

## Mycoflora, Proximate Composition and Nutritional changes during the Storage of *Oryza sativa*.

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### Abstract

The mycoflora and their effects on the proximate and nutritional composition of sundried rice were investigated after a 20-week period of storage. Seven fungi were isolated from the rice sample by dilution, direct plating and washing methods. They were *Aspergillus flavus*, *Aspergillus niger*, *Absidia* spp, *Fusarium* spp, *Rhizopus* spp, *Saccharomyces cerevisiae* and *Aspergillus fumigates*. The proximate analysis in g/100g revealed that moisture and protein contents increased during storage; moisture from 7.84 to 11.72 and protein from 11.45 to 12.56. While the ash, fat, fibre and carbohydrate contents reduced significantly throughout the storage period: ash (4.51 to 4.25), fat (2.04 to 1.57), fibre 10.27 to 8.52 and carbohydrate (63.72 to 61.28). The minerals (mg/100mg): Na, K, Ca, Mg, Zn, Fe, Mn and P decreased significantly during storage. Na (41.26 to 38.50), K (97.55 to 90.43), Ca (5.36 to 4.25), Mg (3.78 to 3.20), Zn(3.54 to 3.12), Fe (2.18 to 2.00), Mn (1.25 to 1.02) and P (23.33 to 18.55). It was observed that the storage condition and rice nutrients encouraged fungal growth; thereby reducing the seeds weight and affecting their economic value. Some of these fungi produce toxins which are injurious to health when consumed. Hence, depletion of rice nutrients as a result of fungal contaminants would be prevented by storing rice in environmental condition unsuitable for fungal growth.

**Key words:** Fungi, Proximate, Mineral, Sundried-rice, Stored rice

### 1.0 Introduction

Rice (*Oryza sativa*) is an important cereal plant belonging to the grass family Poaceae (Vaughan *et al.*, 2003). It is one of the two cultivable species of its genus, and originates from Asian countries including India, Thailand and China; the other species *Oryza glaberrima* (African native rice) is mainly grown in West African countries (Linares, 2002). The unculturable and wild rice varieties contain favourable genes responsible for their resistance to drought and pest. Nigeria is the highest producer of rice in West Africa (Singh *et al.*, 1997). The varieties produced in Nigeria are *O. glaberrima*, 'Ofada, grown in Ofada town in the South west region of the country and new rice (NERICA), a hybrid of the *O. sativa* and *O. glaberrima* (WARDA, 2008). These species are grown in upland, lowland and swampy areas popularly called 'fadama' in Nigeria. Several varieties of rice are grown in Abakaliki, in Nigeria including 'Faro 14', Mass, Faro 52, Sipi, Awilo, Canada etc (Oko & Ugwu, 2011). Though *O. glaberrima* is appreciated for its taste and culinary qualities by some citizens (Agnoun *et al.*, 2012), its taste may become offensive to some; also its stony nature and tedious method of preparation prior to consumption making its rate of human consumption low in Nigeria. However due to the inability of local production to meet up with its demand which has been soaring at a very fast rate over the years, *O. sativa* having a higher production turnover has since been introduced to West Africa and rapidly replacing the African native rice. Nigeria has since become the second largest importer of rice in the world (Sowunmi, 2014). *O. sativa* is an important staple diet, providing half of the world's population with more than half of daily calories (Guisse, 2010).

*O. sativa* was not a staple grain many years ago in Nigeria; it was affordable to wealthy people and considered as festive food to the middle class. However its good milling characteristics, taste and ease of preparation encouraged its increased rate of importation over the years; it has become affordable and rapidly gaining popularity among the poor (Adeyeye *et al.*, 2000). Rice is mainly cultivated for its highly nutritious seeds consumed by human. Rice is prepared either for domestic consumption or commercial purpose. Rice composition per 100gram of the edible portion of milled rice contains 13.7g moisture, 6.8g protein, 0.5g fat, 0.2g fibre, 0.345 calories, 0.16g phosphorus, 0.06mg riboflavin, 1.09 mg essential amino acids, 78.2g carbohydrate, 0.7mg iron, 70mg magnesium, and 0.14 mg copper (OECD, 1999). Generally, rice can be cooked and eaten with soup or stew; or prepared into various dishes such as, coconut rice, jollof rice, fried rice and 'tuwo', a popular dish in northern Nigeria. The rice seeds are cooked till they become soft, pounded and made into smooth small mounds, and served with various soups (Jackson, 1999). The rice bran is utilized for fertilizers, animal feeds and vegetable oil production (Gautam *et al.*, 2012). Raw rice may also be ground into flour and used for various beverages such as rice milk, rice wine and 'amazake', a low alcohol Japanese drink made from fermented brown

rice. Rice like other cereals before being prepared into foods, go through series of processing including, harvesting, threshing, dehusking, milling, drying, storage and marketing. Dehusking reveals the brown bran and germ that underlie the seed coat collectively refer to as brown rice. Extra-milling or polishing removes the germ, revealing the whiter rice. Sun-drying reduces the moisture content of paddy rice; solar radiation heats the grains as well as the surrounding air, increasing the rate of water evaporation from the grains (Gummert *et al.*, 2004). All the stages involve natural conditions; hence seeds are often contaminated by microorganisms majorly with fungi (Abdullah *et al.*, 2000).

Rice storage is a means of providing a uniform and its constant supply. It is also intended to protect it from weather and various pests; preventing or delaying changes in nutritional value or loss of quality. Commercial dried rice is usually kept in large quantities in bags and barns and when stored in suitable condition such as high temperature and humidity, it may encourage fungal contamination. The study area, Ado-Ekiti in Ekiti State, Southwestern Nigeria was chosen because a high percentage of residents are mostly salary earners and prefer rice which is easily prepared for breakfast to ensure punctuality to workplace. Recently, the rate of influx of people increased in the town because of increased establishment of government tertiary institutions in the state and has resulted in increase in rice-consuming population of rice. Also, being the state capital, most employed people reside there because of rate of development compared to other surrounding towns and villages. Since rice is highly affordable and consumed by the majority in the state, information on the mycoflora that commonly affect it on storage need to be well documented. Public awareness on expected diseases in people when such contaminated rice are consumed should be pronounced. Therefore this work investigated mycoflora associated with stored rice in Ado-Ekiti, Nigeria and their deteriorative effect on its nutritional value.

## **2.0 Materials and Methods**

### **2.1 Collection of Sample**

Rice sample was obtained from a seller in the King's market, in Ado-Ekiti, Nigeria. The sample was collected after two weeks the grains were processed and thereafter sundried for one week. The dried rice was further stored in an insect-free container, carefully labelled and kept in the well ventilated laboratory for 20 weeks in the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria.

### **2.2 Isolation of Fungi**

The mycoflora of stored rice was isolated using the following methods;

#### **2.2.1 Dilution Plate Method**

The dilution plate method of Pittan Hocking (2009) was employed whereby 1 gram of the stored rice was placed aseptically in sterile distilled water and thoroughly shaken. One ml of mixture was pipetted into 9 ml of sterile distilled water in test tube; and serially diluted in series of test tubes containing sterile distilled water. One ml each of aliquots of  $10^{-2}$  and  $10^{-3}$  was introduced into molten Potato Dextrose Agar (PDA) plates supplemented with 100g/ml chloramphenicol. The plates were gently swirled for uniform mixing, allowed to solidify and incubated at ambient temperature for 5 to 7 days. The fungi growths were observed for the fruiting bodies. Hyphae tips of each fungus were transferred successively until pure cultures were obtained.

#### **2.2.2 Direct Plate Method**

Of the sundried rice sample, ten grains were examined randomly for the presence of mould according to the methods of Amusa (2001). Their surfaces were sterilised with ethanol and washed in sterile distilled water. Using a sterile spatula, the sterilized grains were aseptically placed on PDA plates and incubated at 28°C for one week. The hyphae tips of fungi cultures were successively sub-cultured on freshly prepared PDA plates until pure colonies were obtained. The isolates were identified based on their colour, mycelia structure, shapes and presence of fruiting bodies

#### **2.2.3 Washing Method**

One gram of the stored dried rice was weighed into 10 ml of sterile distilled water in a beaker and mixed thoroughly. Few drops of the suspension were inoculated on sterile potato dextrose agar plates containing 100g/ml chloramphenicol using the spread plate technique and incubated at 28°C for 7 days.

### **2.3 Identification of Fungi**

Seven fungi were isolated from the stored rice sample and identified by standard mycological techniques based upon gross cultural, macroscopic and microscopic morphological features that could not be identified by this manner were further identified using slide culture and needle mount technique. The fungi were initially identified based on their cultural and morphological characteristics before being subjected to microscopic

identification. The following reference were consulted to check identification Moubasher (1993), de Hoog et al.(2000), Samson et al.(2004) and Pitt & Hocking (2009). The yeast was identified with subculture on corn meal agar + Tween 80 and CHROMagar Candida (Becton and Dickinson, Spark, MD)

#### 2.4 Proximate Analysis

Sample of the stored sun-dried rice were analysed by the standard procedures AOAC (2005) for ash, crude fibre, moisture and fat. The nitrogen was determined by Micro-Kjeldahl method as described by Pearson (1976) and the percentage nitrogen was converted to crude protein by multiplying 6.25. The carbohydrate content was estimated by the difference in value obtained when all the chemical composition values were subtracted from 100%. All determinations were in triplicates and values of each constituent were expressed in percentage.

#### 2.5 Mineral Analysis

The stored rice sample was analyzed for the minerals; calcium, magnesium, sodium, zinc, iron, copper, potassium, phosphorus and molybdenum using AOAC (2005) methods. The rice sample was ashes at 550°C and dissolved in volumetric flask containing distilled water, deionized with a few drops of concentrated HCl. Sodium and Potassium were determined by using a flame photometer (Model 405 Corning, UK), using NaCl and KCl to prepare the standards. Phosphorus was determined by Molybdate method. All the remaining metals were determined by atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk CT, USA). All determinations were in triplicates.

### 3.0 Results and Discussion

Direct plating was used to isolate surface fungal contaminants, while the washing and dilution methods were used to isolate both the external and internal fungi contaminants. Table 1 gives the summary of the type of fungi obtained from the stored rice. Seven fungal species belonging to five genera were isolated namely: *Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus niger*, *Absidia* spp, *Fusarium* spp, *Rhizopus* spp and *Saccharomyces cerevisiae*. It was noted that while most of the fungi were isolated using all the three methods of isolation, *Aspergillus fumigates* and *Aspergillus niger* were not isolated with the washing method; and *Fusarium* spp and *Absidia* spp were not isolated using the dilution method.. It was observed that the predominant fungi belong to the genera *Aspergillus*. This finding supports that of Amadi & Adeniyi (2007), who reported the presence of *A. flavus*, *A. niger*, *A. oryzae*, *A. terreus*, *Fusarium* sp, *Rhizopus stolonifer*, *P. italicum*, and *P. spirulosum* in stored grains; millet, rice and maize in Ilorin, Nigeria, with *Aspergillus* having a higher occurrence. It is also in agreement with Makun *et al.*, (2014) who reported the predominance of *Aspergillus flavus* in stored rice in Niger state, Nigeria; other fungi isolated were *A. niger*, *Mucor* spp, *Rhizopus* spp, *Alternaria*, *Fusarium* spp, *A. parasiticus* and *Penicillium* spp. The finding also confirms Fagbohun & Faleye (2012) who reported the presence of *Aspergillus* sp. Moreso, the result also supported the report of El-Shanshoury *et al.*, (2014) who revealed the predominance of *Aspergillus* spp in stored cereals, wheat, rice and maize in Egypt.

Fungal pathogens are the major cause of reduction of quality of stored rice when rodents and insects are under control. Stored rice is prone to microbial attack especially when there are favourable environmental conditions such as moderate temperature and high humidity. Reduction in quality and sometimes quantity of stored grain is unavoidable (Oh *et al.*, 2007). Fungi in stored food are divided into two groups namely the 'field fungi' and the 'storage fungi'. Most at times it is difficult to distinguish between the two as fungal growth may start both in the field and during storage. Species of *Aspergillus*, *Rhizopus* and *Penicillium* have been reported as storage fungi which infect crops on the field and may persist and proliferate in storage resulting in increased fungal and mycotoxin contamination with increased duration of storage (Yaseen *et al.*, 2011; Makun *et al.*, 2007; Taligoola *et al.*, 2004). Small quantities of fungal spores are usually present before harvesting of grains; and as the grains are being stored in bags, small quantities of spores contaminates the grains during handling (IRRI, 2006). These spores increase and germinate rapidly in high temperature and moisture.

Fungi can cause food spoilage, biodeterioration, seed discolouration, reduction in weight of rice seeds (Yaseen *et al.*, 2011), resulting in musty odour and consumers acceptability (Aboaba & Amasike, 1991). They also produce various toxins (mycotoxins) as secondary metabolites during crop growth, harvest and storage. The effect of these toxins has been documented and is a major threat to public health (Makun *et al.*, 2009) and adverse economic implication (Wu, 2008). *Aspergillus flavus* produces aflatoxins, especially aflatoxin B<sub>1</sub> one of the most potent naturally carcinogen and nephrogen that may lead to nervous disorderliness (Scott, 1994). The contamination of stored cereals by *Aspergillus niger* in tropical region is disturbing as it produces ochratoxins such as Ochratoxin A which causes allergic reactions in human, kidney and liver impairment in man and animal especially pigs (Gautam *et al.*, 2011; Carlos *et al.*, 2004). Also, *Fusarium* spp produces fumonisins, and on consumption; it has been linked to neutral tube defects and oesophageal cancer in man (Anaissie *et al.*, 2009).

Some of the fungi isolated are also associated with diseases such as endocarditis, endophthalmitis, otomycosis, keratitis, infarction, neuroperia and hepatocellular carcinoma (Crawford & Kumor, 2005). These fungi secrete extracellular hydrolytic enzymes that are capable of utilizing these stored products (Amadioha, 1998). Aside aflatoxin, *Penicillium* sp also produces toxins including such as patulin and citrinin. Patulin is neurotoxin while citrinin is nephritic (Makun *et al.*, 2009). *Rhizopus* sp and *Absidia* sp are members of Zygomycetes that are responsible for mucormycosis, especially the life threatening corticosteroid therapy, diabetic ketoacidosis and neutropenia (Kameswaran & Raghunandhan, 2009) particularly in immune-compromised patients (Scharf and Soliman, 2004). The isolation of *Saccharomyces cerevisiae* is not surprising due to its ubiquity nature. Though it is not considered as a pathogenic fungus, it has been reported rarely as a cause of opportunistic infection due to the low activity of 'Phospholipase B' a virulence factor in pathogenic fungi (Ghannoum, 2009).

The results of proximate composition (mg/100g) of fresh rice and sundried rice after 20- weeks storage are summarized in table 2. The ash, carbohydrate, fat and fibre contents of the rice were observed to reduce after it was sundried and stored; ash (4.51 to 4.25), carbohydrate (63.72 to 61.28), fat (2.04 to 1.57) and fibre (10.27 to 8.52). The reduction in ash, fat and fibre content of the stored rice as the storage time progressed is similar to the findings of Fagbohun *et al.*, (2011), who reported a regression in the same three contents of sundried melon seeds (*Citrullus vulgaris*) throughout 20 week storage. The decline in the fibre content suggests the action of the enzymes produced by the fungi present in the grain. The insoluble fibre of grain speed passage of food through the gut and alleviates constipation. The dietary fibre content is lowest in milled rice and continual decrease when stored is highly insignificant; a diet low in fibre can be a contributory factor to irritable bowel syndrome (Oko *et al.*, 2012). The decrease in ash content indicates loss of minerals of the sundried rice as the storage period progressed. Most fungi have high lipolytic activity and fats and oils in grains are readily broken down into free fatty acids and partial glycerides (Bothast). Reducing action of the lipolytic enzymes produced by the fungi may be responsible for the decline in the fatty content of the rice. The outcome on carbohydrate value of this study differed from the findings of Edeogu *et al.*, (2007), who reported an increase in the carbohydrate content of rice (75.37 to 76.37) in Ebonyi state. Also the value was lower than that reported by Oko & Ugwu (2011) when the carbohydrate content of five rice varieties in Abakaliki were analysed. The decline in carbohydrate values during storage period suggests that storage fungi broke down the non-reducing carbohydrate into reducing ones, subsequently utilizing them for their growth and development (Awadh *et al.*, 1998). The major role of carbohydrates is the provision of (glucose) energy to living cells for the growth and metabolism. However, the moisture and crude protein contents increased with storage period. Moisture increased from 7.84 to 11.82, with mean value of 9.89 and standard deviation of 2.09, while crude protein increased from 11.45 to 12.56 with mean value 12.10 and standard deviation of 0.94. The result is similar to the findings of Samir *et al.*, (1998) who reported the moisture content of stored dried maize/maize products to be within 9-19%. Rice grain is hygroscopic and its moisture content will tend to equilibrate with surrounding air especially in high temperature and relative humidity of humid in tropical climate (IRRI). Also grains respire in storage leading to the production of heat, CO<sub>2</sub> and moisture (Lee, 1999). High moisture enhances microbial growth and therefore shorten the shelflife of stored rice (Weinberg *et al.*, 2008). It was also noted that the crude protein of the rice increased during storage. As the carbohydrate is being utilized in respiratory processes, protein increases mathematically; proteolytic enzymes produced by fungi can modify the protein in grains by hydrolizing it into polypeptides and amino acids. Daharty *et al.*, 1970 reported that protein content was slightly higher in flours damaged samples than the corresponding flours from sound wheat protein with the use of Kjeldahl method. The fungi structure and spores may also have contributed to the protein of the rice sample. However, the decrease in carbohydrate and increase in moisture content is a confirmation of the work of Jood *et al.* (1996).

The rice sample was analysed for mineral and reported in mg/100g as summarized in table 3. The mean concentration in mg/100mg of the minerals analysed in the dried rice sample is in the following order: K>Na>P>Ca>Mg>Zn>Fe>Mn. It was noted that the values of all the minerals decreased significantly with storage period. Na decreased from, 41.26 to 38.50, K decreased from, 97.55 to 90.43, Ca, 5.36 to 4.25, Mg, 3.78 to 3.20, Zn, 3.54 to 3.12, Fe 2.18 to 2.00, Mn 1.25 to 1.02 and P 23.33 to 18.55. The result of the mineral analysed is in agreement with the work of Ekundayo & Idzi (2012) who reported reductions in mineral contents of shelled melon seeds. The reduction in mineral contents confirmed the decrease in ash contents of the rice (Babarinde *et al.*, 2010). Na and K are essential and major minerals needed for the normal function of body cells. The gradual decrease in the value of stored dried rice in this study is different from the findings of Ijabadeniyi and Adebolu (2005) who accounted for increase from 1.07 to 4.71 in stored sorghum. According to Wise (1983), the ratio of Na/K should be 0.6. Any ratio value above may be a predisposing factor to hypertension. However, the ratio of sodium to potassium value of the sundried rice throughout the storage period ranged from 0.42 to 0.43, which would not lead to hypertension when consumed as food. Also magnesium ion is essential as it must



attach to ATP in order to function; Na/K pumps are powered by ATP. Twenty-five percent of ATP is needed for continuous pumping of Na/K pumps in human body cell, while 70% ATP is required in heart brain and kidney. Therefore, if ATP and magnesium are deficient and intracellular K/Na is low, cells voltage becomes low and these organs function abnormally. Phosphorus is a vital mineral essential for the normal cellular function and reduction, consumption of rice is significant because inadequate dietary intake can lead to risk of cardiovascular stroke. Calcium is essential for bone formation and development; and rate of its absorption is associated with amount of Phosphorus present. Ca/P is 0.230 both before and after storage; the length of storage of rice does not affect Ca absorption rate. The low ratio will not encourage strong bone development. Rice is a poor source of micronutrients such as iron and zinc; zinc is involved in more bodily function than other minerals. Increase in concentration of zinc in rice grain will reduce its deficiency. Iron is essential element for formation of haemoglobin; the red pigment of the blood. Non heme iron is obtained from grains, vegetable and fruits (Racheal & Dayles, 2009). Iron deficiency includes dizziness, tiredness and mental confusion. The Magnesium value decrease is different from the findings of Mamiro *et al.* (2001) who reported an increase 0.21 – 2.29 in stored dried sorghum. Grains are major source of manganese. The decrease in manganese of rice in this study is dissimilar to the work of Peplinski *et al.*, (1989) who accounted for increase in manganese of stored rice from 0.91 to 2.55. Manganese is an essential micromineral needed for bone formation and specific reactions related to amino acid, carbohydrate and fat metabolism. Only a small portion is absorbed by the body, the rest are excreted via the bile. Although its deficiency may contribute to few clinical symptoms, a clinical deficiency has not been clearly linked with poor dietary intake of the mineral. However, limited studies on induced manganese depletion in human had revealed scaly dermatitis and hypocholesterolemia in the test individuals.

#### 4.0 Conclusion

This study showed fungal invasion and significant loss of nutritive components of the rice in storage in spite of the fact that the rice was sundried to a safe level before storage. These fungi are deleterious as they are producers of spores and mycotoxins whose effects on human health are critical and demand great concern. Proper drying of grains before storage would minimize microbial contamination. It is also recommended to adopt effective storage management in keeping rice and other grains in environmental condition that would discourage the growth of fungi and other microorganisms. Hygiene must also be taken into consideration during harvesting and processing of grains for storage to prevent microbial contamination. Hence, food safety in public and maintenance of nutritional and economic value of such foods is ensured.

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**Table 1.** Fungal Composition of stored sundried rice sample using the three methods of isolation.

Name of fungi	Direct plate	Dilution plate	Washing method
<i>Aspergillus flavus</i>	+	+	-
<i>Aspergillus fumigates</i>	+	+	-
<i>Aspergillus niger</i>	+	+	+
<i>Absidia spp</i>	+	-	+
<i>Fusarium spp</i>	+	-	+
<i>Rhizopus spp</i>	+	+	+
<i>Saccharomyces cerevisiae</i>	+	+	+

+ = Isolated (Present)      - =Not Isolated (Absent)

**Table 2:** The summary of the proximate composition of the stored sundried rice during storage.

Duration of storage (Week)	Ash	Moisture	Crude protein	Fat	Fibre	Carbohydrate
0	4.51	7.84	11.45	2.04	10.27	63.72
4	4.45	9.38	11.77	2.03	9.89	62.40
8	4.48	9.54	12.14	2.01	9.84	62.15
12	4.47	10.35	12.26	1.98	9.22	61.97
16	4.36	10.54	12.44	1.89	8.76	61.71
20	4.25	11.72	12.56	1.57	8.52	61.28
Mean	4.42	9.89	12.10	1.92	9.41	62.19
SD	0.22	2.09	0.94	0.40	1.55	1.86
CV	4.98	21.13	7.80	20.83	16.5	3.04

Key: SD (Standard Deviation) CV (Coefficient of Variation)



**Table 3:** The summary of the mineral analysis (mg/100mg) of sundried rice during 20 weeks of storage

Duration of storage (Week)	Na	K	Ca	Mg	Zn	Fe	Mn	P
0	41.26	97.55	5.36	3.78	3.54	2.18	1.25	23.33
4	40.67	96.48	5.28	3.65	3.49	2.15	1.22	23.28
8	40.64	95.75	5.23	3.60	3.44	2.11	1.20	22.67
12	39.89	92.56	5.20	3.54	3.28	2.08	1.16	21.50
16	39.66	91.85	5.16	3.48	3.25	2.05	1.08	20.42
20	38.50	90.43	4.25	3.20	3.12	2.00	1.02	18.55
Mean	40.10	94.10	5.08	3.54	3.35	2.09	1.20	21.62
SD	0.97	6.41	0.90	0.42	0.33	0.14	0.23	4.18
CV	2.41	6.81	17.7	11.86	9.85	6.69	18.83	19.33

**Key:** SD (Standard Deviation), CV (Coefficient of Variation)

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