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Impact of Milling on the Microbiological Quality of Yam Flour in Southwestern Nigeria

[1]Y.M. Somorin, [1]M.O. Bankole, [1]A.M. Omemu and [2]O.O. Atanda

[1]Department of Microbiology, University of Agriculture, P.M.B 2240, Alabata Road, Abeokuta, Ogun State, Nigeria

[2]Department of Foodservice and Tourism, University of Agriculture, P.M.B 2240, Alabata Road, Abeokuta, Ogun State, Nigeria

Corresponding Author: Y.M. Somorin, Department of Microbiology, University of Agriculture, P.M.B 2240, Alabata Road, Abeokuta, Ogun State, Nigeria Tel: +234-8050901065

ABSTRACT

The impact of the milling process on the microbiological quality of yam flour produced from dried yam chips was investigated. Dried yam chips samples were procured from markets in Lagos, Ogun and Oyo states, southwestern Nigeria. Total viable bacterial count for *Dioscorea rotundata* (white yam) flour milled across the three locations range from 2.5×10^5 to 4.33×10^5 cfu g^{-1} while *D. alata* (water yam) flour ranged from 2.03×10^5 to 4.72×10^5 cfu g^{-1}. Yam chips milled in the market had significantly higher ($p<0.05$) total viable bacterial count compared to those milled in the laboratory. Milling machines at Ibadan market harboured the significantly highest microbial count (2.1×10^3 cfu cm^{-1}). All the yam flour samples milled in the market had *Bacillus megaterium* and *Staphylococcus saprophyticus*. *Fusarium oxysporum, Aspergillus niger* and *Rhizopus nigricans* were isolated from both white yam flour and water yam flour. Milling introduced some fungi known to produce mycotoxins into the yam flour. Milling yam chips into flour in the machines available at the markets increased the microbiological contamination of the yam chips by between 10^1->10^2 folds due to some unhygienic practices observed during the milling and this has implications for the microbiological quality and safety of the yam flour meal consumed. Educating processors of yam flour on the importance of regular cleaning of milling machines and avoiding collection of flour spilled on the floor into the lot to be consumed will assist in ensuring that best practices are complied with and consumers have access to safer yam flour.

Key words: Milling machine, yam chips, yam flour, microorganisms, hygiene, food safety

INTRODUCTION

Yams (*Dioscorea* spp.) constitute an economically important staple food in tropical and subtropical regions of the world. Yam tubers have high carbohydrate contents (Kouassi *et al*., 2009) and are also sources of proteins, fats, vitamins and minerals for many people. Production and consumption of yam is predominant in West and Central Africa (Otegbayo *et al*., 2005). Yam, *Dioscorea* spp., is the second most important root tuber crop in Africa, after cassava, with the production of cassava being about 22% more than that of yam. Nigeria is the largest producer in the world with over 35 million metric tonnes (FAOSTAT, 2010) with Kogi state being one of the major yam producing states (Ekunwe and Orewa, 2007).

The nutritional composition of yam is mainly starch but has varying levels of proteins, lipids, minerals and most vitamins except Vitamin C (Huang *et al*., 2007). The most preferable method of consuming yam is by boiling and pounding because this gives it a soft texture and allows for easy swallowing. In Nigeria, yam is also processed into various staple, intermediate and end-products. In West Africa, yam could be processed into flour which is used to prepare amala, boiled and eaten or pounded (Otoo and Asiedu, 2009). Infection of yam by microorganisms could be at any stage in its growth, from seedling stage through to postharvest (Amusa *et al*., 2003). Yams are subjected to several diseases. Some fungal species have been associated with the deterioration of yam tubers during storage (Okigbo and Ikediugwu, 2000). Therefore, to overcome the high perishability of yam, seasonal nature of yam production and also to serve as a preservative method, yams are processed into dried chips (Hounhouigan *et al*., 2003).

Drying can improve the shelf life of the tubers which have high moisture content and a unique process have been developed in Nigeria and Benin Republic (Akissoe *et al*., 2001). *Dioscorea rotundata* (white yam) are mostly used for this process because they are thought to give the best "amala", however, it has also been shown that tubers from *D. alata* can give "amala" as appreciated as the one produced from white yam (Ukpabi *et al*., 2008).

Usually, yam chips are sold out directly to consumers who in turn mill the chips into yam flour, sieve through a 1 mm wire mesh and stir in boiling water to form "amala" (Bankole and Mabekoje, 2004). The quality of the dried yam chips varies from location to location and from processor to processor (Mestres *et al*., 2004). Though information on microbiological safety of the dried chips from white yam has been reported, there is no information on the microbiological quality of chips from water yam and the impact of the milling practices on the microbiological quality and safety of the yam flour is not available. This study was undertaken to investigate the microorganisms associated with dried yam chips from white yam and water yam and examine the influence of the milling process on the microbiological quality of the resulting yam flour.

MATERIALS AND METHODS

Collection of samples: This study was conducted in 2009/2010. Dry yam chips samples were collected from Kuto market in Abeokuta, Ogun State; Mushin market in Lagos State and Oja-Oba market in Ibadan, Oyo State. Two kilogrammes each of white yam and water yam were collected aseptically into sterile airtight containers at five different sales points within each market. The samples were milled in milling machines available in the markets and from each sample; two flour samples weighing 200 g each were obtained. This was done in the three markets except for Kuto market where only white yam chips were available during the sampling. In total, 10 samples of white yam flour were taken in each of the three markets and 10 samples of water yam were collected from Mushin and Ibadan. Another set of all the yam chips collected was milled in sterilized milling machines in the laboratory.

The samples were labeled and transported at ambient temperature to the laboratory immediately after collection. Swabs of milling machines used for yam flour production were also collected prior to the milling of the samples. All samples were kept at 4°C pending microbiological analysis.

Microbiological analysis: The total viable bacterial count of the yam flour samples and the milling machines were determined by the pour plate method procedure. All microbiological media used were prepared according to the manufacturer's instructions.

Ten grams of yam flour samples were homogenised with 90 mL of 0.1% sterile peptone water in screw capped flasks by means of horizontal and vertical agitation for a few minutes to obtain the

10^{-1} dilution. The swabs of the five milling machines used in milling yam chips in each of the locations were dipped into 10 mL of 0.1% sterile peptone water in screw capped flasks and agitated as described earlier. Further ten-fold serial dilutions were made up to 10^{-5} for colony count.

One mL volume of each dilution was over-poured with 10-15 mL of Plate Count Agar (Difco, USA) for bacterial culture and Potato Dextrose Agar (LABTEC) amended with 60 μg mL^{-1} chloramphenicol (PDAC) for fungal culture. Triplicates of each set-up were made. All inoculated plates of Plate Count Agar were incubated at 30°C for 48 h while the inoculated PDAC plates were incubated at 25°C for 3-5 days. The colonies were counted and recorded. The different colonies on the plates were isolated, purified and stored on Nutrient Agar (NA) (LABTEC) slants for further characterization and identification.

The bacterial classifications were made using a series of cultural and biochemical test based on methods described by Ochei and Kolhatkar (2008). Cultural and morphological identification of mould isolates was according to structure of mycelium, conditions of branches, presence of conidiophores, sclerotia and shape as compared with Barnett and Hunter (1987), Pitt and Hocking (1997) and Samson *et al.* (2004).

Statistical analysis: The mean of total viable bacterial count obtained from the yam flour samples was subjected to analysis of variance (ANOVA) and Duncan Multiple Range Test to separate the means and it was determined at the 5% probability level using SPSS 16.0 for Windows (SPSS Inc., NY).

RESULTS

Commercial milling machines used in milling yam flour had microorganisms which ranged from 1.32×10^{3} cfu cm^{-1} in Abeokuta to 2.1×10^{3} cfu cm^{-1} in Ibadan, with the total viable bacterial count differing significantly ($p<0.05$) in the markets (Table 1). *S. epidermidis, B. megaterium, Aspergillus flavus, Rhizopus oryzae, A. niger* and *P. oxalicum* were the commonly isolated microorganisms in the commercial milling machines.

The total viable bacterial count of the yam flour ranged from 3.85×10^{3} to 4.72×10^{5} cfu g^{-1}, irrespective of the location of collection and milling machine employed. The flour harboured varying microbial loads with the flour milled with laboratory milling machine recording the lowest total viable bacterial count of 3.85×10^{3} (Table 2) while the highest was 4.72×10^{5} cfu g^{-1} (Table 2), milled from a commercial mill at Ibadan.

Table 1: Total viable bacterial count and Microorganisms isolated from milling machines used in yam flour processing

Location	Total viable bacterial count (cfu cm^{-1})	Bacteria isolated	Fungi isolated
Abeokuta	1.32×10^{3c}	*Enterobacter aerogenes*,	*Aspergillus flavus*, *A. niger*,
		Bacillus megaterium,	*Penicillium oxalicum*,
		Staphylococcus epidermidis,	*A. fumigatus*,
		S. saprophyticus,	*Fusarium verticillioides*,
		Klebsiella oxytoca	*Rhizopus oryzae*
Ibadan	2.1×10^{3a}	*S. aureus*, *S. epidermidis*,	
		B. badius, *Corynebacterium* spp.	
		B. megaterium,	ND
Mushin	1.63×10^{3b}	*K. pneumoniae*, *B. megaterium*, *S. epidermidis*,	*R. oryzae*, *P. oxalicum*, *A. niger*,
		Proteus mirabilis, *Corynebacterium* spp.	*A. flavus*, *P. citrinum*, *Mucor* spp.

ND: Not determined. Means within column with the same letter are not significantly different ($p>0.05$)

Table 2: Total viable bacterial count and Microorganisms isolated from white yam flour and water yam flour

	Laboratory milled				Commercially-milled			
	White yam		Water yam		White yam		Water yam	
Sample location	Total viable bacterial count (cfu g^{-1})	Bacteria isolated	Total viable bacterial count (cfu g^{-1})	Bacteria isolated	Total viable bacterial count (cfu g^{-1})	Bacteria isolated	Total Viable bacterial count (cfu g^{-1})	Bacteria isolated
Ibadan	3.85×10^{3b}	*B. megaterium* *K. oxytoca*, *S. aureus*, *P. aeruginosa*,	7.5×10^{3a}	*B. megaterium*, *K. oxytoca*, *S. aureus*, *P. aeruginosa*	4.32×10^{5a}	*B. badius*, *B. megaterium*, *S. saprophyticus*	4.72×10^{5a}	*B. badius*, *B. megaterium*, *S.saprophyticus*, *Corynebacterium* spp
Mushin	1.81×10^{4a}	*S. aureus*, *Proteus mirabilis*, *Pr. vulgaris*, *B. megaterium*, *Enterobacter cloacae*	2.0×10^{4a}	*Ps. aeruginosa*, *Pr. mirabilis*	2.5×10^{5a}	*Escherichia coli*, *B. megaterium* *En. aerogenes*, *S. saprophyticus*, *K. pneumoniae*, *S. aureus*,	2.03×10^{5a}	*S. aureus*, *En. aerogenes*, *S.saprophyticus*, *B. megaterium*
Abeokuta	1.88×10^{4a}	*B. megaterium*, *K. oxytoca*, *S. aureus*, *Pr. vulgaris*, *En. cloacae*, *Corynebacterium* spp, *Ps. aeruginosa*	ND	ND	4.33×10^{5a}	*B. megaterium*, *K. oxytoca*, *S. saprophyticus*, *S. epidermidis*, *Corynebacterium* spp, *Edwardsiella tarda*, *B. badius*	ND	ND

ND: Not determined. Mean values within a column with the same letter are not significantly different ($p>0.05$)

Table 3: Fungi isolated from water yam flour and white yam flour

Sample location	White yam flour	Water yam flour
Abeokuta	*Fusarium oxysporum, Aspergillus niger*	ND
Ibadan	*F. oxysporum, A. niger, A. fumigatus*	*F. oxysporum, A. niger*
Mushin	*R. nigricans, A. niger* *A. flavus, F. oxysporum,* *Penicillium citrinum, P. oxalicum*	*Rhizopus nigricans, A. niger*

ND: Not determined

For white yam flour milled in the laboratory, Abeokuta samples had the highest mean total viable bacterial count of 1.88×10^4 cfu g^{-1} whereas the Ibadan samples had the lowest count with 3.85×10^3 cfu g^{-1} (Table 2). Samples from Ibadan had a significantly higher ($p<0.05$) count compared to samples sold in Mushin and Abeokuta. Market-milled white yam flour from Abeokuta had highest mean count of 4.33×10^5 cfu g^{-1}, Ibadan samples had 4.32×10^5 cfu g^{-1}, with the Mushin samples having the lowest count (2.5×10^5 cfu g^{-1}) (Table 2). The total viable bacterial count did not differ significantly ($p>0.05$) in samples from the three locations. Water yam flour from Mushin that was milled in the laboratory had a higher total viable count compared to those from Ibadan, however, this difference was not significant ($p>0.05$) (Table 2).

The diversity of bacteria isolated from white yam and water yam flour milled in the laboratory are shown in Table 2. The bacteria isolated from white yam flour and water yam flour were similar except for *Corynebacterium* spp. and *Proteus vulgaris* which were not found in the water yam flour. Generally, the total viable bacterial count of laboratory-milled yam flour was significantly lower ($p<0.05$) than that of yam flour milled at the commercial milling machines available in the markets. Yam flour milled at Mushin market had the lowest total viable bacterial count of 2.5×10^5 cfu g^{-1} for white yam and 2.03×10^5 cfu g^{-1} for water yam.

Staphylococcus aureus, Enterobacter aerogenes, S. saprophyticus, Bacillus megaterium, B. badius and *Corynebacterium* spp. were found to commonly contaminate both water yam and white yam flour and in addition *Edwardsiella tarda, Escherichia coli, S. epidermidis* and *K. pneumoniae* were found only on white yam flour. *Fusarium oxysporum, Aspergillus niger* and *Rhizopus nigricans* were found in both white yam flour and water yam flour, however, *Aspergillus fumigatus, Aspergillus flavus, Penicillium citrinum* and *P. oxalicum* were additionally isolated from white yam flour (Table 3).

The microorganisms commonly isolated from all the yam flour samples irrespective of variety, location and milling procedure were *B. megaterium* and *A. niger* (Table 2 and 3). White yam flour had more types of microorganisms than water yam flour.

DISCUSSION

This microbial diversity could be as a result of the cross-contamination from other food items since the mills are also used for other food items. Residue build-up in milling machines could also constitute a significant source of microbiological contamination (Berghofer *et al*., 2003).

The high microbial count might be due to microorganisms already present on the dry yam chips from where the flour was obtained, the method of milling and the milling machine used. This is in agreement with Babajide *et al*. (2006) and Djeri *et al*. (2010) who had observed the presence of bacteria on dry yam chips from different processing sites in Nigeria and Togo, respectively. The procedure whereby raw yam exhumed from the soil is peeled and cut into chips to be sun-dried on broom-swept cemented floors or on mats may predispose dry yam chips to soil and other environmental contamination.

All the commercially milled yam flour samples exceeded the recommended levels by International Commission on Microbiological Specifications for Foods (1998). This indicates contamination of the yam flour by microorganisms in the milling machines and the unhygienic practices by the processors. *Bacillus* spp. was abundant in both yam flour types and this was similar to a previous report in fruit juices where *Bacillus* was the most diverse organism (Addo *et al*., 2008). Milling machines used are often not specifically used for dry yam chips alone but for other food materials such as various dry cereals which may harbour other various microorganisms. Also, these milling machines are not sanitized regularly and hence, a high possibility of cross-contamination. Some processors crush the yam chips directly unto the cemented floors instead of into buckets or containers. The crushed materials are then swept and gathered together from the floor before grinding into flour.

Processors in Mushin prevented the flour from spilling on the bare ground and thus eliminating that point of contamination. Proper and hygienic processing practices have been related to the quality of the yam chips. The practices employed during the milling stage of processing are thus very important to the safety of the yam flour. In a related study by Darman Roger *et al*. (2007), low hygienic quality was also reported in cassava-based products indicated by total aerobic count as high as 56×10^5 cfu g^{-1}.

Some of the isolated organisms are indicators of faecal contamination and they could have contaminated the yam chips and flour during the parboiling stage and handling of the yam chip and flour (Ehiri *et al*., 2001). Similarly, the yam chips could have been contaminated from the practice of spreading yam chips on bare grounds and thus contact with the soil during drying.

Some of the isolated fungi are important in food safety as they have been reported to produce mycotoxins which have varying implications for health and the economy especially in developing countries. *Aspergillus, Fusarium* and *Penicillium* are the most important mycotoxigenic genera of fungi and they were recovered from the samples used in this study. Similar results were reported by Gnonlonfin *et al*. (2008) who found species of *Aspergillus*, *Fusarium* and *Penicillium* contaminating dry yam chips in Benin Republic. Moulds isolated from yam flour in this study were also similar to those reported by Jimoh and Kolapo (2008) in yam chips and other foodstuffs obtained from markets in Ibadan, Nigeria. Though the incidence of the mycotoxigenic fungi is low in this study, the possibility of mycotoxin contamination of the yam flour cannot be ruled out.

Some microorganisms isolated from this work have been reported to produce toxins which could lead to several disease conditions in man. For example, species of *Bacillus* have been found to produce cereulide which is heat-stable and produces a vomiting-type syndrome (Hoton *et al*., 2009). Food poisoning cases involving vomiting and seizures soon after the consumption of yam flour meal has been reported in families in Nigeria with high severity in children (Adedoyin *et al*., 2008; Adeleke, 2009). In recent times, food poisoning with similar symptoms due to consumption of yam flour meals have been on the increase in Nigeria. Similar cases of outbreaks of food poisoning involving families have been reported (Dierick *et al*., 2005; Shiota *et al*., 2010). Furthermore, some of the moulds isolated from this work have been reported to produce mycotoxins which have been implicated in immune suppression, liver cancers and even death (Probst *et al*., 2007; Williams *et al*., 2004).

CONCLUSION

This study highlights the unhygienic practices employed during the processing of yam chips into yam flour and also the contribution of the milling process not only to increasing the bacterial load

of the yam flour but also introducing other organisms that could be pathogenic into the food product. This is evident in the higher microbial counts, between 10^1->10^2 folds, found in the yam flour samples milled in the markets and the wide diversity of microorganisms which have been reported to be pathogenic to man and agents of food spoilage. The milling process is a critical point in the production of yam flour and could determine the quality of the yam flour presented to the consumers. This study will help food regulators in Nigeria to understand the situation of practices employed by millers of yam flour and the possible health risks associated with such unhygienic practices especially in the wake of the increasing food poisoning cases reported due to consumption of yam flour meals. Processors of yam flour should be educated on the importance of regular cleaning of their milling machines and avoid the collection of flour spilled on the floor into the lot to be consumed by man. Also, inspection of processing units by responsible government agencies will ensure that the best practices are complied with and consumers will have access to safer yam flour.

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