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Effects of Processing *Triticum* spp on Total Aflatoxins and Its Distribution into Wheat Products

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Authors' contributions

This research article is an excerpt from Ph.D. thesis designed by author JRW who was a post graduate student under the supervision of the authors CMZW, IOA and JBA. The student wrote the manuscript, performed the analysis and managed the literature searches. The auhors supervised every stage of the research. All the authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aflatoxins are carcinogenic. The presence of such mycotoxin in food and feed could pose health threat to both human and animals.

Aims: The aim of this research were to study the distribution and the fate of total aflatoxins from in-process aflatoxin contaminated wheat into products.

Place and Duration of Study: The study was carried out in a low tonnage 100 kg/h processing unit from northern Nigeria from February - November, 2016.

Methodology: Standard methods were adopted to determine some proximate compositions of wheat and roller mill processed wheat products (wheat flour, semolina, brown flour and bran). ELISA was employed to detect and quantify total aflatoxin from the samples.

Results: Tempered wheat presented higher moisture content (15.5%) followed by wheat flour (14.8%) and least in bran (12.42%). Highest ash values was recorded in bran (1.55 dm%), followed by raw wheat (1.31 dm%), damped wheat (1.18 dm%), brown flour (0.84 dm%), wheat flour (0.64 dm%) and least in semolina (0.50 dm%). Mean percentage protein content decreased gradually

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from the raw material (15.18%) to wheat flour (12.34%). This represents 18.71% reduction in protein content during processing at 70% extraction. The effects of wheat flour extraction rate and total aflatoxin retention presented positive correlation. As the extraction rate increased, the aflatoxin retention also increased with increasing ash content. At 60% extraction, aflatoxin retention was 2.0% indicating decontamination of 98.0% total aflatoxins with corresponding 0.57%dm ash content. Increasing the extraction rate from 60 – 75%, the ash content and the total aflatoxin retention increased from 0.57 - 0.82%dm and 2.0 - 56.59% respectively while total aflatoxin decontamination decreased from 98.0 – 43.41%.

Conclusion: This research has demonstrated that aflatoxin contaminated wheat used for processing could redistribute aflatoxins into wheat products thus the need for strict surveillance of raw material by processors and regulatory bodies during importation and at factory level to safeguard incidence of aflatoxicosis in consumers.

Keywords: Triticum; extraction; aflatoxin; mycotoxin.

1. INTRODUCTION

Aflatoxins are difurocaumarins derivatives, with oxygenated pentaherocyclic structure. They are teratogenic and carcinogenic [1,2]. Aflatoxin B_1 (AFB_1) is a common occurring mycotoxin and the most potent hepatocarcinogen known to mammals and thus been classified by the International Agency of Research on Cancer (IARC) as Group 1 carcinogen [3].

Other researchers have reported the occurrence of aflatoxins in wheat flour [4,5] and wheat flour products such as bread [6,7].

Wheat flour processing is standardized, and involves the following steps; screening or cleaning (removing impurities such as stone, broken grains and chaff from the wheat), followed by damping or tempering (the grains are wetted through addition of calculated amount of clean water to toughen the outer layers and mellow the starchy endosperm in order to facilitate separation [8] and also to raise the moisture of the grains to about 15%. This is succeeded by incubation for 18-24h or higher depending on the specie of the wheat. Finally, grinding the tempered wheat in three stages; break release (the tempered grain is punched by specialized fluted rollers), scratching (endosperm of the wheat grain scratched off the bran), and size reduction through grinding followed by grading (seiving).

Wheat and wheat flour have been used for preparation of certain foods such as bread, cakes, dough nuts and spaghetti etc. It is necessary to understand the dynamics of total aflatoxins distribution during the milling process and the likely aflatoxin miniatures left over in finished wheat products for human consumption. Therefore the aim of this research was to determine the fate and distribution of total aflatoxins from in-process aflatoxin contaminated wheat into products.

2. MATERIALS AND METHODS

2.1 Source and Cleaning of Wheat Samples

A sample (100 kg) of American wheat *cultivar* was obtained from four wheat flour mills that operate within northern Nigeria. The wheat was cleaned using China 350 kg/h screening machine which removes impurities such as dust, stones, chaffs and broken wheat.

2.2 Tempering of Raw Wheat before Processing into Wheat Flour

The wholesome wheat (20 kg) was tempered with precisely calculated amount of purified water according to the formula shown below by authors [9] with modification to achieve moisture level of 15.5 % as it is practiced in Nigeria wheat flour mills. Another batch of 50 kg of wheat was also tempered in a similar manner as described. However, the later was spiked with known quantity of aflatoxin B_1 (44.0 µg/kg) obtained from Helica Biosystems Inc. U.S.A and the total aflatoxin determined using Enzyme Link Immunosorbent Assay (ELISA). The damped grains were mixed separately to obtain homogenous samples. The two batches of samples were incubated at ambient temperature (27±2°C) for 24 h in a clean 100 L rubber container with cover. The tempered samples were given second water addition based on the moisture of the first sample when the desired moisture was not achieved.

Water added (mL) = (DM – MC)/(100 – DM) X QS

Where:

DM = desired moisture content
MC = original moisture content of raw wheat
QS = quantity of sample tempered

2.3 Determination of Some Proximate Values

The percentage moisture, ash and protein contents were determined according to standard methods [10]. The moisture was calculated based on weight difference and expressed as percentage. The ash content was calculated based on dry matter basis.

2.4 Determination of the Fate and Distribution Pattern of Aflatoxins during Processing

Fifty kilogram (50 kg) of the tempered wholesome wheat spiked with aflatoxin was divided into five portions. The first portion was milled at 75% extraction (75% wheat flour + 25% bran), the second sample was milled at 70%, while the third and forth portions was milled at 65% and 60% extractions respectively using China batch wheat mill with capacity of 100 kg/h. The other portion of tempered wheat spiked with aflatoxin from above were milled separately at 75% extraction to obtain various wheat products; wheat flour, semolina, brown flour and bran. The varied extraction rates was obtained by successive adjustment of the roller distance (aperture) from each other by bringing the rotating fluted rolls much closer to each other to create varied particle sizes. The milling machine consisted of pair of fluted rollers rotating oppositely that punch open tempered whole grain (break release). The fluted rolls scratches the wheat endosperm from the bran and the endosperm ground and graded with different sizes of standard nytal sieve automatically by the system into wheat products. The raw wheat and wheat products samples from each extraction rate were withdrawn aseptically with the aid of sterile spatula for determination of moisture, ash and total aflatoxin contents.

2.5 Detection and Quantification of Total Aflatoxins Using ELISA Kits

Total aflatoxins were determined from the wheat and wheat products using ELISA kits obtained from biosystem Inc, USA and were used according to manufacturer's instruction.

3. RESULTS AND DISCUSSION

The proximate compositions determined from this research (Table 1) showed that the mean moisture, ash and protein contents of in-process wheat into wheat products presented tempered wheat with the highest moisture content (15.52%) and least in bran (12.42%). Although the optimum milling moisture of soft wheat is 14 – 15.5% and 15.5 - 17% for hard wheat [11], authors [12] reported reduced ash content during high moisture content wheat extracted flours. The major product wheat flour presented mean moisture (14.12%). The mean percentage protein content decreased gradually from the raw material (15.18%) milled into wheat flour (12.34%). This represents 18.71% reduction in protein content. The report of [13] reported a similar decline in protein content of flour, leading to reduced amount of gluten, and a shortened period of dough development during bread processing. All the wheat flour mills from which the raw material was collected process wheat grains into wheat flour between 72-75% extractions. The high moisture levels exceeding 10 % obtained in this study could favour aflatoxin production as reported by [14] when aflatoxigenic strains are present with ventilation. The decline in protein content from this work might have been due to the high extraction rate, the sheer between the grinding rollers and generated heat emanating from the grinding rolls leading to denaturation of the proteins. The 18.71% reduction in protein content obtained in this study is higher than the 8% reported by [15]. The vast difference could be due to the different extraction rates, wheat *cultivar* used and nature of machineries employed for the processing. While nowadays wheat mills uses fluted rollers for grinding and smooth rolls to achieve very high extraction rates, the olden day mills uses hammer mills. Thus the higher the extractions rates the greater the likelihood of starch and protein damage.

Table 2 presents total aflatoxin reduction during processing aflatoxin contaminated wheat into wheat flour with respect to ash content. The unrefined wholesome America wheat *cultivar* presented ash content of 1.20%dm and the total aflatoxin content of the spiked wheat grain was 44.0 µg/kg. At 60 % extraction (60% wheat flour + 40% bran) the percentage ash recorded was (0.57%dm). This result represents total aflatoxin reduction by 98.0%, while the total aflatoxin retention was 2.0% (Table 3). This level of flour extraction (60%) represents significant level of aflatoxin decontamination which could be due to dilution of the toxin into the products and or due to high percentage of separated bran which has been linked to majorly contain the toxins. A four times reduction factor was observed by [16] from raw material processing to finished products. This work has demonstrated that while the extraction rate increased from 60 – 75%, the ash content and the total aflatoxin retention increased from 0.57 - 0.82 %dm (Table 2) and 2.0 - 56.6% (Table 3) respectively. Consequently, the aflatoxin decontamination decreased from 98.0 – 43.4% (Table 2) for the same extraction rate. Positive correlation exists between the extraction rate and total aflatoxin retention. As the extraction rate increases, the total aflatoxin retention increases with increasing ash content. The work of [24] also showed the degree of contamination with mycotoxin increases as the ash content increases as a result of high extraction.

High ash content in wheat flours is mostly accompanied by the presence of dusty bran introduced into the wheat flour due to over scraping of the endosperm from the bran. The explanation to this has been made earlier by [17] that moulds and aflatoxins are majorly situated outside the bran. Thus aflatoxins could easily be introduced into high extraction wheat flours due to the over scraping of the bran which was evident from this research. An assessment of ash content of wheat products at 70% extraction with respect to 15.5% tempering showed that as the moisture increases from 12.5 - 15.5%, the ash content reduced for all the products; wheat flour, semolina, brown flour and bran (Table 4). This is due to the 15.5% tempering when given appropriate incubation period for the bran to get toughened preventing crushing by the roller mills, while the endosperm mellow and thus, easier for extraction and separation leading to low bran in the products likewise very low ash and total aflatoxins as seen in Table 2.

In a separate study other researchers have reported that mycotoxins are concentrated in germ and bran fractions in dry milling process [18-22]. An earlier measure of ash was reported by [25] that most polyphenol oxidase (PPO) is located in bran. For this reason the determination of polyphenol oxidase activity could be vital tool to measure bran contamination.

Fractions used for food especially wheat flour showed least amount of total aflatoxins. This observation is similar to the reports of the authors [23,20] who observed this while studying the effect of industrial processing on the distribution of fumonisin B_1 in dry milled wheat fractions.

This work has provided framework for the understanding of the distribution pattern of total aflatoxins when contaminated wheat is processed into wheat products at different extraction rates. While bran had the highest aflatoxin retention (42.10%), followed by brown flour (20.68%), wheat flour (15.68%) and least by semolina (5.68%) (Table 5). The distribution of aflatoxins in this study was seen to reflect on the amount of aflatoxin contaminated dusty brand introduced during the extraction of the products from the grain. Since the aflatoxins are situated outside the bran [17] the higher the dusty bran, the more aflatoxin levels in the product. Although cleaning processes before milling and other food processing exercise could reduce aflatoxin levels of the grains [16], there was that redistribution and dilution of the products by pneumatic transport system leading to aflatoxin contamination.

Table 1. Mean±S.D. of some proximate composition determined on in-process wheat and products at 70% extraction

Sample	Moisture (%)	Ash contents (%)	Proteins (%)
Wheat	12.55±0.22[a]	1.31±0.41[b]	15.18±1.08[a]
Damped wheat	15.52±0.14[a]	1.18±1.00[b]	14.67±1.02[a]
Wheat flour	14.12±0.15[a]	0.64±0.11[c]	12.34±0.13[a]
Semolina	14.60±0.21[a]	0.50±1.11[c]	12.89±0.22[a]
Brown flour	13.5±1.12[a]	0.84±1.23[c]	12.40±0.06[a]
Bran	12.42±0.23[a]	1.55±0.66[d]	0.55±0.02[c]
p-value	0.993	0.009	1.000

Mean±S.D. Anova, Duncan Multiple Ratio test (DMRT). Significant at P=.05

Table 2. Total aflatoxin reduction during processing aflatoxin contaminated wheat into wheat flour with respect to ash content

Extraction rate (%)	Ash (% dm)	Total aflatoxins (μg/kg)	Total aflatoxin decontamination (%)
0	1.20	44.00	0.00
60	0.57	0.90	98.00
65	0.61	1.70	96.14
70	0.68	15.00	65.91
75	0.82	24.90	43.41

Results are means of duplicate samples

Table 3. Fate of total aflatoxins during wheat flour extraction from contaminated *Triticum* sp

Extraction rate (%)	Total aflatoxins (μg/kg)	Aflatoxin retention (%)
0	44.00	100.00
60	0..90	2.00
65	1.70	3.86
70	15.00	34.09
75	24.90	56.59

Results are means of duplicate samples

Table 4. Ash content of wheat products at 70% extraction with respect to 24 h tempering

Moisture (%)	Ash content (%dm)			
	Wheat flour	Semolina	Brown flour	Bran
13.50	0.76	0.64	0.92	1.44
14.00	0.73	0.59	0.89	1.40
14.50	0.71	0.56	0.86	1.45
15.00	0.67	0.52	0.86	1.51
15.50	0.64	0.49	0.85	1.56

Results are means of duplicate samples

Table 5. Distribution pattern of aflatoxins in wheat products during processing at 70 % wheat flour extraction (%)

Product	Aflatoxin content (μg/kg)	Percentage retention (%)
Wheat	44.0	100.00
Wheat flour	6.90	15.68
Semolina	2.50	5.68
Brown flour	9.10	20.68
Bran	18.50	42.10

Results are means of duplicate samples

4. CONCLUSION

The protein content showed 18.71% reduction during processing of American wheat *cultivar* into wheat products. Processing aflatoxin contaminated wheat presented total aflatoxin decontamination of 98.0%, while the total aflatoxin retention was 2.0 μg/kg at 60% extraction. This work has demonstrated that while the extraction rate increased from 60 – 75 %, the ash content and the total aflatoxin retention increased from 0.57 - 0.82%dm and 2.1- 56.6% respectively. The aflatoxin decontamination decreased from 98.0 - 43.1% for the same extraction rate.

Tempered wheat milled at 70% extraction presented this trend of percentage ash content: as the moisture increases from 12.5 - 15.5%, the ash content reduced for all the wheat products: wheat flour, semolina, brown flour and bran. It was easier for extraction and separation of products at 15.5% moisture than at 12.5% tempering.

This research has demonstrated redistribution of total aflatoxins from the aflatoxin contaminated wheat used for processing into wheat products. There is need for processors to screen *Triticum* spp for aflatoxins before use for processing into products to safeguard toxication in consumers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Khanafari A, Soudi H, Miraboulfathi M. Biocontrol of *Aspergillus flavus* and aflatoxin B1 production in corn. Iranian Journal of Environmental Health Science and Engineering. 2007;4(3):163-168.
2. Liu Yan, Wu Felicia. Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. Environmental Health Perspectives. 2010; 118(6):818.
3. [IARC] International agency for research on cancer. Aflatoxins some naturally occurring substances: Food items and constituents, heterocyclic amines and mycotoxins. IARC scientific Publications. IARC, Lyon. 1993;56.
4. Gashgari Rukaia M, Yassmin M. Shebany, Youssuf A. Gherbawy. Molecular characterization of mycobiota and aflatoxin contamination of retail wheat flours from Jeddah markets. Food Borne Pathogens and Disease. 2010;7(9):1047:1054
5. Bhat MY, Fasal M. Effects of *Aspergillus flavus* metabolites on wheat seed germination and seedlings. Arab Journal of Plant Protection. 2011;139-140.
6. Chelae AM, Abayeh OJ, Jideani IJ. Effects of cooking and fermentation on the fate of aflatoxin B1and B2 in contaminated millet and fresh cow milk. Nigeria Food Journal. 2003;21:125-128.
7. GumusT, Arici M, Daglioglu O, Velioglu M. Fate of aflatoxins during bread making Journal of Food Science and Technology. 2009;46(4):384-387.
8. Heuzé V, Tran G, Lebas F, Noblet J, Lessire M, Baumont R, Renaudeau D. *Wheat bran*. Feedipedia, a programme by INRA, CIRAD, AFZ and Cauvain, Stanley P. & Cauvain P. Cauvain. Bread Making. CRC Press. 2015;540. ISBN 1- 85573-553-9
9. Rashid Maliha, Samina Khalil, Najma Ayub, Waseem Ahmed, Abdul Ghaffar Khan. Categorization of A*spergillus flavus* and *Aspergillus parasiticus* isolates of stored wheat grains in to aflatoxinogenics and non- aflatoxinogenics Pak. J. Bot. 2008;40(5):2177-2192
10. AOAC. Association of official Analytical chemists Official Methods of analysis 13th Edition Arlinton, VA. 1980;26:414-434
11. Posner ES, Hibbs AN. Theory of tempering wheat for milling. Pages 110-114 in: Wheat Flour Milling. E. S. Posner and A. N. Hibbs, eds. AACC International: St Paul, MN; 1997.
12. Meera Kweon, Ron Martin, Edward Souza. Effect of tempering conditions on milling performance and flour functionality. Cereal Chemistry. 2009;86(1):12–17
13. Małgorzata Warechowska, Agnieszka Markowska, Józef Warechowski, Antoni Miś, Agnieszka Nawrocka. Effect of tempering moisture of wheat on grinding energy, middlings and flour size distribution and gluten and dough mixing properties. Journal of Cereal Science. 2016;306–312.
14. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. American Journal of Clinical Nutrition. 2004;80:1106 –1122.
15. Waiver CM, Chen PH, Rynearson SL. Effects of milling on trace elements and protein content of oats and barley. Cereal Chem. 1981;58(2):120-124
16. Lloyd B. Bullerman, Andreia Bianchini. Stability of mycotoxins during food processing. International Journal of Food Microbiology. 2007;119:140–146.
17. Radiana Tamba-Berehoiu, Popa Nicolae-Ciprian, Popescu Stela, Cristea Stelica, Culea Rodica, Tamba-Berehoiu Suzana. Distribution of some toxic contaminants in the milling products, during the milling process. Romanian Biotechnological Letters. 2009;15(3):5281 - 5286
18. Aldrich AJ. The effects of processing on the occurrence of ochratoxin A in cereals. Food Additives and Contaminants. 1996;13(Suppl 27):27–28.
19. Scudamore KA, Banks JN, Guy RCE. Fate of ochratoxin A in the processing of whole wheat grain during extrusion. Food Additives and Contaminants. 2004;21(5): 488–497.
20. Brera C, Debegnach F, Grossi S, Miraglia M, Effect of industrial processing on the distribution of fumonisin B_1 in dry milling corn fractions. Journal of Food Protection. 2004:67(6):1261–1266.

21. Herrera M, Juan T, Estopaña G, Ariño A. Comparisoxynivalenol, ochratoxin A and aflatoxin B1 levels in conventional and organic durum semolina and the effect of milling. Journal Food Nutrition Research. 2009;48(2):92-99.
22. Giménez I, Herrera M, Escobar J, Ferruz E, Lorán S, Herrera A, Ariño A. Distribution of deoxynivalenol and zearalenone in milled germ during wheat milling and analysis of 674. Journal of Food and Nutrition Research Toxin Levels in Wheat Germ and Wheat Germ Oil. Food Control. 2013;34(2):268-273.
23. Park DL. Effect of processing on aflatoxin. Advances in Experimental Medicine and Biology. 2002;504:173–179.
24. Schollenberger Margit, Helga Terry Jara, Sybille Suchy, Drochner W, Muller HM. Fusarium toxin in wheat flour collected in an area in southwest Germany. International Journal of food Microbiology. 2002;72(1-2):85-89.
25. Fuerst EP, Anderson JV, Morris CF. Polyphenol oxidase in wheat grain: Whole kernel and bran assays for total and soluble activity. Cereal Chem. 2006; 83:10-16.

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