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Full Length Research Article

FERMENTATION AND BAKING REDUCED AFLATOXIN B_1 RETENTION OF WHOLE WHEAT FLOUR LEAVENED WITH *SACCHAROMYCES CEREVISAE* ISOLATED FROM SOME CEREAL BASED FERMENTED FOODS

*[1] J.R. Wartu, [2] C.M.Z. Whong, [2]I.O. Abdullahi and [2]J.B. Ameh

[1]Department of Microbiology, Faculty of Science, Kaduna State University, Kaduna, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria

*Corresponding Author Email Address: reubjw@gmail.com Phone: +2348037348233

ABSTRACT
Aflatoxins are the most common occurring mycotoxin and most potent hepatocarcinogen known to mammals. The aim of this research was to isolate *Saccharomyces cerevisae* from cereal based fermented foods (*ogi, madidi and kunun-zaki*) for leavening bread and to study aflatoxin B_1 reduction potential of solid state fermentation and baking of whole wheat flour dough into leavened bread. Proximate values were determined using standard techniques. While alpha amylase activity of the whole wheat flour was determined by Hagberg falling number (HFN), the viscoelastic property and dough strength of the flour was determined using Chopin alveograph. Enzyme Link Immunosorbent Assay technique was employed to detect and quantify aflatoxin B_1 from the samples. The alpha amylase of the whole wheat flour was 240 seconds, while the viscoelastic property of the flour was 230 joules. The aflatoxin level during fermentation of the wheat flour dough showed that within 60, 90 and 120 minutes of fermentation at 35±2°C/2 h, the percentage aflatoxin loss increased as follows; 10.3. 34.5 and 55.2 % respectively. Conversely, the total aflatoxin retention decreased in this order; 89.7, 65.5 and 44.8% respectively. As the fermentation time increased, the percentage aflatoxin loss increased and the percentage aflatoxin retention decreased. The baking profile of the fermented dough also showed that as the baking temperature increased from 180, 200 and 220°C the aflatoxin loss increased from 58.6, 62.1 and 72.4 % and the aflatoxin B_1 retention also decreased 41.4, 37.9, and 27.6% respectively. Fermentation and baking of whole wheat flour indicated that more than half of the toxin was eliminated but, some miniatures of toxins were still present in the fermented dough and the baked bread. This study showed that the *Saccharomyces cerevisiae* isolated from *ogi* has strong potential for leavening and was comparable to the commercial baker's yeast. The use of wholesome raw material for bread production is advocated, because, the left over miniatures of aflatoxin in the finished products could be a health threat to consumers of bread produced from contaminated whole wheat flour.

Keywords: *Dough, fermentation, baking and aflatoxin retention*.

INTRODUCTION

The major microbial metabolite challenge associated with bread is total aflatoxins (B_1, B_2, G_1 and G_2) contamination. Aflatoxins are difuranocaumarin derivatives that are mutagenic and carcinogenic. Aflatoxin B_1 is the most toxic and thus been classified by the International Agency of Research on Cancer (IARC) as Group 1 carcinogen (IARC 1993; Hwang and Lee, 2006). Preston and Williams (2005) explained that aflatoxin B_1 (AFB_1) is bio-converted to its more damaging form, by the action of cytochrome genes. The bio-converted form then binds DNA at a specific codon within the *TP53* tumor-suppressing gene, mutating it to inactivate its cancer-protective actions (Sporn *et al*., 1966; Scaife, 1971; Preston and Williams, 2005). Other workers have reported the incidence of aflatoxins in maize (Hell *et al*., 2000), wheat flour (Gashgari *et al*., 2010; Bhat and Fazal, 2011) and wheat flour product such as bread (Chelae *et al*., 2003; Gumus *et al*., 2009). Wheat flour is a free flowing wheat product with particle size ranging from 90 – 180µm (NIS 2004). Wheat (*Triticum* sp Link) (Shewry, 2009; Donner *et al*., 2000) is a cereal grain. Unrefined wheat grain contain some nutritional elements; B_1, B_2, B_3, E, folic acid, calcium, phosphorus, zinc, copper, iron, and fiber (Belderok *et al*., 2000). Apart from carbohydrates, the mealy endosperm of wheat contains fats (1.5%) and very high proteins level (13-18 %), albumins and globulins (Šramkova *et al*., 2009). Wheat contains two major proteins in form of gluten complex; glutenins and gliadins (Duarte *et al.,* 2010). American wheat *cultivar* with high quality gluten content has been majorly used in Nigeria as a raw material to produce white wheat flour and whole wheat flour. Both flours have been used as a raw material for production of leavened bread. Bread is nutritious, being a product of wheat flour.
The aim of this research was to study aflatoxin B_1 reduction potential of solid state fermentation during dough development and baking of whole wheat flour leavened with commercial yeast (safeinstant yeast) and *Saccharomyces cerevisae* isolated from fermented cereals for dough leavening into whole wheat bread.

MATERIALS AND METHODS

Isolation and Identification of *Saccharomyces cerevisae* from some Nigeria Cereal Based Fermented foods

The yeast *Saccharomyces cerevisae* was isolated from some cereal based Nigeria fermented foods (*orgi, madidi* and *kunun-zaki*) as follows: Sabouraud`s Dextrose (SDA) was prepared according to manufacture instruction and supplement with chloramphenicol for selective enumeration of yeast. Ten (10) gram of each food sample was homogenized in 90 mL of sterile peptone water. Then one (1.0 mL) of the supernatant was inoculated using spread plate techniques. Pure culture was made by sub culturing. Microscopic examination of the isolates was carried out using the method of Thais and Danilo (2002).

Colonial Morphology
Distinct colony morphology of the pure isolates on solidified Sabouraud`s Dextrose Agar (SDA Oxoid) was observed visually for the following feature; color, edge, opacity, and elevation.

Microscopic Identification
The technique adopted by Ijah *et al*. (2015) was used for the microscopic identification of yeast based on cultural characteristics using lactophenol cotton blue staining. Briefly, a drop of lactophenol cotton blue stain was placed on a clean grease-free slide using a dropper. Then a film of the 72 h old fungal mycelium was aseptically picked from the pure culture plate using a sterilized inoculating needle. The mycelium was emulsified and teased in the drop of the lactophenol cotton blue stain and then covered carefully with a cover slip to prevent trapping of air bubbles. The slide was then mounted on light compound microscope and observed under 40X objective lens of compound light microscope.

Carbohydrate Utilization Test.
The carbohydrate utilization test was performed according to the methods adopted by Mohammed *et al*. (2022) with little modifications using the broth (peptone: 10 g; NaCl: 5 g; phenol red: 0.018 g; distilled water: 1000ml; carbohydrate 10 g) with inverted Durham tubes in the broth. Exactly 5% each of maltose, sucrose, fructose, galactose, glucose, lactose, and xylose sugar was poured separately in 100mL of sterile deionized water and sterilized by pasteurizing at 62^{o}C for 30 min. The media were inoculated with each of the yeast isolates from the fermented cereal and incubated for 72 h. The color change from red to yellow indicated the fermentation using carbon sources.

Taxonomic identification
The isolates belonging to *S. cerevisiae* species were identified by the carbon and nitrogen assimilation tests and fermentative capacity test as described by Vaughan-Martin and Martin (1993) and Sanni and Loner (1993).

Quality properties of whole wheat flour
Proximate composition of whole wheat flour was carried out according to the methods described by APHA (1999). Other quality properties of whole wheat flour determined were as follows;

Determination of Alpha amylase using Hagberg Falling Number
Seven gram of whole wheat flour was weighed and mixed with 25 mL of distilled water in a glass falling number tube with a stirrer and shaken to form slurry. The slurry was heated in the system boiling water bath at 100^{o}C and stirred up and down constantly. The starch gelatinizes and forms a thick paste. The time the stirrer takes to drop through the paste was recorded as the falling number value. The falling number instrument analyzes viscosity by measuring the resistance of a flour and water paste to a falling stirrer. Falling number results were recorded as an index of enzyme activity in a wheat or flour sample and the results were expressed in time as seconds. A falling number above 300 seconds indicates minimal enzyme activity and good quality flour. A low falling number below 250 seconds indicates substantial enzyme activity. The level of enzyme activity measured by the Falling Number test affects product quality.

Determination of Visco-Elastic and Dough Strength using chopin Alveograph
250 grams of whole-wheat flour was mixed with a normal saline to form a dough using chopin alveograph. Five 4.5 cm circular dough patties were formed and then rested in the alveograph in a temperature-regulated compartment at 25°C for approximately 20 minutes. Each dough patty was tested individually. The alveograph blows air into a dough patty, which expands into a bubble that eventually breaks. The pressure inside the bubble was recorded as a curve on graph paper. The alveograph determines the gluten strength of dough by measuring the force required to blow and break a bubble of dough. The results include P Value, L Value, and W Value. Stronger dough requires more force to blow and break the bubble (higher P value). A bigger bubble means the dough can stretch to a very thin membrane before breaking. A bigger bubble indicates the dough has higher extensibility, that is, its ability to stretch before breaking (L value). A bigger bubble requires more force and will have a greater area under the curve (W value). The Alveograph test provides results that are common specifications used by flour millers and processors to ensure a more consistent process and product. The alveograph is well suited for measuring the dough characteristics of weak gluten wheat. Weak gluten flour with low P value (strength of gluten) and long L value (extensibility) is preferred for cakes and other confectionery products. Strong gluten flour will have high P values and is preferred for breads.

Wheat Flour dough Development with Natural Aflatoxin
Wheat samples of American wheat cultivar were bought from wheat sellers within Kaduna central market, Nigeria. The sample was divided into two portions. The first part was used for determination of proximate composition while the moisture of the second part was raised to 15.5 % according to the method described by Wartu *et al*. (2017) and incubated in jute sack at room temperature (27±2^{o}C) for ten days to develop moulds and aflatoxins. The contaminated wheat was sun dried at the room temperature and was ground into whole wheat flour. The total aflatoxin content of the whole wheat flour was determined using ELISA

***Sacharomyces cerevisae* Culture Development**
Pure cultures of the isolated *Saccharomyces cerevisae* was obtained through sub culturing and was inoculated into 250 mL conical flasks containing sterile yeast extract, peptone, and sucrose according to Tika *et al* . (2017) with some little modifications. The flasks were kept in shaker incubator at 37◦C for 72 h and yeast pellets were collected after centrifugation at 10,000 for each fermented cereal.

Dough Leavening and Bread Development
Dough was prepared by adding 1.0 g sodium chloride to the 50.0 g total aflatoxin contaminated whole wheat flour. Four and half (4.5 g) of sucrose was also added and mixed for 5 min. Then 0.55 g of safeinstant yeast (positive control) was added and clean water was added slowly while mixing for 5 min to form consistent dough. The procedure was repeated with the *Saccharomyces cerevisae* isolated from *ogi, madidi* and *kunun-zaki* and cultivated as described by Tika *et al*. (2017) and another similar setup without yeast added (negative control). The total aflatoxin contaminated dough was fermented in six graduated clean fabricated iron measuring cylinders each. The rate of fermentation process was assessed by noting the rise of the dough mixture kept in the

graduated clean measuring cylinder and was fermented at 35 ± 2°C for 2 h. Samples for total aflatoxin determination was withdrawn at intervals of 30, 60, 90 and 120 min. The fermented dough was baked separately in an electric oven at the following temperatures: 180, 200 and 220°C for 20 minutes.

Extraction of Total Aflatoxin from naturally contaminated wheat, whole wheat flour, dough and Bread

Extraction of total aflatoxins from dough and bread was done according to the kits manfacturer`s instructions adopted by Wartu *et al*. (2015). Firstly, the aflatoxin extraction solvent, 70 % methanol was prepared by weighing 70 mL of methanol mixed with 30 mL of distilled water. Then twenty (20 g) representative samples were weighed separately and placed in a clean bottle with cover. Hundred (100) mL of the extraction solvent methanol was added to each. The samples were then mixed by gentle shaking to disperse the samples in the extraction solvent. The particles were then allowed to settle and were filtered through no. 1 filter paper. The filtrate at this stage was used for aflatoxin assay using ELISA.

Quantification of Aflatoxin B_1 using Direct Competitive ELISA kits.

Aflatoxin was determined from the whole wheat flour dough and bread using ELISA kits obtained from Helica Biosystem Inc, USA and was used according to manufacturer's instruction.

RESULTS AND DISCUSSION

Proximate values determined on whole wheat flour and the aflatoxin reduction potential of fermentation during dough development and baking of whole wheat flour dough leavened with *Saccharomyces cerevisae* isolated from fermented cereal products and commercial yeast was assessed. The proximate values determined on the whole wheat flour samples were within the recommended limit by Standards Organization of Nigeria and National Agency for Food and Drug Administration and Control (Table 1). ``W`` Value (230 joules) obtained from the alveograph test is the area under the curve. It is a combination of dough strength (P value) and extensibility (L value) and expressed in joules. Even though the higher the W index, the longer the leavening time, the whole wheat flour sample used for this experiment indicates that it is a strong gluten wheat. Also from the same Table, the alpha amylase detection indicated that the falling number of the whole wheat flour used was 240 seconds. Normally, a low falling number below 250 sec indicates high enzyme activity, most often as a result of sprout damaged wheat probably because of the long incubation period before milling. Consequently bread of such might be sticky and slicing difficult. Conversely, a high falling number above 300 sec indicates very little enzyme activity and no sprouting. Such flours require additional enzyme addition. The falling number obtained in this study 240 seconds depicts flour with substantial enzyme activity.

The carbohydrate utilization determined on the isolates from the fermented cereals is shown in Table 2. The probable yeast isolated was *Saccharomyces cerevisae.*

The *Saccharomyces cerevisae* isolated from *ogi* was the fastest. The isolates from *madidi and kunun-zaki* showed similar rise but slower than the former. Isolate from *ogi* was comparable to that by the commercial yeast even though the commercial yeast was still better in terms of leavening and thus higher bread volume in mm than the isolated yeasts. There was a significant difference between the whole wheat flour primary dough and the two hours final dough. Similarly, the breads produced using safeinstant dry commercial yeast and the isolated *S.cerevisae* on one hand with the control. The cross-sectional examination of the various bread leavened with the various isolates have comparable crumb texture and structure were similar to the dough leavened with safeinstant yeast. However, the negative control had hard texture and was compact with very low volume being the least.

The reduction of aflatoxin B_1 during fermentation of whole wheat flour dough is presented in Figure 1. The unfermented dough had initial aflatoxin B_1content of 29.0 µg/kg. Following 30 minutes fermentation at 35±2°C, the aflatoxin B_1 level remained unchanged. The toxin retention reduced from 29.0 µg/kg to 26.0 µg/kg after 60minutes fermentation and the percentage aflatoxin B_1 reduction recorded was 10.3 %. This value increased to 34.5 % aflatoxin loss after 90 minutes incubation and the aflatoxin retention decreased to 19.0 µg/kg. Significant aflatoxin B_1 reduction (55.2 %) was registered at 120 minutes fermentation which represents 44.8 % retention. There was positive correlation between the fermentation time of the dough and the toxin loss; as the fermentation time increased, the percentage aflatoxin B_1 loss increased and the percentage retention also decreased.

The baking profile of the fermented dough also showed positive correlation between the baking temperature and the percentage aflatoxin B_1 loss (Figure 2). As the baking temperature increases from 180 – 220°C the toxin reduction increased from 55.2 – 72.4 % and the retention also decreased. Half of the aflatoxin B_1 content of the fermented dough was further decontaminated through baking period's to77.4 % aflatoxin loss. The 55.2 and 77.4 % aflatoxin loss due to fermentation and baking respectively obtained from this study is higher than the result obtained by Gumus *et al*. *(*2009) who reported 33.7% maximum fermentation of dough and 70.4% during baking. Another study by El-Tawila *et al*. (2003) reported 41.17% total aflatoxin absorption after dough baking into bread. Though most of the products of wheat flour undergo baking (bread, cakes,) and frying (dough nuts, rolls), aflatoxins are very stable to heat in dry state up to their melting points (Shapira and Paster, 2004; Rodrigues *et al.,* 2007). However, Ananth and Farid (2003) documented that heating aflatoxin contaminated food in the presence of moisture (Mendez- Albores *et al*., 2009), leads to destruction of aflatoxins over a period of time. This destruction leads to opening of the aflatoxin lactone ring with the possibility of decarboxylation and loss of the methoxy group from the aromatic ring at elevated temperatures. This process could be the reason behind the significant decontamination of the aflatoxins from the whole wheat flour dough during fermentation and baking. The variations in different levels of decontamination during fermentation and baking reported by different researchers could be attributed to different varied baking temperatures and the length of fermentation periods. The types of aflatoxin studied also could create the differences in result because aflatoxin types have different melting point temperatures. However, the aflatoxin miniatures left over in the bread samples could still be a health threat to consumers. It is therefore necessary to control aflatoxin contamination of the raw material before use for production of products such as bread.

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Table 1: Properties of whole wheat flour

S/N	Parameters	Determined Values
1.	Moisture (%)	13.4
2.	Dry gluten (%)	11.3
3.	Ash (%)	1.08
4.	pH	6.3
5.	Protein (%)	11.0
6.	Fats (%)	0.70
7.	Free fatty acid	0.11
8.	W (joules)	230
9.	Alpha amylase (sec.)	240

Table 2: Sugar utilization test.

Cereal Based Food	Bud	Biochemical Characteristics							Probable Organisms
		Fructose	Sucrose	Maltose	Lactose	Glucose	Xylose	Galactose	
ogi	+	+	+	+	+	+	+	+	*S. cerevisae*
kunun-zaki	+	+	+	-	+	+	+	+	*S. cerevisae*
madidi	+	+	+	+	+	-	+	+	*S. cerevisae*

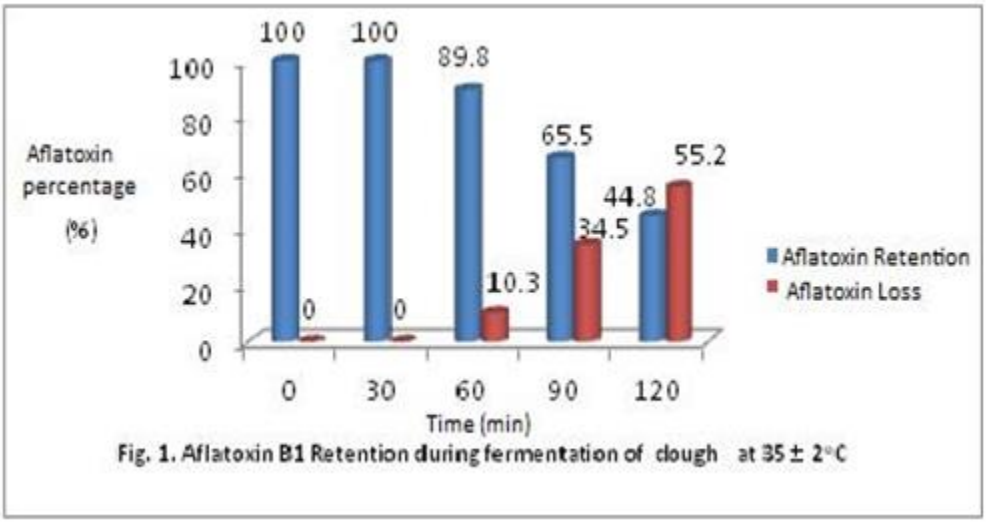

Fig. 1. Aflatoxin B1 Retention during fermentation of dough at 35 ± 2°C

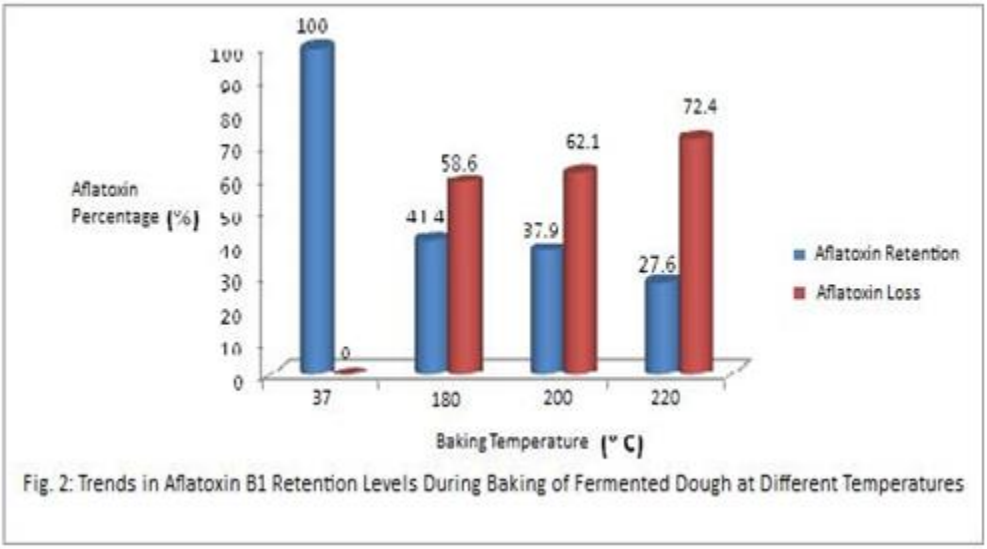

Fig. 2: Trends in Aflatoxin B1 Retention Levels During Baking of Fermented Dough at Different Temperatures

Conclusion

This study showed that the *Saccharomyces cerevisiae* isolated from ogi could be used as a commercial baker's yeast.

The retention of aflatoxin B_1 during fermentation of wheat flour dough showed that at 60, 90 and 120 minutes of fermentation at 35°C/2h, the percentage aflatoxin reduction increased as follows; 10.3. 34.5 and 55.2 % respectively. Conversely, the total aflatoxin retention decreased in this order; 89.7, 65.5 and 44.8 % respectively. As the fermentation time increased, the percentage aflatoxin loss increased and the percentage aflatoxin retention decreased. The baking profile of the fermented dough also showed that as the baking temperature increased from 180 – 220°C the total aflatoxin loss increased from 58.6 – 72.4 % and the aflatoxin retention also decreased. This work has demonstrated that though more than half of the toxin was eliminated during fermentation and baking, but it is not efficient in the decontamination of aflatoxin B_1.

Competing interests

The authors declare that they have no competing interests

Authors' contributions

JRW, who was a student under the below professors conceived the study, drafted the manuscript which happen to be an excerpt from PhD work. Collection of samples and aflatoxin detection using ELISA was carried out under strict supervision and coordination of Professors CMZW, IOA. and JBA. The manuscript was proof read by the professors and corrections were effected by JRW. Final manuscript was approved by all authors.

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