Jewel Journal of Scientific Research (JJSR) 6(1&2): 27–33, 2021
©Federal University of Kashere-Nigeria (Print ISSN: 2384 – 6267, Online ISSN 2756-651X)
jjsr.fukashere.edu.ng

Mycoflora and Nutritional Composition of Wheat (*Triticum aestivum* L) Grains Sampled in Lagos, Nigeria

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Abstracts

The continuous evaluation of food quality in terms of its nutritional and microbiological status is central to the validation of measures for hazard and critical control point system. This study, was therefore, aimed at determining the mycoflora and the nutritional composition of wheat grains collected from major wheat markets in Lagos, Nigeria. A total of 220 samples were collected from different sellers at different time between June 2012 and December 2015 according to the standard recommended technique of international commission for microbiological specification for foods. The samples were subsequently analyzed for both nutritional and microbial composition using standard recommended techniques. Results obtained depicts different fungal species in the following respective order viz; *Penicillium chrysogenum* (22.5%), *Aspergillus flavus* (17.5%), *Fusarium solani* (15.8%), *Aspergillus niger* (12.5%), *Aspergillus fumigatus* (12.0%), *Rhizopus stolonifer* (10.0%), *Alternaria alternata* (6.2%) and *Trichoderma atroviridae* (3.5%) while both the chemical and the proximate compositions of the examined wheat grains exhibited significant disparity but without any significant variation in the patterns of fungal loads. Our results consequently emphasized the importance of wheat as proxy indicator of nutrient rich foods for the malnourished but should be stored under good storage conditions to circumvent the growth and survival of spoilage fungi.

Keywords: Wheat, Mycoflora, Contaminated, Contamination

Received: 20th Feb., 2021 *Accepted:* 19th May, 2021 *Published Online:* 30th June, 2021

Introduction

Wheat is an important source of vegetable protein that is cultivated world-wide with about 607 million tons produced in 2007, making it third most-produced cereal after maize (784 million tons) and rice (651 million tons) (FAO, 2007). In Nigeria, wheat is cultivated in Taraba, Jos and Obudu (Asamudo *et al*., 2017) and their importance in making flour for leavened,, steamed breads, biscuits, cookies, cakes, breakfast cereal, pasta, noodles and couscous have also be recognized (Cauvain and Cauvain, 2003). Some other people have also recognized the imperativeness of this staple vegetable food in brewery industry

especially during their fermentation to make alcoholic beer, vodka and even bio-fuel (Palmer, 2001; Neill, 2002). In agriculture, wheat has been used as a forage crop for livestock and their straw have been used as fodder for cattle and other like animals (Smith, 1995). Generally, the rich composition of catalytic elements in wheat and its flour coupled with its functional ingredients such as fibre, phytochemicals, minerals, and other essential amino acids make wheat one of the most acceptable cereal crop (Musaige *et al*., 2007; Dewettinck *et al*., 2008). However, the vulnerability of wheat grains to fungal attack both at pre and post-harvest and even during processing is a growing public health problem and this may further exacerbate fungal proliferation and hence leading to food poisoning (Birk *et al*., 2003, Čonková *et al*., 2006; Sabillon, 2014). This food poisoning on the other hand, has become a very topical subject eliciting great deal of public concern to many people all over the world (Mamajoro, 2009) because of their potentials involvement in condemning several agricultural products in addition to their responsibility in causing substantial effects in stored food stuffs including discolouration, production of off-odours, deterioration in technological quality among other effects (Basilico *et al*., 2001; Magnolia *et al*., 2006) and mycotoxin contamination (Jayeola and Oluwadun, 2010; Thomas and Ogunkanmi, 2014). The mycoflora and intrinsic factors that might contribute to the deterioration of wheat grains are very important in order to ensure food safety since consumption of wheat and wheat products is fast gaining ground in Nigeria due to its health benefit. This study was, therefore aimed at investigating the mycoflora and the nutritional composition of marketed wheat grains sourced in Lagos, Nigeria.

Materials and Methods

Collection of Samples

A total of 220 wheat grains were purchased from different markets in the five (5) administrative divisions of Lagos State viz; Ikeja, Ikorodu, Lagos, Epe, and Badagry divisions. These samples were collected separately in pre-sterilized aluminium pan and transported to the laboratory within 24hours of its collection. On getting to the laboratory, wheat grains were segregated into healthy and diseased groups based on the following parameters discoloration, weight loss, and visible mold growth among other factors

Isolation and Identification of fungal mycoflora: The diseased grains were surface sterilized with 0.1% solution of mercury chloride ($HgCl_2$), rinsed in sterile distilled water before being plated on potato dextrose agar (PDA) in triplicates. The plates were incubated at room temperature (27 ± 2^0C) for 48 hours. Purification of the isolates was done, by subsequent sub-culturing into fresh sterile media until pure cultures were obtained. Microbiological identification of fungal isolates were carried out using both macro-morphological (Maren, 2002), and micro-morphological characteristics (Pitt and Hocking, 1997), while molecular identification was done as described earlier (Fredricks *et al*., 2005; Pryce *et al*., 2003).

Chemical and Proximate analyses of wheat grains

The chemical analysis of wheat grains was determined using the method described by Nout *et al*. (1989) while the proximate analysis was estimated following the recommended method of the Association of official Analytical chemists as described by Adeniran and Ajifolokun (2018)

Results

The PCR amplification of the 18S ribosomal RNA gene of the fungal isolates obtained from wheat yielded a single fragment of an approximately between 700 and 900bp with a BLAST search delineating percentage similarity of between 97-100% . The identified fungal isolates were *Penicillium chrysogenum* (22.5%), *Aspergillus flavus* (17.5%), *Fusarium solani* (15.8%), *Aspergillus niger* (12.5%), *Aspergillus fumigatus* (12.0%), *Rhizopus*

stolonifer (10.0%), *Alternaria alternata* (6.2%) and *Trichoderma atroviridae* (3.5%). Table 2 shows the species identification by both Microbiological and Molecular methods for the isolated filamentous fungi. Except for 78 *Penicillium chrysogenum,* 63 *Fusarium solani*, 14 *Trichoderma atroviride*, 25 *Alternaria alternata* and 20 *Rhizopus stolonifer* that were misidentified *as Penicillium verrucosum, Fusarium moniliforme, Trichoderma species and Rhizopus species* respectively, all the remaining 200 isolates were correctly identified by the microbiological method. Conversely, the comparator molecular technique successfully identified all the isolated organisms resulting in 100% correct diagnosis. The number of discrepant species identified by the microbiological method represents a total of 200 fungal strains belonging to five different genera to connote approximately 50% of the total isolated organisms.

The table 3 below depicts the occurrence of fungi in the marketed wheat grains obtained from different sources in Lagos State, Nigeria. All the sample investigated harbors *Aspergillus flavus* and *Penicillium chrysogenum* while *Fusarium solani* was isolated from samples from nine locations, *Aspergillus niger* isolated from samples from eight locations, *Aspergillus fumigatus* occurs in sample from five different markets, though to varying degree of distribution pattern. Also, *Alternaria alternata* and *Trichoderma atroviride* was found in the samples from Mushin and Somolu markets respectively.

Table 1: Molecular Identification of Fungal Isolates from Wheat

Culture number	**Species**	**Ga18**	**%**
FW1	*Aspergillus niger*	AY214445.1	97
FW2	*Aspergillus flavus*	AY214445.	100
FW3	*Aspergillus fumigatus*	FJ878717.1	98
FW4	*Penicillium chrysogenum*	L76153.1	98
FW5	*Fusarium solani*	KJ573076.1	98
FW6	*Trichoderma atroviride*	FJ904855.1	98
FW7	*Alternaria alternata*	AY354228.1	98
FW8	*Rhizopus stolonifer*	HM051076.1	100

Ga18= Gene Bank accessions for 18S rRNA gene, % = percentage similarity of sequence

Table 2: Identification of filamentous Fungi by Microbiological and Molecular Methods

	Number (%) of isolates in category					
	Microbiological method			**Molecular method**		
Species	**CI**	**MI**	**NI**	**CI**	**MI**	**NI**
Aspergillus niger	50	0	0	50	0	0
Aspergillus flavus	70	0	70	0	0	0
Aspergillus fumigatus	48	0	0	48	0	0
Penicillium chrysogenum	12	78	0	90	0	0
Fusarium solani	0	63	0	63	0	0
Trichoderma atroviride	0	14	0	14	0	0
Alternaria altenata	0	25	0	25	0	0
Rhizopus stolonifer	20	20	0	40	0	0
Total	200(50%)	200(50%)	0(0)	400(100)	0(0)	0(0)

KEY: CI- Correct identification, MI- Misidentification and NI- No identification

Table 3: Occurrence of fungi found associated with marketed wheat in Lagos State, Nigeria.

	LOCATIONS													
Organism	**AG**	**OSH**	**MSH**	**YB**	**SOM**	**OY**	**ID**	**M12**	**SR**	**0B**	**IKY**	**IKD**	**EP**	**BGR**
Aspergilus flavus	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penicillium Chrysogenium	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fussarium solani	+	+	-	-	-	+	+	+	+	+	-	+	-	+
Aspergillus fumigatus	+	+	-	-	-	-	-	+	-	-	+	-	-	+
Aspergillus niger	+	-	+	-	+	+	+	+	+	-	-	-	+	-
Rhizopus stolonifer	-	-	-	+	-	+	+	+	-	+	-	+	-	-
Alternaria alternata	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Trichoderma Atrovidae	-	-	-	-	+	-	-	-	-	-	-	-	-	-

AG – Agege, Osh – Oshodi, MSH – Mushin, SOM – Somolu, YBA – Yaba, OY –Oyingbo, ID – Ido, M12 – Mile12, SR – Sura, OB – Obalande, IKY – Ikoyi, IKD – Ikorodu, EP – Epe and BGR – Badagry.

Table 4: Chemical and proximate composition of *wheat seeds* sampled from Lagos, Nigeria

Chemical and Proximate Parameters	**Mean±SD**
Moisture content (%)	3.8±0.03
Ash content	2.8±0.21
Fat (%)	3.1±0.01
Protein (%)	13.8±1.21
Carbohydrate(%)	74.6±2.01
Magnesium (%)	0.74±0.21
Phosphorus (%)	0.86±0.11
Iron (%)	0.36±0.01
Potassium (%)	1.30±0.12
Calcium (%)	1.91±0.01
Copper (%)	1.31±0.17
Manganese (%)	8.35±1.72
Zinc (%)	9.34±2.15
Molybdenum (%)	1.99±0.11
Chromium (%)	1.92±0.02
pH	6.4±1.31
aw	0.92±0.01

The above table connotes the chemical and proximate composition of wheat grains sampled from Lagos, Nigeria. As shown in this table, the sampled wheat grains contain varied levels of both proximate and chemical compounds with carbohydrates being the most significantly represented (74.6±2.01%). Protein and Fats are other proximate that shows some level of representation in the following order of respects (13.8±1.21%) and (3.1±0.01%). Some chemical parameters including magnesium, phosphorus, iron, potassium, calcium, copper, molybdenum and chromium were also found while both pH and water activity were found to be (6.4±1.31) and (0.92±0.01) respectively.

DISCUSSION

The importance of spoilage fungi in the deterioration of food has been emphasized (Schawn and Wheals, 2004; Ogiehor and Ikenebomeh, 2006; Thomas *et al.,* 2012; Thomas and Ogunkanmi, 2014). In this study, the main filamentous fungi isolated were *Penicillium chrysogenum*, *Aspergillus flavus*, *Fusarium solani*, *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer* , *Alternaria alternata* and *Trichoderma atroviridae*. The presence of these xerophilic moulds in food represents significant changes in the organoleptic, microbiological and nutritional quality of such food (Ogiehor and Ikenebomeh, 2005; Magnolia *et al.,* 2006). From public health perspective, growth of these organisms in food suggests an imminent danger to the consumer especially when their secondary metabolites have been produced in the food (Otteneder and Majerus, 2000; Thomas *et al.,* 2021).

Aspergilli which were the most predominant in this study are among the most abundant soil mycoflora (Bennett and Klich, 2003). Among the isolated *Aspergilli, Aspergillius niger and Aspergillus flavus* were the most prevalent to confirm them as the major contaminants of wheat grains in the studied regions. Their presence as the most predominant indicates that the practices associated with the processing and post processing handling of wheat grains including spreading on the floor, mats, displaying in open bowl in the markets as well as use of various packaging materials to haul finished products from rural to urban areas (Ogiehor and Ikenebomeh, 2005) might have exacerbated their wide distribution. Some of these fungal mycoflora has been reported to be notorious producers of certain mycotoxins including citrinin, patulin and even ochratoxins and are now known to cause tremors, coagulopathy and enteritis (Ojo, 2003). The good representation of protein by wheat in this study emphasized higher representation than other cereal studied by FAO (1999) where it was found that brown rice contain 7.3%, sorghum 8.3%, rhye 8.75%, maize 9.8%, wheat 10.6%, barley 11.0% and pearl millet 11.5%. However, the wheat samples analyzed in this study have lower fat contents. The pH values found in this study fall within the range at which toxigenic moulds can grow to further affirmed the reasons for wide contamination of wheat grains by several filamentous fungi. The observed pH of wheat samples in this study corroborated the report of Thomas *et al*. (2014) who further linked pH range above 2.0 -11.2 as being influential to growth of some toxigenic spp of *Aspergillus, Penicillium and Fusarium* (Jayeola and Oluwadun, 2010). The a_w of the wheat samples analyzed ranges from 0.89 to 0.99 and it has been reported that minimum a_w value for most spoilage molds growth in food is 0.80 and 0.61 for xerophilic molds (Safefood, 2014). The fact that this important cereal crops were also rich in calcium is enough to emphasize their importance in controlling blood pressure induced as a result of calcium deficiency. This is because one study found a reduction in systolic blood pressure of 0.34mm/Hg per 100g of calcium consumed daily and reduction in diastolic blood pressure of 0.15mm/Hg per 100g of calcium consumed daily (Beto, 2015). Hence, wheat might be found to be beneficial to people with hypertension due to their high calcium content. A study found modest zinc supplement (5.7mg/day) results in increased

growth rate (Roohani *et al.,* 2013) indicating that grains with high zinc content might benefit children under active growth. In conclusion, Our results consequently emphasized the importance of wheat as proxy indicator of nutrient rich foods for the malnourished but should be stored under good storage conditions to circumvent the growth and survival of spoilage fungi.

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