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Performance Evaluation of a Centrifuge Type Grain Cleaning Machine and Its Effect on Chemical and Microbial Quality of Sorghum and Millet Grains

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Abstract: Cleaning is an important unit operation in food processing. However, cleaning of some cereal grains including millet and sorghum prior to processing is tedious due to their small sizes. In this study, the cleaning efficiency of a locally fabricated centrifuge-type grain cleaning machine was tested and compared to the traditional method of cleaning. The centrifuge cleaning machine consists of an outer main frame which is stainless steel, cylindrical drum with a hundred percent opening discharge pipe on the lower side, an inner 250 μm perforated stainless steel cylindrical sieve, a motor-powered transmission stirrer unit with two bats placed adjacent at ninety degrees to each other. Color measurement of grain samples was done with a Lovibond Tintometer Colorimeter. The microbial load – enterobacteria, coliform, *Escherichia coli*, aerobic mesophiles, yeast and *Staphylococcus aureus* was analyzed using the AOAC (2012) methods. HPLC was used to quantify the aflatoxin levels - aflatoxins B1, aflatoxins B2, aflatoxins G1 and aflatoxins G2. The time and cost which initially took 8 h using two laborers at a fee of GHC100.00, was reduced to 3 h with just one laborer costing GHC50.00. Skinning damage to millet and sorghum caused by locally fabricated machine was determined to be 2.1% and 4.6% respectively. The results of the study showed that the cleaning machine could be adopted for small scale washing of millet and sorghum grains for foods such as *fura*.

Keywords: Grain Cleaning Machine, Sorghum, Millet, Aflatoxins, Microbial Population

1. Introduction

Sorghum and millet are among the major staple food grains in the semi-arid tropics of Africa, Latin America, and Asia for over 750 million people [1]. These cereal grains contain high amounts of amino and fatty acids, essential vitamins, and mineral contents [2].

Cleaning is one of the proven methods to improve cereal grain quality and safety before use [3]. Cleaner grains are expensive and are in high demand in the agricultural processing industry [3].

Despite the importance of these cereal grains and the role it

plays in the livelihood of people living in Ghana, the issue of quality and safety continues to be of concern [4]. The traditional or manual cleaning of millet and sorghum, are done extensively before processing [5]. This procedure is tedious, time consuming, labour intensive and largely inefficient. To address these challenges, a wide variety of machines have been developed for cleaning cereal grains. Some of these machines function by means of air blown through a set of sieves using gravitational forces or suction to get rid of impurities. Others utilize coarse vibrating sieves that separate impurities from clean grains based on size [6]. However, with the advent of efficient cleaning and separating mechanisms, the use of centrifugal force in cleaning systems has become popular. In

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this study, sorghum and millet grains were cleaned with a locally fabricated centrifuge cleaning system using water.

The focus of this study was to test the efficiency and effectiveness of a locally fabricated centrifuge-type grain cleaning machine that uses water and to evaluate its performance on microbial load and mycotoxin levels of millet and sorghum.

2. Materials and Methods

2.1. Materials

Centrifuge cleaning machine

The centrifuge cleaning machine consists of a main frame, stainless steel cylindrical drum, power transmission and stirring unit and a discharge pipe. The individual parts are described as follows:

- The vessel has two components: a perforated stainlesssteel cylindrical drum and a solid vessel which houses the inner vessel and it is surrounded water;
- 2) A 1.5 hp variable electric motor housed in a perforated steel cage for operational safety;
- 3) Vertical stirrer.

Raw Materials

Millet and sorghum were purchased from certified grain distributors in an open market in Accra. The initial moisture content of the grains was recorded as 9.9 % and 10.8% (w.b) for sorghum and millet respectively.

2.2. Methods

Initial Testing of the Centrifuge Cleaning Machine

The cleaning machine was filled with 30 L of water after which the centrifuge system was switched on. 10 kg sample was poured into the inner perforated vessel and operated for 10 mins. The floating material was scooped off and the water was drained into a collector vessel.

To evaluate the mechanical washing and skinning efficiencies, experimental tests were carried out at a local agroprocessing company in Accra. Initial testing of the machine was carried out to check for proper functioning of all machine components. The rotational speed (in rpm) of stirrer, power requirement and consumption, as well as alignment and correct levelling of parts were obtained while the machine was operating at no load. Performance of the machine in terms of capacity, efficiency and damages were evaluated and recorded. Washing efficiency and skinning damage were estimated using a method described as follows; [7].

Washing Efficiency = $\frac{\text{weight of food sample after washing}}{\text{weight of food sample before washing}} x100$ Skinning Damage = $\frac{\text{weight of skinned product after washing}}{\text{total weight of product after washing}} x100$

2.3. Analytical Methods

2.3.1. Determination of Aflatoxins B_1 , B_2 , G_1 and G_2

Aflatoxins (B₁, B₂, G₁ and G₂) were determined based on CEN official method EN14123 (2007). Twenty-five gram (25 g) of millet or sorghum powder were extracted with 200 mL methanol in distilled water at a ratio of 4:1 respectively and 5 g NaCl. The mixture was homogenized for 3 min (i.e., 3000 rpm for 2 min and at 3500 rpm for 1 min) and filtered through Whatman No. 4 filter paper. Sixty milliliters (60 mL) of phosphate buffered saline (PBS) were added to 10 mL of filtrate and then the mixture was stirred.

Immunoaffinity columns specific for aflatoxins were preconditioned and used for solid phase extraction. Columns were fitted to a vacuum manifold and antibodies in the column activated by passing 10 mL of phosphate buffer saline through it at a flow speed of 3 mL/min. The whole filtrate-PBS mixture (70 mL) was loaded onto the activated immunoaffinity column and allowed to drain by gravity. The columns were washed at three cycles, each with 5 mL of distilled water at a flow rate of 5 mL/min. Air was blown through the column to get rid of all wash solvent molecules with a vacuum pump. Elution of aflatoxins was done in two steps into a 5 mL volumetric flask with 0.5 mL of methanol (highest grade) and then with 0.75 mL of methanol after one minute. Air was blown through the column to collect all eluates. Distilled water was used to adjust the volume of eluate to 5 mL and the eluate vortexed. Thereafter, 2 mL was

pipetted into HPLC vials for quantification.

2.3.2. Aflatoxin Quantification by HPLC

Agilent High Performance Liquid Chromatography system (HPLC 1260 infinity series, Agilent, USA) with a quaternary pump and fluorescence detector was used for aflatoxins quantification. Data acquisition and quantification was done using Chem station (OpenLab edition, Agilent). The equipment was set at an excitation wavelength of 360 nm and an emission wavelength of 440 nm and the column compartment (X-bridge column: 250 mm x 4.6 mm, i.d., 5 μm) temperature regulated at 35°C. The mobile phase was a mixture of water: methanol: acetonitrile at ratios of 65:20:15, respectively and isocratic delivery mode employed at a flow rate of 1 ml/min with an injection volume of 10 µL. Pyridinium hydrobromideperbromide (PBPB) solution was used for post column derivatization and the run time set at 10 min. Six-point calibration was made using pure aflatoxin standard solution at concentrations of 5 ppb, 10 ppb, 15 ppb, 20 ppb, 25 ppb and 30 ppb and linearity accepted at 0.99 or 99% for calibration curve.

2.3.3 Limit of Detection/Quantification (LOD/LOQ)

Limit of detection and quantification (LOD/LOQ) of the HPLC used were estimated by developing a calibration curve around the least calibration standard concentration. The LOD and LOQ were calculated as;

LOD = 3 x standard deviation/slope.

 $LOQ = 3 \times LOD$.

2.4. Microbial Analysis

Samples of sorghum or millet (10 g) were homogenized in 90 mL of sterile diluent (0.85% NaCl, 0.1% Peptone, pH 7.2) in a stomacher (Lab. Blender, Seward Medical, and Model 4001) for 30 s, and determinations made from ten-fold dilutions. Yeast was enumerated by the pour plate method according to ISO 21527-1; 2008 using Dichloro-Rose-Bengal-Chloramphenicol Agar (Oxoid CM; Oxoid Ltd., Basingstoke, Hampshire, UK), 1% chloramphenicol supplement was added to suppress bacteria growth, at pH 6.5. The plates were incubated, un-inverted at 25°C for 120 h. Aerobic Mesophiles were enumerated by the pour plate method on plate count agar (Oxoid CM 325; Oxoid limited Basingstoke, Hampshire, UK) [8]. Plates were incubated by inversion at 30°C for 72 h. Coliform and E. coli were enumerated by the pour plate method, [9], using tryptone soy agar (pH 7.3) (Oxoid CM 131) overlaid with violet red bile lactose agar (Oxoid CM107), pH 7.4, incubated at 37°C for 24 h for coliform and 44°C for 24 h for E. coli. Colonies suspected to be coliforms were confirmed using brilliant green bile broth, pH 7.4 (Oxoid CM31) incubated at 37°C for 24 h and E. coli on EC broth, pH 6.9 (Oxoid CM 853) followed by tryptophan water incubated at 44°C for 24 h according to NMKL Method No. 125 [10]. Salmonella was enumerated according to NMKL. No. 71, [11] using buffered peptone water broth (BPW) (Oxoid) pH 7.4, incubated at 37°C for 24 h, 0.1 mL of BPW was sub-cultured into 10 mL Rappaport-Vassiliadis broth and incubated at 42°C for 24 h and streaked on xylose-lysine-desoxycholate (XLD agar) medium at 37°C for 24 h. Staphylococcus aureus was determined by spread plate on Baird-Parker agar (Oxoid, CM 275) with egg yolk telllurite emulsion (SR 54) and blood agar

base (Oxoid, CM 55). These media were incubated at 37°C for 48 h. *S. aureus* population was confirmed using biochemical tests [12].

2.5. Colour

Color measurement of grain samples was done with a Lovibond Tintometer Colorimeter (Model F) optical sensor based on Lovibond RYBN system. A glass cell containing grain was placed above the light source, covered with a white plate and RYBN values were recorded. The instrument was calibrated against a standard red-colored reference tile (Ls = 25.54, as = 28.89, bs = 12.03) before use.

2.6. Statistical Analyses

IBM SPSS 25.0.1 (IBM Inc., New York, USA) was used to analyze obtained data for measured parameters. The results were presented as means \pm SD. Dependent sample t-test (paired t-test) was used to determine the significant difference between the means at 95% confidence level.

3. Results and Discussions

Initial testing of centrifuge cleaning machine

The initial testing results of the centrifuge cleaning machine is summarized in table 1. The ratio of weight of millet before and after washing gave a washing efficiency of 83.7%. Results from research carried out on potato washing gave cleaning efficiency between 83.22% and 93.82% [6]. This corresponds with the results of the present study. The lower rotation speed (14-15 rpm) used in the experiment may be responsible for the differences in efficiencies recorded for the present study which used a speed of 40 rpm [6].

Table 1. Initial parameters testing output of grain washer.

DADAMETED (4., -£24-4-)	RESULTS		
PARAMETER (Av. of 3 tests)	Sorghum	Millet	
<i>MC</i> ₁ of product before washing (Av. of 3 products % wb)	9.91	10.82	
Weight of product before washing (kg) W_1	10	10	
RPM	40	40	
Duration of test (min)	5	5	
Volume of water used $W_w(L)$	118	118	
Immediate weight of product after washing, straining (kg) W_2	11.45	12.50	
MC ₂ of product after washing (% wb)	23.20	32.55	
Weight of product at MC_1 after washing W_3 (kg)	8.37	8.58	
Immediate weight of skin (of product) after washing (kg) W_4	0.53	0.26	
MC_3 of skin (of product) before washing %wb [equal to MC_1]	9.91	10.82	
MC_4 of skin (of product) after washing (%wb)	43.04	49.22	
Retention time (s) [equivalent to 1 drum rotation]	0.67	0.67	
Cleaning efficiency (%) $[(W_3/W_1) x 100]$	83.7	85.8	
Skinning Damage/Efficiency (%) [(W ₄ /W ₂) x 100]	4.6	2.1	

Skinning damage (SD) occurs due to rubbing action among the grains itself or between the grains and revolving drum [13]. The average skinning efficiency for millet and sorghum were found to be 2.08% and 4.59% respectively. Research carried out on carrot [13] recorded higher skinning efficiency between 5.80 to 8.50%. The difference in skinning efficiencies may be attributed to the lower rotation speed of

25 rpm at which the experiment was carried out [14].

Hence, an increase in skinning damage may be compensated for, by reducing the speed of rotation of the machine from 40 rpm to 25 rpm. A reduction in the frictional forces within the product as well as between the product and drum due to slower speeds may lower the skinning damage of food samples.

3.1. Aflatoxins

The international agency for research on cancer has classified aflatoxins as a group-1 carcinogen hence its occurence in cereal grains including millet and sorghum are of major health concern to consumers [15]. The aflatoxin levels in both fresh uncleaned and cleaned sorghum, including its separated debris, were below detection limit. The instrument detection limit was 0.20 μ g/kg, 0.17 μ g/kg, 0.26 μ g/kg and 0.36 μ g/kg for aflatoxin B₁, aflatoxin B₂, aflatoxin G₁ and aflatoxin G₂, respectively. The non detection of aflatoxins in sorghum samples (fresh and cleaned) showed that the levels in the sorghum were far below the European

union's (EU) maximum limit of 2 μ g/kg for Aflatoxins B₁ alone [3]. This observation however, contrasts reports on high levels of Aflatoxins B₁ (ranging from 6-16 μ g/kg) in over 25% of 67 sorghum samples from various markets in Togo [17]. Levels exceeding EU's limit of 2 μ g/kg for Aflatoxins B₁ in sorghum was reported in 2018 [18]. The results from previous studies showed sorghum, like most cereal grains, is a good substrate for the growth of *Aspergillus spp*, the moulds responsible for producing aflatoxins [19]. The non detection of aflatoxins in sorghum samples in this study therefore could be attributed to good storage and postharvest handling practices which may have prevented the growth of these *Aspergillus spp* molds [20].

Table 2. Aflatoxin levels in traditional and mechanized millet (µg/kg) before and after cleaning.

Cereal Sample		AFB1	AFB2	AFG1	AFG2
Raw millet Traditional processed	Uncleaned	31.64	24.12	18.23	3.68
millet	Cleaned	12.43±0.01°	11.95±0.006°	5.48 ± 0.006^{c}	2.79±0.017 ^a
	Debris	25.56±0.01 ^b	25.26 ± 0.006^{b}	15.84±0.006 ^b	1.70 ± 0.012^{d}
Mechanized processed millet	Cleaned	8.42 ± 0.01^{d}	10.50 ± 0.012^{d}	4.50 ± 0.012^{d}	1.83±0.017°
	Debris	27.89 ± 0.01^{a}	36.60 ± 0.012^{a}	16.60 ± 0.015^{a}	2.73 ± 0.006^{b}

Cleaning of millet samples in the centrifuge system resulted in a significant reduction of aflatoxin B₁ levels in the cleaned millet samples. Aflatoxin B₁ levels reduced from 31.64 µg/kg in the uncleaned millet to 12.43 µg/kg in the traditional processed millet representing a 60.7% reduction. There was a 73.3% reduction representing 8.42 µg/kg, after uncleaned millet was cleaned in the centrifuge system. Similarly, aflatoxin G₁ levels reduced from 18.23 µg/kg to 5.48 µg/kg in the traditional cleaning. For the mechanized cleaning, aflatoxin G₁ reduced from 18.23 µg/kg to 4.50 μg/kg. Aflatoxins G₂ levels decreased from 3.68 μg/kg to 1.83 µg/kg after washing of the millet in the cleaning machine and 2.79 μg/kg after manual washing. Aflatoxins B₁ levels in millet debris (MD) was measured to be 27.89 µg/kg (Table 2). The significant reduction of aflatoxin levels in the cleaned millet when compared to the uncleaned millet, can be attributed to the centrifuge cleaning system. The different physical properties of mold-damaged seed grains compared to non-damaged grains is exploited to separate them by density segregation [7]. In the centrifuge cleaning system, by means of density segregation, the mold infested millet floated on the water in the system which was scooped away. Since these molds are responsible for aflatoxin production, the

absence of molds in the millet may have lead to low aflatoxin levels in the cleaned millet. It has been pointed out that, a mere separation of discolored and mold infested grains from the lot could reduce aflatoxin levels from 40-80% [21]. This showed most aflatoxin contamination in seed grains come from damaged grains and its debris and it was evident in this study when the debris of millet (both mechanized and traditional) measured the highest levels of aflatoxins B_1 . Aflatoxins B_2 is established as the dihydroxy derivative of aflatoxin B_1 and so under favourable conditions, aflatoxins B_1 may be converted to aflatoxins B_2 [22]. This could have been the reason behind the high levels of aflatoxins B_2 in the mechanized processed millet samples.

Cleaned samples were significantly different for both traditional and mechanized processed millet in all aflatoxin levels. However, the cleaning equipment reduced aflatoxin levels further than the traditional processing.

3.2. Microbiological Population

The microbiological safety of cereal products begins with the state of raw materials. Molds and *salmonella* were not detected in any of the grain samples before and after cleaning.

Table 3. Microbiological population (log cfu/g) in traditional and mechanized millet before and after cleaning.

Cereal Sample	-	Entero-bacteria	Coliform	E. coli	Aerobic mesophiles	Yeast	S. aureus
Raw millet Traditional	Uncleaned	6.68	6.54	6.38	6.65	9.38	3.08
Processed Millet	Cleaned	2.65±0.006°	2.95 ± 0.006^{c}	2.48 ± 0.006^{c}	5.79 ± 0.006^{b}	2.3 ± 0.017^{c}	2.7 ± 0.012^{c}
Processed Millet	Debris	5.3±0.015 ^b	5.26 ± 0.006^{b}	5.84 ± 0.012^{b}	$5.70 \pm 0.006^{\circ}$	6.26 ± 0.006^{b}	5.43 ± 0.006^{a}
Mechanized Processed	Cleaned	0.5 ± 0.006^{d}	0.5 ± 0.006^{d}	0.5 ± 0.012^{d}	2.83 ± 0.006^{d}	1.21 ± 0.012^{d}	1.4 ± 0.006^{d}
Millet	Debris	7.48 ± 0.012^{a}	6.6 ± 0.012^{a}	6.6 ± 0.023^{a}	6.73 ± 0.017^{a}	7.83 ± 0.017^{a}	3.68 ± 0.023^{b}

The samples, irrespective of its form, are significantly different for each microbial test. The traditional cleaning process does little in reducing microbial count in the millet samples. The cleaning equipment significantly reduced the

microbial load for all microbial test. This indicates a high food safety index. The mechanized cleaning was efficient compared with the traditional process.

Cereal Sample Entero-bacteria Coliform E. coli Aerobic mesophiles Yeast S. aureus Uncleaned $6.68\pm0.017a$ 6.54±0.029a 6.38±0.017a $6.65\pm0.012b$ 9.38±0.029a 3.08±0.023a Sorghum 2.65±0.016b 2.95±0.029b 2.48±0.016b 5.79±0.029d 2.30±0.023c 2.70±0.029b Cleaned Uncleaned 2.48±0.017c ND ND 6.73±0.023a 3.83±0.023b $2.68\pm0.035b$ Millet ND 5.83±0.023c 1.21±0.012d 2.40±0.029c Cleaned ND ND

Table 4. Microbiological population (log cfu/g) in sorghum and millet before and after cleaning.

Microbial population of sorghum was found in the ranges $(3.1 - 9.4 \log \text{cfu/g})$ and millet $(2.3 - 5.8 \log \text{cfu/g})$ which indicates that the microbial population found in sorghum is higher than that of millet (Table 4). The cleaning equipment reduced the microbial load significantly in both sorghum and millet grains. Sorghum and millet counts of Enterobacteria, coliform, E. coli, aerobic mesophiles, S. aureus were all below the 105 Cfu/g, set by Ghana Standards Authority (GS 955-2018). In both cereals, whereas enterobacteria, coliform, E. coli and yeast counts reduced after cleaning, aerobic mesophile and S. aureus population recorded a mere reduction of less than one log cycle. This indicates that the equipment is not efficient in reducing aerobic mesophile count in the cereal grains tested which could be attributed to stirring which incorporates air into the water for cleaning.

3.3. Colour

Several studies have reported a relationship between grain colour and grain quality [17, 23]. Table 6 presents the color results for the traditional and mechanized processed grains.

Table 5. Color scores for traditional and mechanized processed millet grains.

Cereal Sample	-	Red	Yellow
Traditional processed	Cleaned	1.3±0.114°	1.0±0.114°
millet	Debris	3.0 ± 0.120^{b}	1.5±0.120 ^b
Mechanized	Cleaned	0.8 ± 0.114^{d}	0.8 ± 0.114^{d}
processed millet	Debris	3.3 ± 0.120^{a}	1.6 ± 0.120^{a}

There was a significant difference in both colours for traditional and mechanized cleaned millet. Mechanized cleaned millet was significantly lighter in all colors than the traditional cleaned millet with values 0.8 and 1.3 in red, 0.8 and 1.0 in yellow respectively.

Table 6. Color scores for raw and cleaned sorghum and millet grains.

Cereal	Sample	Red	Yellow	Total
Carahum	Uncleaned	2.0±0.01a	1±0.01 ^a	3.0
Sorghum	Cleaned	1.0 ± 0.17^{b}	1 ± 0.01^{a}	2.0
MC11-4	Uncleaned	2.0±0.01a	0.8 ± 0.01^{b}	2.8
Millet	Cleaned	0.8 ± 0.01^{c}	1 ± 0.06^{a}	1.8

There was no significant difference in red colour for both uncleaned millet (2.0) and sorghum (2.0). The same was recorded for yellow colour in cleaned sorghum and millet. Cleaned sorghum was significantly lighter in red color than uncleaned sorghum with values of 1.0 and 2.0 respectively (p < 0.05), an indication showing most of the debris contributing to the dark color in the sorghum samples were removed during cleaning.

The degree of lightness of the yellow colour in sorghum however did not change after cleaning, an indication of low debris in the sorghum samples. The redness color scores for both millet and sorghum reduced significantly (p < 0.05) after the cleaning process. The millet redness intensity reduced from 2.0 to 0.8 whereas that of sorghum reduced from 2.0 to 1.0. Research on sorghum associated high lightness and low redness color in sorghum to mean low levels of tannins in the sorghum [23]. Therefore, the cleaning process of this present study could result in grains of low tannin levels considering the high lightness and reduced redness color scores. Tannins in grains adversely affects the grain's metabolism energy and protein utilization [24, 25] hence, its reduction is beneficial for processing and nutrients availability.

4. Conclusion

This study examined the cleaning efficiency and skinning damage of a locally fabricated centrifuge-type cereal grain cleaning machine and its effect on some quality indices of sorghum and millet grains. The cleaning efficiency of the machine and its associated skinning damage on the cereal grains were different. The cleaning efficiency and skinning damage were 83.7% and 4.6%, 85.8 and 2.1% correspondingly for sorghum and millet. The chemical and microbial quality of the grains improved after cleaning with the machine. The aflatoxin levels and microbial population in cleaned millet were lower than the uncleaned grains. The study has shown that the centrifuge-type cleaning machine is efficient for these grains and may be adopted for use in small scale processing.

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