Research Article

Filamentous Fungi in Selected Processed Indigenous Flours Sold in, Kumasi, Ghana

Benjamin Tetteh Awaitey[1], Felix C Mills-Robertson[2*]

[1]Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[2]Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

*Corresponding author: Felix C Mills-Robertson, Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Tel: +233208970091; Email: mirobfc2011@gmail.com

Abstract

This study evaluated the filamentous fungi present in selected locally processed indigenous flour sold in the Kumasi Metropolis of Ghana using standard microbiological methods. Results from this study showed that dry cassava (kokonte) flour recorded mould count ranging from $1.70 \times 10^5 \pm 0.15$ cfu/g to $4.03 \times 10^5 \pm 0.35$ cfu/g while maize flour had mould count ranging from no observable growth count to $1.18 \times 10^6 \pm 0.18$ cfu/g. Total plate count showed contamination levels between no observable growth count to $9.1 \times 10^6 \pm 0.25$ cfu/g for the maize flour samples, while for the dry cassava (kokonte) flour, counts ranged from $7.8 \times 10^6 \pm 0.30$ cfu/g to $4.64 \times 10^6 \pm 3.18$ cfu/g. Moisture analysis revealed percentage moisture content of 12.4%±0.15 to 19.7% ± 0.12 for the maize flour samples and 10.9% ± 0.27 to 16.9% ± 0.56 for dry cassava (kokonte) flour. Coliforms test indicated negative for seven of eight (7/8) maize flour samples and six out of eight (6/8) for dry cassava (kokonte) flour samples bought from the various markets. From the study, thirteen mould species belonging to five genera were isolated from the various flour samples. Ten different species were isolated from the dry cassava (kokonte) flour while thirteen different varieties from the five genera were isolated from the maize flour.

Keywords: Moulds; Dry Cassava (Kokonte) Flour; Maize Flour

Introduction

The high incidence of post-harvest food losses, arising mainly due to inadequate food storage and preservation technologies, is a major issue affecting the quality of food in West Africa, where seasonal food shortages and diseases resulting from nutritional deficiency are still a major concern [1]. Study has shown that fruits, vegetables, roots and tubers contribute to nearly 50% of perishable food commodities while grains such as maize, sorghum, millet, rice and cowpeas contribute to about 30% of food loss after harvest in West Africa [1]. Factors that contribute to these losses may include; inappropriate food processing technologies, poor harvesting and inefficient post-harvest handling practices, bad roads, moribund rail systems and many others [1]. In Ghana, issues of post-harvest losses are predominant especially where locally produced crops such as cassava, yam,

maize, rice, beans, and others are hardly processed leading to waste of crops especially during bumper harvest. In order to extend the shelf-life of some of these crops and hence reduce the incidence of postharvest losses, they are processed into flours and other products which may be used by individuals at home or sold on commercial basis. Methods involved in processing these indigenous foodstuffs may, however, expose them to contamination by several pathogens mainly filamentous fungi and some bacteria in addition to contamination from the farm before processing. For instance, most cereal grains can be contaminated by different species of microscopic fungus during it developmental stages [2] and these pathogens may affect the crop resulting in a reduction of the quality of the grain.

Some species may produce mycotoxins that intoxicate both humans and animal upon consumption [2]. Mycotoxin classes known to occur in cereals, including the aflatoxins (AFB1, AFB2 and AFG1, G2), tricotecens, deoxinivalenol (DON) and (T-2 toxin), the fumonisins (FB1, FB2 and FB3), the zearalenone (ZON),

ochratoxin A (OTA) and the ergot alkaloids [3] are known to be carcinogenic. Studies have revealed that majority of these mycotoxins are produced by the genuses *Aspergillus*, *Penicillium* and *Fusariu* [3].

Spores produced by these fungi are very difficult to eliminate due to their stability to high temperature and other harsh environmental conditions, hence the presence of these spores in food poses threat to the health of consumers. In the case of flour, the high-grade types are treated to contain very low or no contamination due to use of advance technologies [4]. However, locally processed indigenous flours may be contaminated by different microbes due to improper food safety practices and as these flours are usually sold on commercial basis, they may result in exposing consumers to several health risks.

The purpose of this study, therefore, was to evaluate the filamentous fungi of some selected processed indigenous flours sold in Kumasi in Ghana.

Materials and Methods

Experimental design

Two types of processed indigenous flours were considered for this study; maize flour and dry cassava (kokonte) flour. These two flours were considered for the study because they are very common and frequently used in food preparation in Ghana. Sixteen samples (eight of each flour) were selected for this study from four different markets (Ayigya market, Central market, Bantama market and Atonsu market) in Kumasi. For each market, two Vendors were selected at random for sample collection and administration of questionnaires and from each Vendor three sample were bought. Samples were packaged into sterile sample bags and brought to the laboratory for analysis. Samples were analyzed on the same day they were bought from the market, but those that were not analyzed same day were stored in the refrigerator at $4°C$. Samples were analyzed in batches, four samples per batch. Two samples (maize flour and kokonte flour) were also processed in the laboratory to serve as controls.

Moisture Content Analysis

Two (2) gram sample was weighed into a Petri dish and placed in an oven at $130°C$ for 2 hours, weighing intermittently until there was no change in weight [5]. The samples were cooled to room temperature in a desiccator at each time before weighing. The moisture content was express as; (Weight loss/ initial weight of flour) x 100%

Microbial Sample Preparation

Working under aseptic condition, ten grams (10 g) of each sample was weighed using a sterile weighing boat and transferred to sterile sample bottles containing 90 mL sterile peptone water [3]. Each sample was vortexed for about 1 minute at moderate

speed and serially diluted to make five dilutions (10^{-1}-10^{-5}) by transferring 1 mL homogenized sample to 9 mL dilution blank, mixing well until the 10^{-5} dilution was obtained. Aliquots (0.1 mL) of these dilutions were used for the study.

Microbial Enumeration

Spread plate method of inoculation was employed in the microbial examination of the samples. From the prepared 10-fold serial dilutions, enumeration of moulds were carried out by the spread plate method on Potato Dextrose Agar containing 100 mg/L of chloramphenicol and 50 mg/L Oxytetracycline to suppress the growth of bacteria [3]. The plates were incubated at $25°C$ for 5 to 7 days. After the appropriate incubation periods, dilutions with 20-200 colonies were selected and manually counted. The number of colony-forming units per gram (cfu/g) of samples was calculated by multiplying the number of organisms by the dilution factor.

Isolation and Identification of Moulds

Three different media for the cultivation of fungi; Potato Dextrose Agar (PDA), Oxytetracycline- Glucose Yeast Extract Agar (OGY) and Dichloran Rose Bengal Chlortetracycline Agar (DRBC) (each containing 100 mg/L of chloramphenicol) were used for the isolation of the moulds. From the prepared dilutions, 0.1 ml of the inoculum was inoculated onto the different media by the spread plate method and the plates incubated at $25°C$ for 5 to 7 days.

Identification of Moulds

Mould cultures were prepared by lifting the mycelia mat of the organism with a sterile inoculation pin into a drop of lactophenol blue on a slide teased (spreading the mat) and covered with a cover-slip and observed under a microscope. Different characteristic features of the isolated organism were observed and used in their identification using the fourth edition of introduction to foodborne fungi [6].

Total Plate Count

Spread plate method of inoculation was used to determine the total plate count of organisms in the samples. From the prepared serial dilutions, enumeration was carried out by the spread plate method on Plate Count Agar. The plates were incubated at $37°C$ for 24 hours. After the appropriate incubation, plates with 30-300 colonies were selected and manually counted. The number of colony- forming units per gram (cfu/g) of samples was calculated by multiplying the number of organisms by the dilution factor.

Coliform Count

Sterile MacConkey broth was prepared in test tubes for the cultivation of coliforms. From the prepared serial dilution, 1 mL of inoculum from the 1:10 dilution (10^{-1}) was transferred into 9 mL of MacConkey broth under aseptic condition. Incubation was

done at 37°C for 24 hours and test tubes which showed change in media colour from red to yellow were recorded as positive.

Statistical analysis

Analysis was done in triplicates in order to minimize the error margin as much as possible. Results obtained were tabulated into Microsoft Excel 2010 and for easy interpretation, the data was subjected to one-way Analysis of Variance (ANOVA) and the significance differences between the means of the various markets determined by using Statistical Package for Social Sciences (SPSS version 20). P-values ≥ 0.05 were considered as statistically not significant.

Results and Discussion

Moisture content of the sample

The average moisture content of the maize flour samples ranged from 12.37%±0.15 to 19.70% ±0.12 while that of cassava flour ranged from 10.93%±0.27 to 16.90%±0.56 as shown in Table 1. The moisture content obtained for the various kokonte flour samples is in agreement with the study result (10.0% to 16.9%) reported by Lokko, (1978). Several researches have been conducted to establish an acceptable moisture content of kokonte flour. Reports show that for a kokonte flour to remain stable, a moisture content of 12% is required [7]. Apart from samples bought from Atonso market (Atm KF1 and Atm KF2) and the control (CKF) which recorded values lower than 12%, the remaining samples had moisture contents higher than 12%. This suggests that the high moisture content may be a contributory factor to microbial contamination since studies have shown that microorganisms require moisture for their growth [8]. Correlation analysis revealed a positive correlation between moisture content and mould count of dry cassava (kokonte) flour. Though high moisture content in foods is known to be a strong influence for growth of microorganisms, the results for the moisture content of Atm KF1, Atm KF2 and CKF from this study suggest that other factors apart from the high moisture content may account for the contamination in the samples.

MARKETS					
FLOUR SAMPLES	**Aym**	**Cm**	**Atm**	**Bm**	**Control**
MF1	15.07±0.19c	14.03±0.18b	13.87±0.12b	13.23±0.09ab	12.37±0.15a
MF2	13.33±0.37ab	19.70±0.12d	13.10±0.25ab	15.50±0.15c	
KF1	12.57±0.34b	14.67±0.23c	10.93±0.27a	15.00±0.06c	11.70±0.17a
KF2	13.03±0.29b	16.90±0.56d	11.03±0.23a	13.00±0.15b	

Key: Aym - Ayigya market, **Cm** - Central market, Atm - Atonso market, **Bm** - Bantama market, **CMF** - Control Maize Flour, **KF-** kokonte flour, **MF-** maize flour, C-control sample, 1 and 2 - Vendors 1 and 2 from the same market. For the same flour sample, means that do not share the same letter (superscript) were significantly different (P<0.05) but those that share the same letter (superscript) do not differ significantly (P>0.05)

Table 1: Percentage Moisture Contents of the Maize and Cassava Flour samples.

Enumeration of Moulds

Results for the enumeration of moulds in the samples have been represented in Table 2. For the dry cassava (kokonte) flour, all the samples were contaminated with moulds, representing 100% moulds contamination, but the levels of contamination varied among the samples. The level of mould contamination among the samples exceeded the acceptable moulds level in food (10^3 cfu/g) as reported by African Organization for Standardization [9]. This is an indication that the dry cassava (kokonte) flour sold on the various markets may be unwholesome and, therefore, pose health risk to consumers. In comparing the results for mould count in the various kokonte flour, with results obtained by Lu et al. [10], all the samples recorded count higher than 6.5×10^5 cfu/g as they reported in their studies except for the samples from Vendor-2 in Bantama market (Bm KF2) which recorded $1.70 \times 10^3 \pm 0.15$ cfu/g. The control sample also recorded count lower than reported. Statistical analysis showed a significant difference between the counts at p<0.05. Enumeration of moulds in the maize flour samples revealed varying degree of contamination among the

samples ranging from no observable mould count as recorded by Atm MF1, Bm MF1, Bm MF2 and CMF to high count of $1.18 \times 10^6 \pm 0.18$ cfu/g as recorded by Cm MF1. Samples from Aym MF2, Cm MF2 and Atm MF2 showed counts that were a little higher than the tolerable level of 10^3 cfu/g, recording $1.67 \times 10^3 \pm 0.30$ cfu/g, $3.40 \times 10^3 \pm 0.30$ cfu/g and $2.97 \times 10^3 \pm 0.30$ cfu/g, respectively. Extremely low counts of mould in the samples as recorded by Atm MF1, Bm MF1, Bm MF2 and CMF is in agreement with results obtained by Adu-Gyamfi and Appiah [11], obtaining mould count of 5.0×10^2 cfu/g. The low count of moulds reported may be due to the dehulling of grains before processing into flour as reported by Victor et al. [12]. It is thought that the microorganisms are usually found on the outer coat of the grains and hence dehulling is a means of reducing contamination. The extent of contamination in samples for Aym MF1 and Cm MF1, however, suggests that the dehulling process may not be a guarantee that samples are absolutely free from contaminants. The high incidence of contamination in these samples may be attributed to factors such as the high moisture content of the flour

samples, the length/period the samples have been on market, the processing method, the hygienic practices employed in processing, and the conditions under which the food commodities were sold on the market.

MARKETS					
FLOUR SAMPLES	**Aym**	**Cm**	**Atm**	**Bm**	**Control**
MF1	3.03×105±0.96a	1.18×106±0.18b	NILa	NILa	NILa
MF2	1.67×103±0.30a	3.40×103±0.17a	2.97×103±0.30a	NILa	
KF1	1.08×104±0.25c	3.20×104±0.32c	1.77×104±0.18c	1.43×104±0.18c	TFTC
KF2	1.90×105±0.25d	4.03×105±0.35e	7.03×104±0.61c	1.70×103±0.15c	

Key: TFTC- Too Few to Count, NIL – No mould count. **Aym** - Ayigya market, **Cm** - Central market, **Atm** - Atonso market, Bm - Bantama market, **CMF** - Control Maize Flour, **KF**- kokonte flour, **MF**- maize flour, C-control sample, 1 and 2 - Vendors 1 and 2 from the same market. For the same flour sample, means that do not share the same letter (superscript) were significantly different ($P<0.05$) but those that share the same letter (superscript) do not differ significantly ($P>0.05$)

Table 2: Load of Moulds on the Samples (cfu/g).

Identification of moulds

Moulds belonging to five genera were isolated and identified from both the maize flour and the dry cassava (kokonte) flour. The different genera included *Cladosporium, Aspergillus, Mucor, Rhizopus* and *Penicillium* (Figure 1). Tables 3 and 4, show the different organisms isolated from the various flour samples from the different markets. These findings are contrary to that of Lu *et al.*, [10] who isolated only two genera; *Aspergillus* and *Penicillium* from their samples. The most predominant organisms isolated from the dry cassava (kokonte) flour were *Aspergillus flavus, Rhizopus stolonifer* and *Mucor hiemalis* while that for the maize flour were *Penicillium crustosum, Rhizopus stolonifer, Cladosporium cladosporioides* and *Aspergillus wentii*. The prevalence of these organisms in the various flour samples may be due to the ubiquitous nature of their spores as mentioned by O'Gorman et al. [13]. Studies have shown that some of these moulds produce mycotoxins which are known to be teratogenic, mutagenic, hepatotoxic, genotoxic and hepato carcinogenic depending on how long an individual gets exposed to the toxin [14].

FLOUR SAMPLE	ORGANISM ISOLATED
Aym MF1	*Mucor racemosis, Cladosporium cladosporioides, Aspergillus flavus, Aspergillus fumigatus, Aspergillus wentii, Aspergillus ochraceus, Aspergillus versicolor, Cladosporium herbarum, Penicillium crustosum, Penicillium camemberti*
Aym MF2	*Aspergillus ochraceus, Aspergillus wentii, Aspergillus fumigatus, Cladosporium cladosporioides, Penicillium crustosum*
Cm MF1	*Rhizopus stolonifer, Penicillium crustosum, Mucor racemosis,*
Cm MF2	*Aspergillus flavus, Rhizopus stolonifer, Cladosporium cladosporioides, Aspergillus wentii,*
Atm MF1	*No observable growth recorded*

Atm MF2	*Mucor hiemalis, Rhizopus stolonifer, Penicillium crustosum, Penicillium viridicatum*
Bm MF1	No observable growth recorded
Bm MF2	No observable growth recorded
CMF	No observable growth recorded

Key: Aym MF1-maize flour from vendor 1 at Ayigya market, **Aym MF2**- maize flour from vendor 2 at Ayigya market, **Cm MF1**-maize flour from vendor1 at central market, **Cm MF2**-maize flour from vendor2 at central market, **Atm MF1**- maize flour from vendor1 at Atonso market, **Atm MF2**- maize flour form vendor2 at Atonso market, **Bm MF1**- maize flour from vendor 1 at Bantama market, Bm MF2- maize flour from vendor 2 at Bantama Market, **CMF**- control maize flour.

Table 3: Identified Moulds on the Maize Flour from the Different Markets.

FLOUR SAMPLE	ORGANISM ISOLATED
Aym KF1	*Aspergillus flavus, Aspergillus wentii*
Aym KF2	*Aspergillus flavus, Penicillium crustosum, Cladosporium cladosporioides, Aspergillus wentii*
Cm KF1	*Rhizopus stolonifer, Aspergillus flavus, Penicillium crustosum, Cladosporium cladosporioides*
Cm KF2	*Rhizopus stolonifer, Cladosporium cladosporioides, Aspergillus flavus, Aspergillus wentii, Mucor hiemalis, Mucor racemosis,*
Atm KF1	*Aspergillus flavus, Rhizopus stolonifer, Mucor hiemalis, Penicillium viridicatum*
Atm KF2	*Aspergillus flavus, Mucor racemosis, Mucor hiemalis, Rhizopus stolonifer*
Bm KF1	*Mucor hiemalis, Rhizopus stolonifer, Aspergillus flavus, Penicillium viridicatum, Aspergillus versicolor, Aspergillus fumigatus*
Bm KF2	*Aspergillus fumigatus, Mucor hiemalis, Mucor racemosis Rhizopus stolonifer*

CKF	*Cladosporium cladosporioides, Penicillium crustosum,*

Key: Aym KF1-kokonte flour from vendor 1 at Ayigya market, **Aym KF2**- kokonte flour from vendor 2 at Ayigya market, **Cm KF1**-kokonte flour from vendor1 at Central market, **Cm KF2**-kokonte flour from vendor 2 at Central market, **Atm KF1**- kokonte flour from vendor1 at Atonso market, **Atm KF2**- kokonte flour form vendor 2 at Atonso market, **Bm KF1**- kokonte flour from vendor 1 at Bantama market, **Bm KF2**- kokonte flour from vendor 2 at Bantama market, **CKF**-control kokonte flour

Table 4: Identified Moulds on the Dry Cassava (kokonte) Flour from the Different Markets.

Total Plate count

Results from the total plate count is an important indication of the hygienic conditions surrounding the food and also shows the effectiveness and efficiency of the food chain process as well as the shelf life of the food [12]. In this study, the total plate count from the kokonte samples were higher (Table 5) than that reported by Lu et al. [10] who recorded 16×10 cfu/g. Only samples from Vendor-2 in Bantama market (Bm KF2) and the Control Kokonte Flour (CKF) recording $7.8 \times 10 \pm 0.30$ cfu/g and $5.63 \times 10 \pm 0.45$ cfu/g, respectively, were a little lower than the results obtained by Lu et al. [10]. For most of the samples, the level of contamination was found to be higher than the recommended level (10 cfu/g) and this is an indication of poor sanitation or problems resulting from the process control or handling of the raw material [12]. For the maize flour samples, except for samples from Bantama market Vendor-1 (Bm MF1) which did not record any growth for total plate count, the remaining samples recorded counts which exceeded the tolerable level. This is an indication that favourable conditions exist within the flour to support the growth of various organisms.

MARKET					
FLOUR SAMPLE	**Aym**	**Cm**	**Atm**	**Bm**	**Control**
MF1	$5.5 \times 10^4 \pm 0.62$a	$4.02 \times 10^5 \pm 3.05$a,b	$3.4 \times 10^3 \pm 0.62$a	NILa	$4.37 \times 10^3 \pm 0.83$a
MF2	$6.4 \times 10^5 \pm 0.30$a,b	$8.7 \times 10^4 \pm 0.96$a	$9.1 \times 10^6 \pm 0.25$c	$3.42 \times 10^6 \pm 2.52$b	
KF1	$3.3 \times 10^6 \pm 2.41$e	$6.4 \times 10^5 \pm 0.78$e	$4.43 \times 10^6 \pm 3.03$e	$4.33 \times 10^5 \pm 3.35$e	$5.63 \times 10^3 \pm 0.45$e
KF2	$4.6 \times 10^5 \pm 0.62$e	$4.64 \times 10^6 \pm 3.18$e	$3.76 \times 10^6 \pm 2.69$e	$7.8 \times 10^3 \pm 0.30$	

Table 5: Total Plate Count (cfu/g) of Organisms Isolated from Both Flour Samples.

Coliform Count

Most of the samples tested negative for coliforms except for maize flour sample bought from Vendor-2 from Atonso market (Atm MF2), dry cassava (kokonte) flour bought from the Central market Vendor-1(Cm KF1) and kokonte flour bought from Vendor-1(Bm KF1) (Table 6). Results from coliform test, which is an indicator of personal hygiene level of flour sellers [12], suggest that the high level of contamination may not necessarily be as a result of poor hygiene practices by flour sellers but other factors such as contamination during harvest and storage of cereal as mentioned by Victor et al. [12].

Sample	Test result	Sample	Test results
Aym MF1	Negative	**Aym KF1**	Negative
Aym MF2	Negative	**Aym KF2**	Negative
Cm MF1	Negative	**Cm KF1**	Positive
Cm MF2	Negative	**Cm KF2**	Negative
Atm MF1	Negative	**Atm KF1**	Negative
Atm MF2	Positive	**Atm KF2**	Negative
Bm MF1	Negative	**Bm KF1**	Positive
Bm MF2	Negative	**Bm KF2**	Negative
CMF	Negative	**CKF**	Negative

Table 6: Coliform Count on Samples.

Conclusion

The studies revealed that most of the flour samples had high moisture content and the level of mould count and total mesophilic microbe present in the flour exceeded the tolerable level indicating that consumers may be at health risk upon consumption of these samples.

References

1. Aworh OC (2008) The Role of Traditional Food Processing Technologies In National Development : the West African Experience. University of Ibadan.

2. Scudamore KA (2005) Identifying Mycotoxins is Paramount in the fight against their spread. J world Grain 23: 36-39.

3. Egbuta MA, Mwanza M, Njobeh PB, Phoku JZ, Chilaka CA, et al. (2015) Isolation of Filamentous Fungi Species Contaminating Some Nigerian Food Commodities. Journal of Food Research 4: 38-50.

4. Doolotkeldieva TD (2010) Microbiological Control of Flour-Manufacture: Dissemination of Mycotoxins Producing Fungi in Cereal Products. Microbiology Insite 3: 1-15.

5. University North Dakota State (2014) Flour Analysis-NDSU Wheat Quality & Carbohydrate Research.

6. Robert A, Ellen S, Jens C, Ole F (1995) Introduction to foodborne fungi (4thed.). The Netherlands: Centraal bureau voor Schimmel cultures.

7. Lokko GP (1978) Aflatoxin contamination of cassava flour (kokonte) processed by traditional methods in Ghana. University of Ghana 1-93.

8. FDA (2015) Safe Practices for Food Processes - Evaluation and Definition of Potentially Hazardous Foods-Chapter 3. Factors that Influence Microbial Growth. Center for Food Safety and Applied Nutrition

9. African Organization for Standardization (2012) ARS 838: Cassava flour --Specification -ars.838.2012.pdf. https://law.resource.org/pub/ars/ibr/ars.838.2012.pdf (Accessed on January 24, 2016)

10. Lu JY, Pace R, Plahar W (1988) A Research Note Survey of the Microbial Quality of Dry Fish, Cassava and Okra in Ghana. Journal of Food Protection 51: 660-662.

11. Adu-Gyamfi A, Appiah (2012) Enhancing the Hygienic Quality of Some Ghanaian Food Products by Gamma Irradiation. Food and Nutrition Sciences 3: 219-223.

12. Victor N, Bekele MS, Ntseliseng M, Makotoko M, Peter C, et al. (2013) Microbial and Physicochemical Characterization of Maize and Wheat Flour from a Milling Company, Lesotho. International Journal of Food Safety 15: 11-19.

13. O'Gorman CM, Fuller HT, Dyer PS (2009) Discovery of a sexual cycle in the opportunistic fungal pathogen Aspergillus fumigatus. Nature 457: 471-474.

14. Fung F, Clark RF (2004) Health effects of mycotoxins: a toxicological overview. Journal of Toxicology Clinical Toxicology 42: 217-234.