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Effect of Fermentation on Aflatoxin Content of Ogi Produced from Mouldy Maize (*Zea mays*)

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Abstract

Mycotoxins are toxins produced naturally by fungi. They are carcinogenic and can affect food quality and safety. Effect of fermentation on aflatoxin content of ogi produced from moldy maize was investigated in this study. Moldy and non-moldy maize grains were fermented and proximate, microbial and aflatoxin content analyses were carried on the samples for 72 hr fermentation periods at 24 hr intervals. Ogi was produced using the standard method of production and dried to give Ogi flour. Results of the proximate analysis show decrease in protein, crude fiber, ash and carbohydrate contents during the fermentation periods. The fat content of steeped non-moldy maize increased (4.32%-4.36%) compared to that of moldy maize (3.94%-4.01%), but they are not significantly different. Microbial analysis showed a reduction in yeast and mold counts, with values ranging from 7.0-0.50 CFU /g × 10^4 in non-moldy maize and 11.45-2.45 CFU/g × 10^4 for moldy maize at the end of 72 hr fermentation. Increased values of Lactic acid bacteria counts were observed at 48hr fermentation for both samples. During fermentation, aflatoxin contents in the moldy grains reduced from initial concentration of 58.00 µg/kg in the raw maize sample to 3.13 µg/kg at 72 hr fermentation period. This study has shown that aflatoxins content in moldy maize can be reduced by natural fermentation processes.

Keywords: Fermentation; Aflatoxin; Ogi; Maize (*Zea mays)*

Introduction

Aspergillus flavus and *Aspergillus parasiticus*, are naturally occurring mycotoxins that are produced by fungi [1]. Aflatoxin B_1 is considered the most toxic and the presence of *Aspergillus* in food products may not always indicate that harmful levels of aflatoxin also are present; it implies a significant risk in consumption. Factors related to environmental and agricultural practices favor mycotoxin contamination of food materials in most of the African continents. Biological decontamination of fungi/mycotoxins by microorganisms has been reviewed in some papers [2-5]; however, there are little research findings on the reduction of aflatoxins by microorganisms during fermentation and its implications. The overall objective of this research is to the effect of fermentation on the reduction of aflatoxin content in moldy maize (Ogi).

Materials and Methods

Sample collection

The white variety of matured moldy and non-moldy maize grain samples (3 kg each) were purchased in a local market (Mushin) in Lagos, Nigeria. Selection of samples was based on the visual appearance of the grains. A portion of the sample was finely milled in an electric grinder for initial analysis of Aflatoxin content while the other portion was kept for ogi processing.

Ogi processing

Both samples of maize grains were cleaned and winnowed to remove foreign matter. The maize grains were further washed to remove other impurities and dust adhering to the grain surface. The grains were then steeped in water almost double the weight of the grains at room temperature for 72 hours in a vessel which softens the kernels in preparation for milling and allows room for some fermentation to take place. The liquor was drained off, the grains were rinsed in fresh clean water, and further wet-milled into a slurry and sieved with a muslin cloth to remove fiber and a portion of the germ, the filtrate was allowed to settle before oven drying into flour.

At 0 hr, 24 hr, 48 hr and 72 hr of fermentation, samples were analyzed for different parameters, such as:

- Proximate composition: using standard procedure [6] (Table 1)
- Total Aflatoxin content: using ELISA method with Agraquant kit
- Microbiological Analyses following the standard procedures

Results and Discussion

Changes in the proximate composition of non-moldy and moldy maize during fermentation

Results indicated that moisture content for both steeped non-moldy and moldy maize throughout the fermentation periods were significantly different ($p = \leq 0.05$). The protein content for both samples decreased from 24 hr till the end of the 72 hr fermentation period. Fermentation resulted only in a marginal improvement in the fat contents for both samples, though not statistically significant. This may be due to increased activity of the Lipolytic enzymes in the fermentation medium which hydrolyzed fat to glycerol and fatty acid [7].

Fermentation significantly decreased the crude fiber content of non-moldy maize during the steeping period from 3.05% (raw maize) to 2.31% (72 hr steeping) and from 2.8% (raw maize) to 2.06% (72 hr steeping) for moldy maize. This decrease in fiber content may be attributed to enzymatic degradation of the fibrous material during fermentation [8]. However, ash content decreased significantly ranging from 1.31% (raw maize) to 0.97% (72 hr steeping) for none moldy

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maize during the steeping period and from 1.23% (raw maize) to 0.62% (72 hr steeping) for moldy maize. Akubor and Chukwu [9], reported decreases in the ash content of fermented oilseeds.

Carbohydrate contents of both maize samples during the steeping period significantly decreased as fermentation progresses. This is in agreement with several researchers [9-11]. The decrease could be attributed to the selective utilization of carbohydrates as an energy source by fermenting microorganisms [12]. Increased activities of α-amylase which hydrolyzes starch to simple sugar could also be responsible for the reduction [13].

Changes in aflatoxin content of non-moldy and moldy maize

Table 2 and Figure 1 shows a decrease in aflatoxin contents of both samples. At 48 hr steeping the now moldy grains has no aflatoxin, similar trend could also be seen in moldy grains, where aflatoxin contents reduced from initial concentration of 58.00 µg/kg in the raw maize sample to 3.1 µg/kg at the 72 hr steeping period, which is still within the maximum acceptable limit of 10.0 µg/kg. The toxicity of the product was significantly reduced after fermenting with a progressive decrease in the pH. This is in agreement with other studies, which clearly show that lactic acid bacteria (*Lactobacillus* strains) involved in natural fermentation efficiently remove aflatoxin from infested raw materials [14,15]. It has been reported that removal of toxins is through non-covalent binding of mutagens by fractions of the cell wall skeleton of lactic acid bacteria [16]. Live microorganisms can absorb either by attaching the aflatoxin to their cell wall components or by active internalization and accumulation [17]. Yeast and LAB cells are known to bind different molecules such as killer toxins and metal ions on complex binding structures on the cell wall surface [18]. Differences between strains of LAB with respect to aflatoxin binding indicate that binding ability is highly strained specific [19]. In some of the earlier studies, LAB is considered to be an inefficient binders of aflatoxin B1 [20,21]. This may be due to the strains used in those studies were binding low amounts of aflatoxins occurs.

Effect of fermentation on the microbial contents of non-moldy and moldy maize grains during fermentation

Results on Table 3 indicated a reduction in the yeast/mold count at 48 hr and 72 hr fermentation period in both samples. In the moldy maize sample, yeast/mold counts decreased to 50% at 48 hr and to 96% at 72 hr of fermentation. The bacterial population increased with an increase in fermentation time in both moldy and non-moldy maize samples. Fermentation resulted in increased Lactic acid bacteria counts at 48 hr for both samples. This increase in Lactic Acid counts as observed in this study is comparable to various studies carried out on fermented cereals [22,23]. The presence of molds at the initial stage of fermentation of maize for Ogi production and the subsequent elimination had been reported previously [24,25]. Mucoraceae fungi have roles in the initial phase of fermentation mostly in saccharification of the substrates [26]. The study also confirmed the major involvement of LAB in Ogi fermentation.

Conclusion

Mycotoxin-contaminated cereals can possibly be used in Ogi production; this, however, does not substitute the use of grains that

Fermentation							
Sample	**Duration**	**Moisture content (%)**	**Crude protein (%)**	**Crude fat (%)**	**Crude fibre (%)**	**Crude ash (%)**	**Carbohydrate (%)**
SNMM	0 hr	9.66 ± 0.02	9.75 ± 0.30	4.32 ± 0.03	3.05 ± 0.01	1.31 ± 0.18	71.86 ± 0.03
SMM		8.09 ± 0.01	9.13 ± 0.10	3.94 ± 0.20	2.80 ± 0.26	1.23 ± 0.04	74.84 ± 0.01
SNMM	24 hr	29.47 ± 0.18	9.67 ± 0.37	4.33 ± 0.25	2.64 ± 0.33	1.13 ± 0.10	52.76 ± 0.12
SMM		33.42 ± 0.14	8.96 ± 0.10	3.95 ± 0.06	2.71 ± 0.50	0.84 ± 0.00	50.12 ± 0.00
SNMM	48 hr	32.62 ± 0.52	9.20 ± 0.10	4.36 ± 0.56	2.52 ± 0.39	1.04 ± 0.10	50.26 ± 0.03
SMM		35.58 ± 0.32	8.43 ± 0.12	3.98 ± 0.21	2.32 ± 0.13	0.75 ± 0.10	48.94 ± 0.13
SNMM	72 hr	33.15 ± 0.05	9.13 ± 0.10	4.36 ± 0.24	2.31 ± 0.01	0.97 ± 0.10	50.11 ± 0.05
SMM		34.73 ± 0.47	8.42 ± 0.10	4.01 ± 0.01	2.06 ± 0.03	0.62 ± 0.38	50.16 ± 0.01

*Mean of triplicate values ± Standard deviation.

Legend:

SNMM-Soaked Non Mouldy Maize

SMM-Soaked Mouldy Maize

Table 1: Proximate composition of non mouldy and mouldy maize during fermentation.

Sample	Fermentation Duration	Aflatoxin content (µg/kg)
SNMM	0 hr	0.77[a]
	24 hr	0.50[ab]
	48 hr	ND[c]
	72 hr	ND[c]

*Values with the same superscript are not significantly

Different (p= ≤ 0.05).

Legend:

SNMM-Soaked None Mouldy Maize

ND-None Detected

Table 2: Aflatoxin contents of none mouldy maize during fermentation.

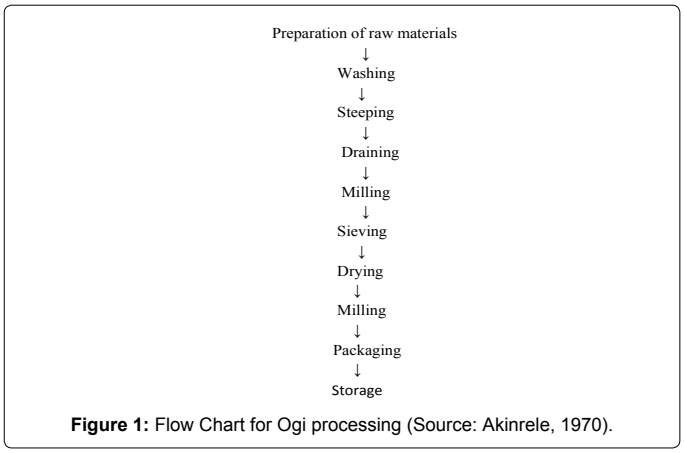

Figure 1: Flow Chart for Ogi processing (Source: Akinrele, 1970).

Samples	Fermentation duration	Lactic acid bacteria count (cfu/g × 10^4)	Total bacteria count (cfu/g × 10^4)	Yeast/Mold count (cfu/g × 10^4)
SNMM	0 hr	ND	0.10 ± 0.10	ND
SMM	0 hr	ND	0.50 ± 0.04	0.10 ±0.10
SNMM	24 hr	9.20 ±0.10	3.70 ± 0.10	7.00 ± 0.40
SMM	24 hr	0.45 ± 0.2	0.97 ± 0.41	11.45 ± 0.10
SNMM	48 hr	30.75 ± 0.39	6.15 ± 0.05	6.19 ± 0.41
SMM	48 hr	30.45 ± 0.04	1.50 ± 0.13	6.25 ± 0.01
SNMM	72 hr	4.80 ± 0.30	2.0 0± 0.40	0.50 ± 0.05
SMM	72 hr	2.95 ± 0.40	ND	2.45 ± 0.50
*Mean of replicate values ± Standard deviation **Legends:** SNMM-Soaked None Mouldy Maize SMM-Soaked Mouldy Maize				

Table 3: Microbial load of none mouldy and mouldy maize during fermentation.

are free of mycotoxin contamination. Further studies may be done on the screening of mycotoxin-degrading microorganisms, and this might lead to the detection of efficient and applicable ones which may be engineered to improve the quality and safety of foods, thereby protect consumer's health. It can be concluded that microorganisms during fermentation of cereals, may be responsible for mycotoxin reduction.

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