< 50% surface discoloration)	1.20	24.00	ND
Clean kernels (No discoloration)	3.45	69.00	ND
Group 2			
Raw Unsorted Kernels (RUK)	5.00	100.00	23.25±0.39
Discoloured kernels (≥ 50% surface discoloration)	0.20	4.00	ND
Discoloured kernels (< 50% surface discoloration)	1.20	24.00	ND
Clean kernels (No discoloration)	3.00	60.00	ND

[1]Values are means ± SD of duplicate determinations
ND = None detected

Sorting performance by Trainee Processors

Aflatoxin results for Processor 1 shows a similar trend as observed for the verification exercise and the training exercise. Total aflatoxins for immature and shriveled kernels registered the highest amount of 321.9µg /kg as compared to a total of 16.34 µg /kg for the raw unsorted kernels. Discoloured kernels registered 0.13 µg/kg for total aflatoxins, testa, and the good kernels did not register any aflatoxins at all. In a study carried out by Galvez *et al* (2003), sound kernels had no aflatoxins or contained low levels (<15 µg/kg) of aflatoxins as against 300 µg/kg of raw materials prior to sorting (Table 4). As was the case in the verification exercise, there was a drastic reduction of the aflatoxin levels in the unsorted peanuts from 16.34 µg/kg to none detected (100% reduction) in the case of Processor 1's training (Table 4), and from a total of 23.22 µg/kg to 0.23 µg/kg (99% reduction) for Processor 2. Again from the results, it was obvious that the shrivelled kernels contributed a great deal to the high level of aflatoxins in the peanuts. This confirms findings by Davidson *et al*., 1982, where nearly 80% of aflatoxin contamination

could be attributed to small and shrivelled kernels. Total aflatoxin level of shrivelled kernels for Processor 2 was 421.74 µg/kg (Table 5). Interestingly, sorted discoloured kernels for both Processor 1 and Processor 2 registered significantly low values for aflatoxins.

These results confirm the study of Awuah & Kpodo (1996), where relatively low levels of total aflatoxins (50% contamination rate – 0.1 µg/kg to 12.2 µg/kg) were detected in the undamaged kernels), while levels from 5.7 µg/kg to 22,168 µg/kg were found in the damaged kernels.

The results of this study is also in agreement with Ndung'u *et al.,* (2013) who concluded that the source of groundnut and the presence of defective nuts were major determining factors in increased aflatoxin contamination in the cottage industry in Kenya. The Ghana Standards Authority has set a limit of 15 µg/kg for total aflatoxins in products meant for human consumption. The European Union however, has a stricter limit of 2 µg/kg for aflatoxin B1 and 4 µg/kg for total aflatoxins meant for direct human consumption. Similarly, Bley-N'dede *et al.,* (2012) showed that prices paid for peanuts, prices received for the commodity, the cost of sorting, and storage are important factors in reducing aflatoxin levels in peanuts.

TABLE 4

Aflatoxin levels in sorted peanut fractions at processor level

Sample	*Fraction*	*Aflatoxins Levels (µg /kg)*[1]				
	(%)	*B1*	*B2*	*G1*	*G2*	*Total*
Processor 1						
Raw unsorted peanuts (RUP)	100.00	16.22±1.71	0.93±0.1	0.04±0.002	ND	16.34±0.60
Sorted shrivelled and Immature kernels	14.00	266.3±23.33	54.94±4.94	0.66±0.1	ND	321.9±9.46
Discoloured kernels	10.1	0.13±0.01	ND	ND	ND	0.13±0.01
Testa	1.40	ND	ND	ND	ND	ND
Good (clean) kernels	72.00	ND	ND	ND	ND	ND
Processor 2						
Raw unsorted peanuts (RUP)	100.00	20.14±1.56	1.87±0.13	1.21±0.16	ND	23.22±0.62
Sorted shriveled and Immature kernels	12.00	203.62±7.52	27.99±4.85	162.46±9.42	27.67±3.18	421.74±6.24
Discoloured kernels	9.20	0.79±0.16	0.1±0.01	0.35±0.07	ND	1.24± 0.08
Testa	1.36	ND	ND	ND	ND	ND
Good (clean) kernels	76.00	ND	ND	ND	ND	ND

[1]Values are means ± SD of duplicate determinations

ND = Not detected

The effectiveness of the sorting procedure in reducing aflatoxin levels to regulatory levels cannot be over-emphasized. Manual sorting of the blanched kernels resulted in significant reduction in the aflatoxin levels in the peanut samples. Blanching proved to be a very good means by which effective sorting can be done to reduce aflatoxin levels in the final products. Immature and shrivelled kernels seem to be more susceptible to aflatoxin contamination than mature kernels. Manual sorting is quite laborious and time-consuming but obviously an effective way of reducing aflatoxin levels in peanuts.

The results also show that the mere presence of the aflatoxigenic moulds does not indicate contamination with aflatoxins as observed in the non-detection of aflatoxins in the discoloured kernels at the training for Processor 1 and Processor 2. In their bid to maximize profits, entrepreneurs may be tempted to add discoloured kernels during processing to increase material balance. Consequently, it may be necessary to investigate possible co-occurrence of aflatoxins and other mycotoxins (e.g. ochratoxin A) in the peanut samples. The discoloration of the kernels indicates possible growth of moulds but not necessarily production of aflatoxins. Complex interactions occur in nature consequently antagonistic relationships may exist to prevent the production of these toxins.

Sorting of nuts would obviously increase costs resulting in reduced profit margins. As a result, industry players with little or reduced or insufficient appreciation of quality and safety of products, will be reluctant to sort unless they are well motivated to produce quality kernels through higher prices for premium quality kernels. Jolly *et al*., (2009) observed low knowledge of aflatoxins among value chain actors in Ghana and Benin. Not much progress has been made in this regard. Therefore, in order to reduce aflatoxin levels and thus promote food safety, there is the need to educate and sensitize the actors and the general public to identify poor quality peanuts to enhance effective sorting.

Conclusion

Manual sorting which involves presorting and further sorting after dehulling and blanching presents an effective way of reducing significantly aflatoxin contamination in peanuts before processing into desired finished products especially with small scale processors. Mechanical sorting is recommended for commercial processors. In all cases, the acquisition of good raw materials would go a long way to increase the material balance and consequently reflect in increased profit margins for processors.

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