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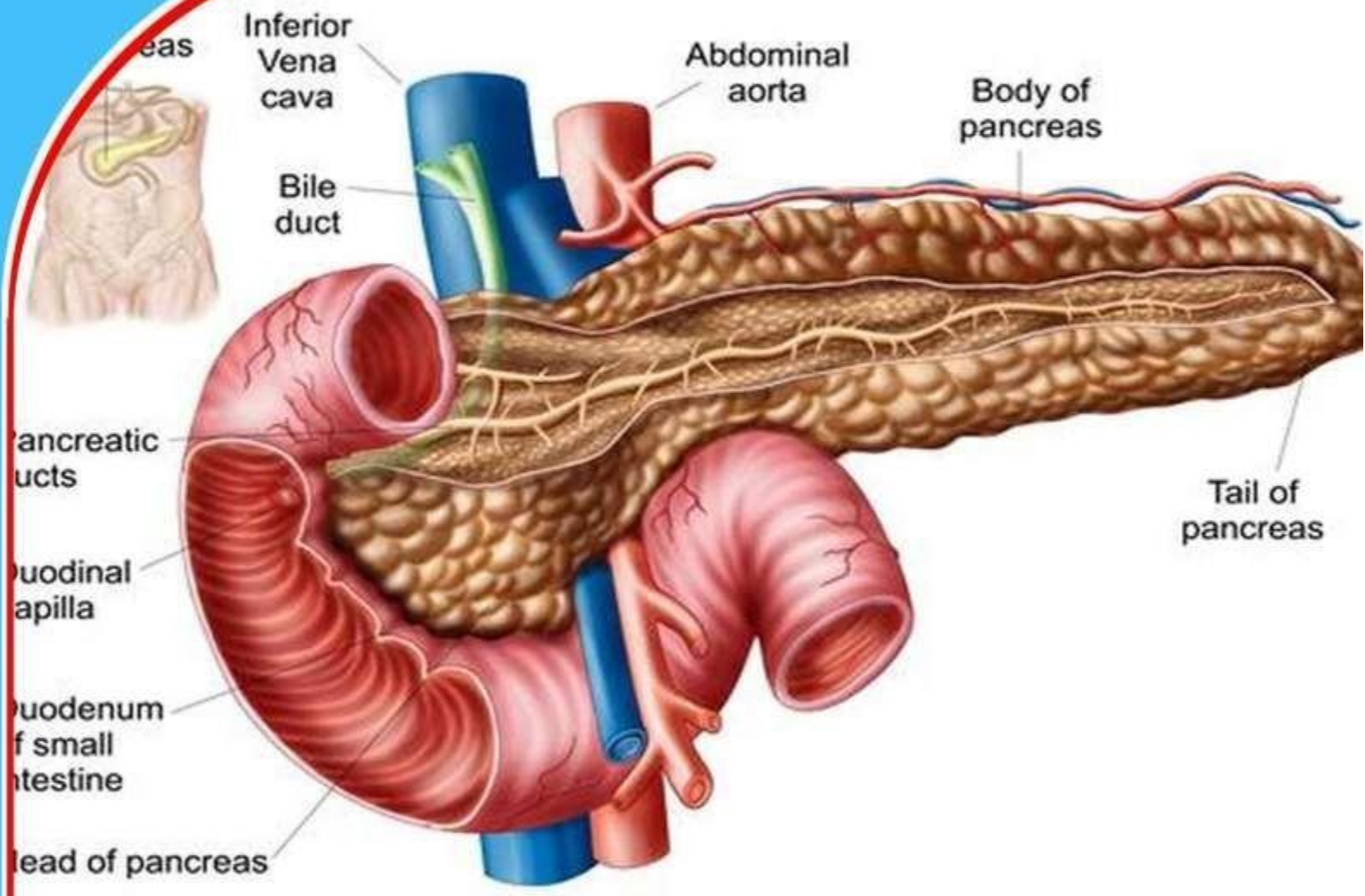

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PROXIMATE AND PHYTOCHEMICAL ANALYSIS OF HEALTHY AND INFECTED MAIZE GRAINS IN ANAMBRA STATE

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Abstract

Four samples of stored maize grains were bought from Awka, Awkuzu, Onitsha and Nnewi. Direct and indirect methods of Fawole and Oso, 1998 were used to isolate the fungi responsible for the spoilage of post harvest maize grains after which four genera were isolated namely: *Curvularia* spp, *Fusarium* spp, *Rhizopus* spp and *Aspergillus* spp. They were identified using Barmette and Haunter, 1987 method of identification. The percentage of occurrence of the fungi from different samples shows that *Aspergillus niger* occured with a frequency of 31.5%, *A. flavus* with 31%, *Rhizopus* spp with 15.25%, *Fusarium* spp with 14.5% and the least *Curvularia* spp with 7.5%. AOAC method of 1999 was used to obtain the nutritional values of the maize grains. Good and spoilt maize grains were used in this research. Spoilt maize grains were used after four months of purchase. Anova were used to compare the nutritional values of both good and spoilt maize samples from the different locations. Six nutrients were obtained from both good and spoilt maize grains namely: crude protein, crude fibre, crude fat, moisture content, ash content and carbohydrate. The results differ in their values for both good and spoilt maize grains. The spoilt maize grains have little protein, fibre and fat and more of ash and carbohydrate. Further research was made on the phytochemical contents of maize and seven metabolites were obtained mamely: oxalate, phytate, tannin, phenol, alkaloid, flavonoid and glycoside. In order to reduce post-harvest grain loss of maize, periodical training of farmers on proper storage facilities are necessary.

Key words: *Proximate analysis, phytochemical analysis, maize*.

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INTRODUCTION

Maize (*Zea mays*) belongs to the family *poaceae*, commonly known as corn in the United States and India, is the third most important cereal grain worldwide after wheat and rice (Golob *et al*., 2004). It is referred to as the cereal of the future for its nutritional values and utilization of its products and by-products (Lee, 1999). The demand for maize has been estimated to increase by 50% from 558 million metric tons in 1995 to 837 million metric tons in 2020 (Martinez *et al*., 2011), fuelled by diverse uses from food processing, animal feed, to ethanol production (FA0, 2006). It is a basic staple food grain for large parts of the world including Africa, Latin America and Asia (Yaouba *et al*., 2012).

Subsequently, the maize is stored while still relatively moist and warm; both warm and high moisture content can result in rapid deterioration of the grains and promote the growth of microorganisms (fungi and bacteria) and insects in the grain (Ekechukwu and Norton, 1999). Maize like order stored products is hygroscopic in nature and tends to absorb or release moisture. Even if properly dried after harvest, exposure to moist and humid conditions during storage will cause the kernel to absorb from the surroundings (Devereau *et al*., 2002), leading to increased maize moisture contents which results in enhanced deterioration.

To maintain high quality maize during storage, maize should been protected from weather (including relative humidity and temperature), growth of microorganisms, and insects (Oyekale *et al*., 2012). According to Campbell *et al*. (2004), the current estimates of the cost of grain loss due to insect and microorganism damage of grain stored in developing countries each year ranged from $500 million to $1 billion. Tuite and Foster (1979) also reported that insects in grain enhance mold development because they increase moisture contents and temperature, and open areas of the grain for attack. Fungal growth in maize is facilitated by hot and humid conditions (Egal *et al*., 2005). It has been reported by several researchers that fungal infestation in maize results in color change, decreases in nutritional values, and reduction of overall quality and quantity of the maize. Major fungi associated with grain storage, including maize are *Aspergillus flavus* and *Fusarium spp* and others. Fungal growth in maize presents a major risk for humans and animals, through production of mycotoxins (especially Aflatoxins).

Maize (*Zea mays*) is a grain grown by farmers in various parts of the world especially Africa. Maize grain losses in Anambra State cause huge economic losses. Its spoilage most times limits its availability to consumers and causes economic waste to farmers. Maize grains are mostly spoiled by fungi. The identification and characterization of these spoilage organisms will go a long way to proffer solution on how this problem of post-harvest spoilage could be solved. Storage materials/system also contributes a lot to spoilage of stored maize grains, farmers are forced to dispose off their produce especially to NCPB at throw away prices and later buy the same at higher prices to meet their subsistence needs. This is because farmers do not have efficient as well as adequate facilities to store their grains after harvesting when the supply is glut. There are a lot of storage capacities in the hands of farmers, only that this great potential has not been adequately exploited especially by the low income small scale farmers (Sasaki, 1995).

Some farmers are therefore forced to store their grains in primitive structures, resulting to huge losses due to contamination and pest damage. This is attributed to the fact that most farmers cannot afford to acquire the efficient modern storage facilities due to financial constraints. Small-scale farmers, in this case, are the most affected. To solve this problem, farmers should be facilitated to buy new grain storage facilities so that they can store their produce which they can later sell at higher prices to make good profits. This is however constrained by the fact that most of these farmers are poor hence may not buy the facilities anyway. To assist farmers NCPB should have assisted the farmers to buy small scale storage facilities like metal silos, through supplying them to farmers at subsidize prices.

This study will help in the acquisition of the knowledge of fungi associated or involved in the spoilage of post-harvest stored maize grains, factors that enhance the spoilage of post-harvest maize grains and the risk involved in the consumption of spoilt maize grains. During storage, several kinds of fungi can remain associated to maize grains either causing their deterioration or simply remain viable to infect germinating seeding. Its spoilage could also limit its availability to consumers thereby causing economic waste to the farmers and the Nation at large.

The aim of this research work is to evaluate fungi responsible for loss of post-harvest stored maize grains in Anambra State, possible ways to curb the spoilage and also to evaluate the nutritional (proximate) contents and phytochmical (metabolites) compostions of the maize grains.

The objectives of this work are to microbiologically isolate and identify fungi responsible for postharvest spoilage of stored maize using four villages as the case study. Know the nutritional content of stored maize. Determine the phytochemical compositions of the grains. Recommend on ways to limit post-harvest maize grain loss.

MATERIALS AND METHODS SOURCES OF MATERIALS

Infected stored maize grains were collected from four different Locations in Anambra State (Awka, Awukuzu, Nnewi and Onitsha). Awka, the capital city of Anambra state with latitude and longitude of 6·12’25ºN7.04’04ºE and 6.20694ºN 7.06778ºE. The city is located 199.1 kilometers (123.7mi) by road directly north of Port Harcourt in the centre of the densely populated Igbo heartland in south east Nigeria. Awkuzu, which lies between latitude 6.12ºN – 6·22ºN and longitude 6·47ºE and 7ºE, it’s about 24km southeast of Awka the capital city of Anambra State
Nigeria. Nnewi, lies on latitude and Longitude 6·1ºN $6^0$55’E / 6.17ºN 6.917ºE and spans over 1,076.9 square miles (2789km^2). Onitsha, lies between latitude and longitude coordinates of 6·10ºN 647ºE / 6.167ºN 6.783ºE, with the city area of 13.97 square miles (36.19km^2), land 13.95 square miles (36.12km^2), water 0.026 square miles (0.67km^2), metro and urban 759 square miles (19765km^2). Location1 is Onitsha, Location2 is Nnewi, Location3 is Awka, Location4 is Awkuzu. The samples was transported in clean, moisture free polythene bags to central laboratory services in National Root Crops Research Institute Umudike, Abia State for microbiological and Nutritional Analysis.

LOCATION OF STUDY

The experiment was conducted in Central Service Laboratory,National Root Crops Research Institute Umudike, Abia State. The study area is located on latitude on 0.5°29N and 07°33E in the rainforest ecological zone of south eastern Nigeria and lies at a mean elevation of 122meters (400ft) above sea level.

METHODOLOGY FOR ISOLATION AND IDENTIFICATION OF POST HARVEST MAIZE GRAINS

Direct and Indirect methods (Fawole and Oso, 1998) were used in isolating and identifying fungi associated with stored maize grains. For direct method, 50 maize grains were surface-sterilized by washing in 70% ethanol solution for 1 minute and rinsed in sterile distilled water 3 times to remove traces of ethanol from these maize. The grains were transferred onto a sterile filter paper for drying. Potato Dextose Agar (PDA) was prepared according to manufactures guide and poured into plates and allowed to solidify. Flamed sterilized forcep was used to place 50 pieces of sterilized maize grains on plates. The inoculated plates were sealed with paper tapes and incubated at 25°C for 3 to 5 days. The inoculated plates were observed daily for mycelia growth on the plated grains. Sterile inoculating needle was rinsed in picking threads of mycelia on the maize grains and were transferred on to a newly prepared PDA plates and were incubated at 25°C for 3 to 5 days for purification. Subsequent sub culturing was done for further purification of the isolates.

The indirect method was equally done through the pour plate method as suggested by Fawole and Oso, 1988. About 50 maize grains were dropped in a gas cylinder containing 50ml of sterile distilled water and 5 drops of tween 20. The cylinder was shaken together vigorously for 5mins. 1ml from the washed water (assay) was serially diluted out by dispensing the 1ml assay into a test-tube containing 9ml of sterile distilled water was shaken together (10^{-1}) 1ml was equally taken from 10^{-1} and transferred into the next test-tube (10^{-2}). The serial dilution continued to 10^{-5}, 1ml from dilution 10^{-2},10^{-3} and 10^{-4} were pour plated for inoculation, allow to solidify, sealed with paper tape and incubated at 25°C for 3 to 5 days.

The plates were observed daily for mycelia growth. The mycelia growth were picked and transferred unto a newly prepared plates which later transferred into an incubator at 25°C for 3 to 5 days. Subsequent sub culturing were done for further purification of the isolates. All the purified cultures (Direct and Indirect) were identified through microscopic examination which was done by physical characteristics of the mycelia like color and structure of the mycelia. Microscopic characteristics through the morphological structure according to (Barmette and Hunter, 1987). A wet mount method (Fawole and Oso, 1988) was done before viewing the pure isolates under $\times 4D$ compound microscope. The morphological structures viewed include sepate or non-sepate mycelia, presence of sporangiophores, fruiting bodies and special organs like rhizoids.

TOTAL VIABLE COUNT

This is known as microbial load. About 1g from sample was weighed out on a sensitive weighing matter scale, 9ml of sterile distilled water was added to it in a gas cylinder, shaken together vigorously and allowed to stand for 5 minutes.

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SERIAL DILUTION

A plastic rack was arranged with sterile test tube containing 9 test tubes filled with 9ml of sterile distilled water. A ten-fold serial dilution was carried out by picking 1ml from the above stock with the aid of a sterile pipette and transferred into the first test tube in the test tube rack and shaken together (10^{-1}). 1ml was taken from 10^{-1} and transferred to next test tube (10^{-2}). The dilution continues to the last test tube. Each tube was shaken vigorously before each transfer.

INOCULATION

A pour plate method (Dhawole and LaMaster, 2003) was used in plating all the samples. About 1ml from dilution (10^{-1}) (the dilution that is not too cloudy or too light) was dropped into sterile 9ml Petri dish with the aid of a sterile pipette. Molten potato dextrose agar was poured into each plate (10ml). The plates were rotated clockwise for easy mix up of the sample and the media. All plates were allowed to solidify on the bench. All plates were duplicated.

INCUBATION

All the solidified plates were transferred into an incubator at 25ºC for 3-5days. Mycelia growths (colonies) were observed daily for counting.

COUNTING OF THE COLONIES

After incubation of the plates, counts of the number of colonies in each plates was done with a hand tally counter (a stopwatch like device for counting) (Dhawale and LaMaster, 2003). A mean of the count was obtained and multiplied with the appropriate diluting factor.

Total Viable Count = Mean Colony Count× dilution factor

METHODOLOGY FOR PROXIMATE AND PHYTOCHEMICAL COMPOSITION OF MAIZE GRAINS MOISTURE CONTENT DERTERMINATION

An empty Petri dish was dried in an oven for about 10 minutes and allowed to cool in a desiccator containing calcium chloride for about 20 minutes and then weighed (W_1). Approximately 2g of the sample was weighed into the Petri dish (W_2) and placed in an oven at 105ºC for 8 hours. It was then brought out, cooled in a desiccator and weighed (W_3). The procedure was repeated until a constant weight is obtained.

CALCULATION

$$\% \text{ MOISTURE} = \frac{W2-W3}{W2-W1} \times 100$$

ASH DETERMINATION

Ash represents the inorganic remains after the organic carbonaceous portion and other volatile components have been oxidized and evaporated away. An empty crucible was fire-polished in

muffle furnace and allowed to cool in a desiccators containing calcium chloride for 20minutes and then weighed (W_1). About 2g of the sample was weighed into the crucible (W_2) and transferred into muffle furnace and heat at 550°C until the sample is completely ash, the crucible was removed and a drop of water was added to expose the unashed portion. The crucible was placed back in the murfle furnace and heated for more 30mins. This was removed and allowed to cool in desiccator after which the crucible with the ash was weighed (W_3).

CALCULATION

$$\% \text{ ASH} = \frac{W3 - W1}{W2 - W1} \times 100$$

CRUDE PROTEIN DETERMINATION

The crude protein content of the sample was determined using the macrokjeldahl method of AOAC (1999). The samples will be digested with concentrated sulphuric acid, using copper sulphate and sodium sulphate as catalyst to convert organic nitrogen to ammonium ions. Alkali will be added and the liberated ammonia is distilled into an excess boric acid. The distillate was titrated with hydrochloric acid or sulphuric acid.

CALCULATION

$$\text{Nitrogen}(\%) = \frac{1.4 \times \text{Titre Volume} \times \text{total volume of digest}}{1000 \times weight of Sample \times Aliquot distilled} \times 100$$

Crude Protein (%) = %Nitrogen x 6.25

CRUDE FAT DETERMINATION

The crude fat was determined using soxhlet extraction method of AOAC (1999). A 500ml fit round bottom flask was washed and dried in an oven for about 25minutes and allowed to cool in a desiccator before it was weighed (W_1). Approximately 5g (W) of the sample was weighed wrapped in a thimble. This thimble and its content were inserted into the extraction column with the condenser. About 350ml of the extracting solvent (n-hexane) was poured into the round bottom flask and fitted into the extraction unit. The flask was heated with the aid of electrothermal heater at 60°C for six hours. Losses of solvent due to heating were checked with the aid of the condenser so that it cooled and refluxed the evaporated solvent. After extraction, the thimble was removed and the solvent salvaged by distillation. The flask and its content were placed on a water bath to evaporate off the solvent. The flask and the residue was transferred to an oven and heated for some minutes to evaporate the remaining solvent and moisture to complete dryness. It was cooled in a desiccators and weighed (W_2).

CALCULATION

$$\% \text{ Crude fat} = \frac{W2-W1}{W} \times 100$$

CRUDE FIBRE DETERMINATIONS

The crude fibre was determined using the gravimetric methods of AOAC (1995). The crude fiber method gives an estimation of insoluble and indigestible food residue which remains after which the sample has been treated under prescribed conditions. It was determined by consecutive treatment with light petroleum, boiling dilute sulphuric acid, boiling dilute NaOH, dilute HCl, alcohol and ether. The insoluble residue was collected by filtration, dried, weighed and ashed to collect mineral contamination.

CALCULATION

$$\% \text{ CRUDE FIBRE} = W \frac{eightofdriedinsolublematter - weightofash}{weightofsamplebeforedefatting} \times 100$$

DETERMINATION OF TOTAL CARBOHYDRATE

The total percentage carbohydrate content was determined by difference of 100 as reported by Yerima and Adamu (2011).

PHYTOCHEMICALS ANALYSIS Alkaloid Determination

Five grams (5g) of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol is added and covered and allowed to stand for 4 hours at 25ºC. This was filtered with Whatmann's filter paper no. 42 and the filtrate is concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide (NH_4OH) was added drop wise to the extract until the precipitate is collected and washed with dilute NH_4OH (1% ammonia solution). Then, filter with pre-weighed filter paper. The residue on the filter paper is the alkaloid, which was dried in the oven (precision electrothermal model BNP 9052 England) at 80ºC. The alkaloid content was calculated and expressed as a percentage of the weight of the sample (Harborne, 1995**).**

Cardiac Glycosides Determination

This will be carried out according to the method described by Osagie (1998). One mililitre (1ml) of extract was added 1ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1ml of 5% aqueous NaOH. It was boiled for 2 minutes (until brick-red precipitate is observed) and the boiled sample was filtered. The weight of the filter paper was taken before filtration. The filter paper with the absorbed residue was dried in an oven at 50ºC till dryness and weight of the filter paper with residue was noted. The cardiac glycoside was calculated in percentage.

Phytate

Phytate contents were determined using the method of Young and Greaves (1940). 0.2g of each of the differently processed corns was weighed into different 250ml conical flasks. Each sample was

soaked in 100 ml of 2% concentrated HCL for 3h. The samples were then filtered. 50ml of each filtrate was placed in 250ml beaker and 100ml distilled water added to each sample. 10ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (111) chloride solution which contained 0.00195g iron per ml. The percentage phytic acid was calculated using the formula:

$$\text{Phytic acid (\%)} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{2}$$

Oxalate Determination by Titration method

This was determine according to Osagie (1998). This determination involves three major steps digestion, oxalate precipitation and permanganate titration.

Digestion

Approximately 2g of sample is suspended in 190ml of distilled water in a 250ml volumetric flask.10ml of 6m HCL is added and the suspension digested at 100ºC for 1 hour. Cool and then make up to 250ml mark before filtration.

Oxalate precipitation

Duplicate portions of 125ml of the filtrate are measured into beakers and four drop of methyl red indicator added. This is followed by the addition of NH_4OH solution (drop wise) until the test solution changes from salmon pink colour to a faint yellow colour (Ph4-4.5). Each portion is then heated to 90ºC, cooled and filtered to remove precipitate containing ferrous ion. The filtrate is again heated to 90ºC and 10ml of 5% $CaCL_2$solution is added while being stirred constantly. After heating, it is cooled and left overnight at 25ºC. The solution is then centrifuge at 2500rpm for 5 minutes. The supernatant is decanted and then precipitate completely dissolved in 10ml of 20% (v/v) H_2SO_4 solution.

Permanganate Titration

At this point, the total filtration resulting from digestion of 2g of flour is made up to 300ml, aliquots of 125ml of the filtrate is heated until near boiling and then titrated against 0.05 M standardized $KMNO_4$solution to a faint pink colour which persists for 30s. The calcium oxalate content is calculated using the formula,

$$\frac{T \times (vme)(Df) \times 10^5}{(ME) \times Mf} (mg/100g)$$

Where T is the titre of $KMno_4$(ml), Vme is the volume – mass equivalent (i.e. 1ml of 0.05m $KMno_4$ solution is equivalent to 0.00225g anhydrous oxalic acid). Df is the dilution factor Vt/A (2.4 where Vt is the total volume of titrate (300ml) and A is the aliquot used(125ml), ME is the

molar equivalent of $KMno_4$ in oxalate (KMo_4 redox reaction) and Mf is the mass of sample used.

Total phenol

The total phenol content of the samples was determined using the method of Barros *et al.* (2007). The extract solution (1 ml) was mixed with Folin and Ciocalteu's phenol reagent (1 ml). After 3 min, saturated sodium carbonate solution (1 ml) was added to the mixture and adjusted to 10 ml with distilled water. The reaction will be kept in the dark for 90 min, after which the absorbance was read at 725 nm (UV-Visible spectrophotometer). Gallic acid was used as the standard and the results was expressed as mg of gallic acid equivalents (GAEs) per g of the sample.

Total flavonoids

The flavonoid content was determined by the use of a slightly modified colorimetry method described previously by Barros *et al.*(2007). A 0.5 ml aliquot of appropriately (2 mg/2 ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % $NaNO_2$ solution. After 6 mins, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 mins, and then 2ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, then the mixture was thoroughly mixed and allowed to stand for another 15 mins. Absorbance of the mixture was determined at 510 nm versus water blank with reference standard prepared with catechin concentrations. The analyses were performed in triplicate. The results was expressed as mg Catechin equivalents per 100g of sample (mg CE/100 g).

Tannin

The Tannin content of the maize grain was determined according to the method of Siddhuraj and Manian (2007). About 1 milliliter of the extract was taken and treated with 0.1g of polyvinyl polyparolylydon in 1ml of distilled water. This was placed inside a refrigerator at 4ºC for 4hours. The sample was removed from the refrigerator and centrifuged for 5mins at 4000rpm. The supernatant (1 ml) was mixed with Folin and Ciocalteu's phenol reagent (1 ml). After 3 mins, saturated sodium carbonate solution (1 ml) was added to the mixture and adjusted to 10 ml with distilled water. The reaction mixture was kept in the dark for 90 min, after which the absorbance was read at 725 nm (UV-Visible spectrophotometer). Gallic acid was used as the standard and the results were expressed as mg of Gallic acid equivalents (GAEs) per gram of the sample. Tannin was calculated using the formula: Tannin = (Total Phenol – Free Phenol).

STATISTICAL ANALYSIS

The data collected were subjected to analysis of Variance (ANOVA) using general linear model option SAS. Test of significance was determined by Duncan's multiple range test 5% level of probability.

RESULTS

IDENTIFICATION OF ISOLATED FUNGI

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Table 1: All fungi isolated from the samples.

S/N	FUNGI	spp
1	*Curvularia*	spp.
2	*Fusarium*	spp.
3	*Aspergillus*	*niger*
4	*Rhizopus*	spp.
5	*Aspergillus*	*flavus*

A total of four genera were isolated from the four samples. Table 1 shows the macroscopy and microscopic view of the fungi.

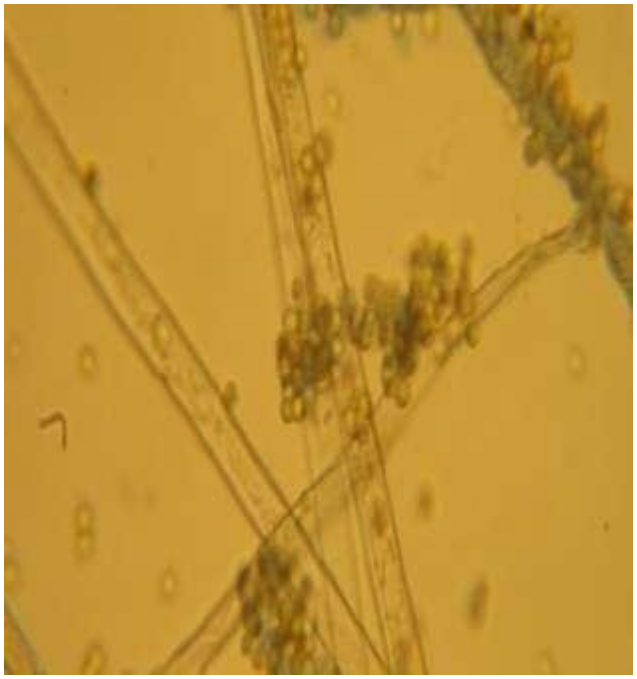

Plate 1:
Macroscopic view of *Aspergillus flavus* **Microscopic view of *Aspergillus flavus***

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Plate 2:
Macroscopic view of *Rhizopus* spp. **Mcroscopic view of *Rhizopus* spp.**

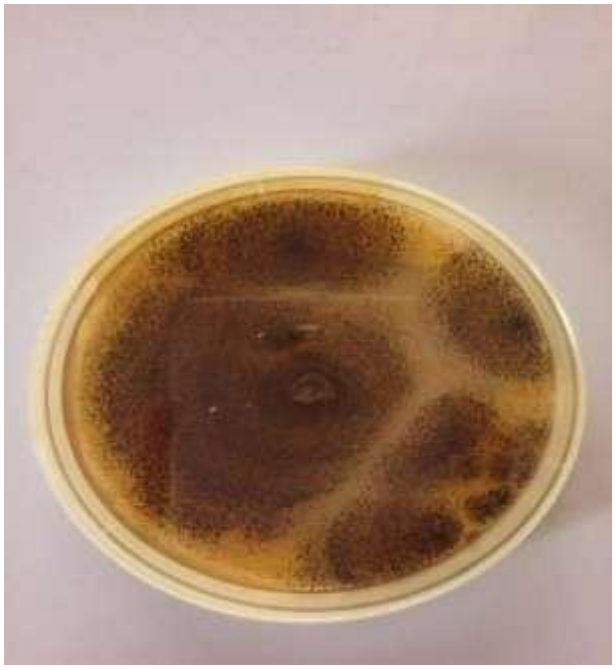

Plate3:
Macroscopic view of *Aspergillus niger* **Microscopic view of *Aspergillus niger***

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Plate 4:

Macroscopic view of *Curvularia* spp. **Microscopic view of *Curvularia* spp.**

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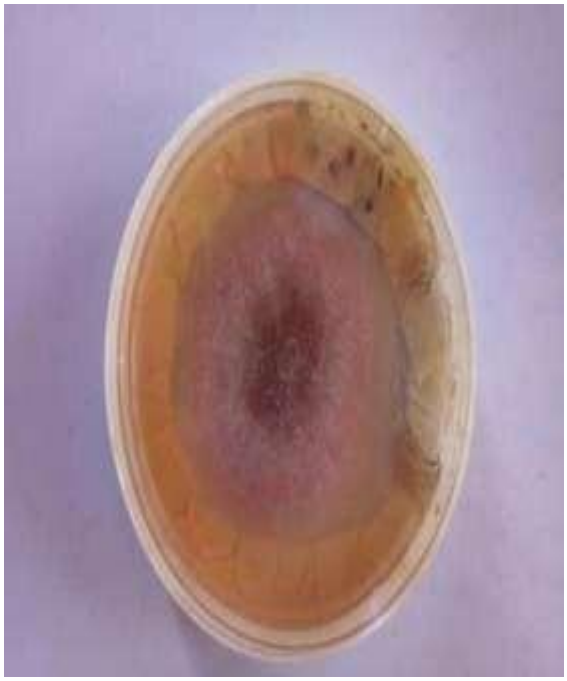

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Plate 5:
Macroscopic view of *Fusarium* spp. **Microscopic view of *Fusarium* spp.**

3.2 PERCENTAGE OCCURRENCE OF ISOLATED FUNGI FROM DIFFERENT LOCATIONS

Table 2: Percentage occurrence of isolated fungi from maize in different locations.

S/N	Locations	*Curvularia*	*Fusarium*	*A. niger*	*Rhizopus*	*A.flavus*
1	Onitsha	16	20	25	18	31
2	Nnewi	-	18	25	12	40
3	Awka	10	-	36	18	28
4	Awkuzu	4	20	40	13	25
	Total	30	58	126	61	124
	Mean	7.5%	14.5%	31.5%	15.25%	31%

Results of the percentage occurrence of isolates from different Locations revealed that *Aspergillus flavus* gave the highest percentage in Location B (40 %) while *Curvularia* spp gave the highest occurrence in Location A (16 %) as seen in Table 2 shown below. As shown in Table 2 for mean and total occurrence, *Aspergillus niger* has the highest occurrence in Locations A,B,C,D (31.5 % mean) while *Curvularia* spp gave the least occurrence among Locations A,B,C,D with (7.5 % mean). There were difference both in Locations and mean calculations among all isolates.

3.3 TOTAL VIABLE COUNT

Table 3: Showing the total viable count of maize grains from different locations.

S/N	LOCATION	TOTAL VIABLE COUNTcfu Colony Forming Unit) (10 log)
1	ONITSHA	1.1×10^2
2	NNEWI	1.9×10^2
3	AWKA	1.3×10^2
4	AWKUZU	9×10^1

The results for TVC which was incubated and observed under temperature of 25°C for 3- 5 days, were serially diluted, growth of colonies were observed and counted with a hand tally counter for each Locations. As shown in Table 3 below, Location B gave the highest colony count of 19, followed by Location C while Location D has the lowest unit count (Table 3).

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3.4 CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF ISOLATES

Table 4: Plate and morphological characteristics of isolated fungi.

COLOR	MORPHOLOGICAL DESCRIPTION	ISOLATE
Darkish Brown  PLATE 6	Conidiophores upright, simple, terminating in a globose or clavate swelling bearing phialide at the apex or entire surface; conidia 1 _celled globose Often variously coloured in mass dry basipetal chain	*Aspergillus niger*

Mycelia are extensive and cotton-like in *Fusarium* **PinkishWhite** culture, condiophores variable, spp

slender and simple, single or grouped into sporodochia; conidia hyaline, macroconidia several-celled, bent at the pointed ends, typical canoe shape.

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Vol.5, Issue 2 No.1, pp 15-43, 2020

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PLATE 7

Dark Brown  PLATE 8	Conidophores brown, mostly simple bearing condia apically or on new sympodia growing points;condiadark,end cells lighter 3 -5 celled,more or less fusiform, typically bent with one of the central cells enlarged.	*Curvularia* spp
White Blackspots  PLATE 9	Colonies grew fast and coarse. Characterized by dark sporangia containing dark to pale spores and a large collumella. At the base of thesporangiophores are root like rhizoids.	*Rhizpous* spp
Green Flavus  PLATE 10	Conidiophores upright, simple, terminating in a globose or swelling, bearing phalide at the apex or radiating from the apex or the entire surface, conidia 1-celled, globose, always colored in mass dry basipetal chains.	*Aspergillus flavus*

European Journal of Biology
ISSN 2709-5886 (Online)
Vol.5, Issue 2 No.1, pp 15-43, 2020

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The isolates were identified using cultural characteristics and morphology according to Barmette and Hunter, Colony morphology (pigmentation and arrangements), motility and general biochemical characteristics and the results were presented in Table 4 above.

3.5 NUTRITIONAL ANALYSIS OF MAIZE GRAINS

Table 5: Nutritional analysis (proximate) of healthy maize grains

PARAMETERS	ONITSHA	NNEWI		AWKA	AWKUZU
Crude proteins (%)	10.5 ± 0.622	10.72 ± 0.311	10.28 ± 0.311		11.16 ± 0.311
Crude fibre (%)	9.05 ± 0.070	5.42 ± 0.035		6.82± 0.671	6.5 ± 0.212
Ash content (%)	1.3 ± 0.282	1.52 ± 0.049		1.25 ± 0.353	1.34 ± 0.113
Moisture (%)	5.87 ± 0.176	5.45 ± 2.050		8.55 ± 0.707	7.81 ± 0.410
Total lipids (%)	4.65 ± 0.282	4.38 ± 0.275		4.45 ± 0.070	3.5 ± 0.141
Total carbohydrate (%)		72.49 ± 2.029		68.4 ± 1.697	69.69 ± 0.961

The nutritional (proximate) composition of maize grains obtained from sample areas for good maize grains are shown in Table 5. It explains carbohydrate as the most dominating nutrient of maize grains in the range of 68-72%, followed by protein in the range of 1011%. A lot of people believe that maize grains do not have another nutrient contents except carbohydrate (starch) but Table 5 gives a full nutritional contents and their range from different Locations. Statistical analysis using Anova shows no significant differences exists between the different Locations.

European Journal of Biology
ISSN 2709-5886 (Online)
Vol.5, Issue 2 No.1, pp 15-43, 2020

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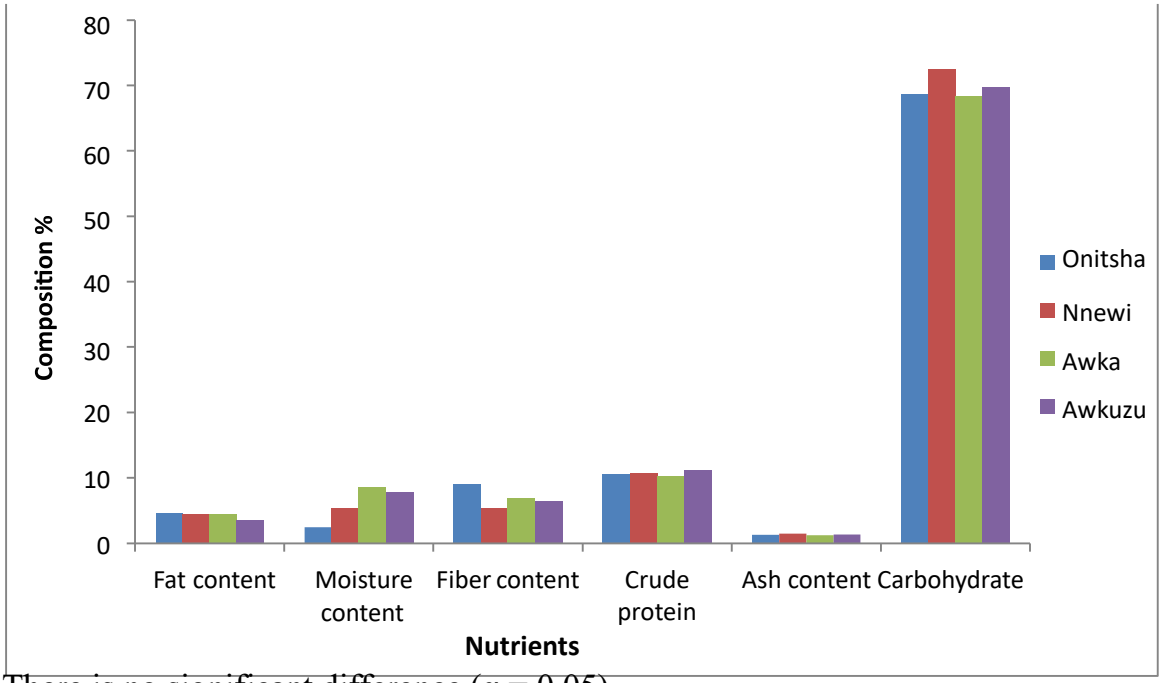

There is no significant difference ($\alpha = 0.05$)

Figure 1: Nutritional composition of good maize grains from different locations

European Journal of Biology
ISSN 2709-5886 (Online)
Vol.5, Issue 2 No.1, pp 15-43, 2020

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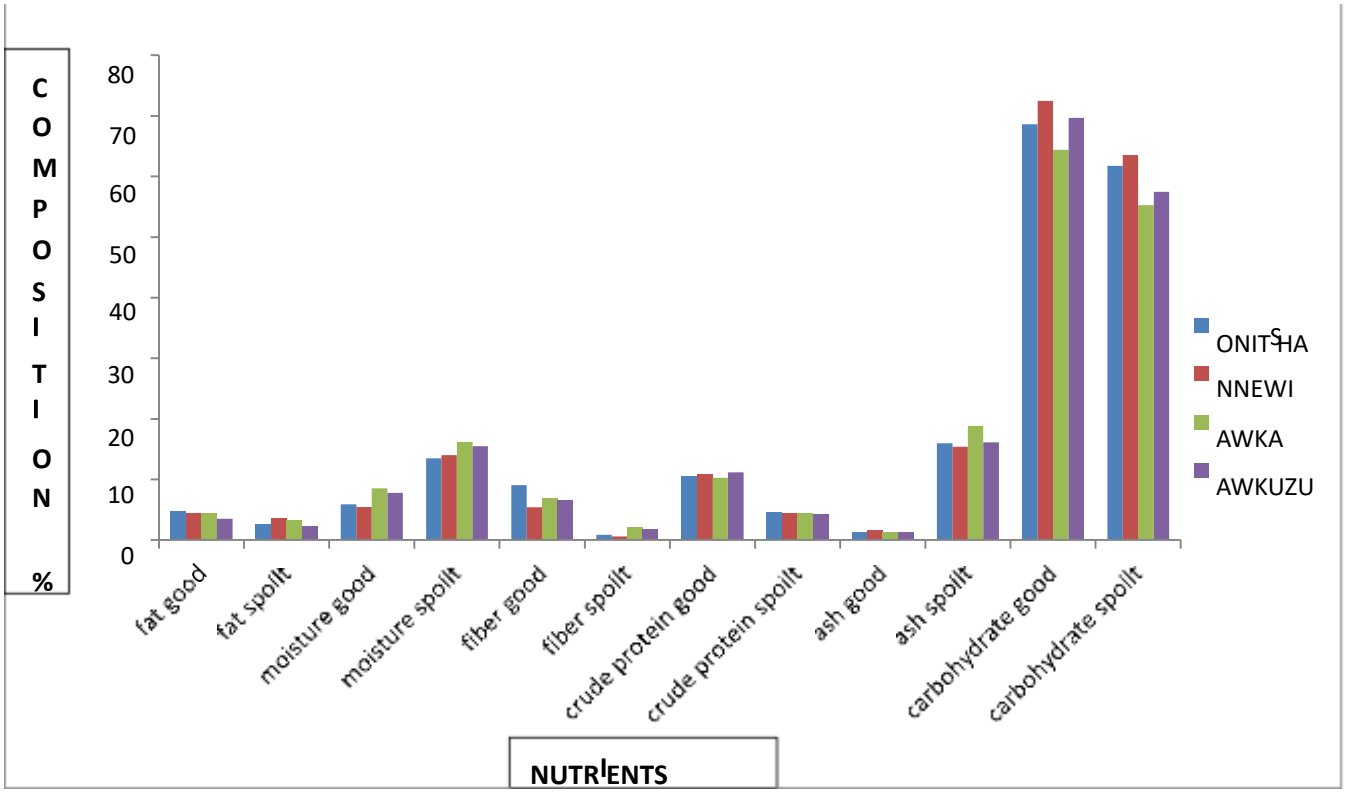

*No Significant difference (α = 0.05)

Figure 2: Nutritional composition of good and spoilt maize grains from onitsha, nnewi, awka and awkuzu.

3.6 NUTRITIONAL COMPOSTION OF SPOILT MAIZE GRAINS

Table 6: Nutritional analysis (proximate) of spoilt maize grains.

PARAMETERS	ONITSHA	NNEWI	AWKA	AWKUZU
Crude proteins (%)	4.55 ± 0.494	4.37± 0.247	4.37± 0.247	4.2 ± 0.497
Crude fibre (%)	0.85± 0.353	0.6 ± 0.000	2.08± 0.176	1.8 ± 0.141
Ash content (%)	15.98 ± 0.466	15.37± 2.411	18.81 ± 0.311	16.13 ± 3.478
Moisture (%)	13.47± 3.719	13.99 ± 0.014	16.16± 0.120	15.48 ± 1.209
Total lipids (%)	3.4 ± 0.282	3.5 ± 0.424	3.3 ± 0.141	2.25 ± 0.131
Total carbohydrate (%)	61.75 ± 3.111	63.54 ± 0.176	55.26 ± 0.643	57.45 ± 2.666

Table 6 above explains the available nutrients of maize (spoilt) grains after 4 months of purchase, there is a random decrease in the nutritional contents as compared to good maize grains in Table 5. It was noted that almost all the nutrients were lost except carbohydrate

European Journal of Biology
ISSN 2709-5886 (Online)
Vol.5, Issue 2 No.1, pp 15-43, 2020

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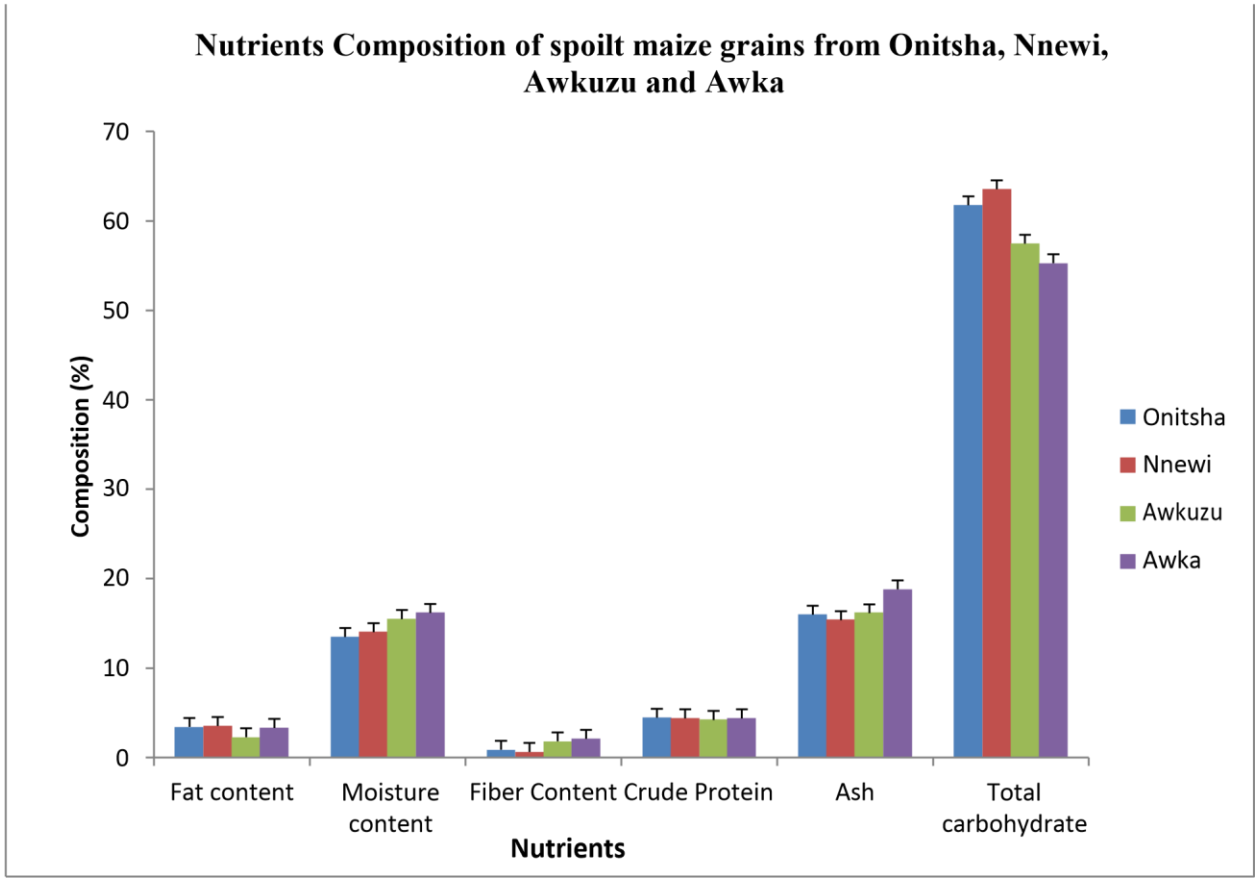
ue to the spoilage observed. Statistical analysis using Anova shows no significant differences exists between the different Locations.



No Significant difference (α= 0.05)

Figure 3: Nutritional contents of spoilt maize grains from onitsha, nnewi, awka and awkuzu.

PHYTOCHEMICAL (metebolites) ANALYSIS

Table 7: Phytochemical compositions of maize grains

PARAMETERS	ONITSHA	NNEWI	AWKA	AWKUZU
Oxalate (%)	10.12 ± 0.190	10.39 ±0.572	10.39 ± 0.190	10.26 ± 0.381
Phytate (%)	3.57 ± 0.028	3.61 ± 0.084	4.01 ± 0.028	3.67 ± 0.000

European Journal of Biology
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Vol.5, Issue 2 No.1, pp 15-43, 2020

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Alkaloid (%)	0.18 ± 0.028	0.22 ± 0.028 0.045 ± 0.004	0.30 ± 0.254 0.047 ± 0.001		0.18 ± 0.084
Tannin (%)	0.046 ± 0.003				0.044 ± 0.001
Total phenol (%)	0.167 ± 0.002	0.165 ± 0.003		0.163 ± 0.007	0.161 ± 0.005

Total flavonoid (%)	0.204 ± 0.014	0.210 ± 0.011	0.204 ± 0.009	0.206 ± 0.014
Cardiac glycoside (%)	3.27 ± 0.070	3.51 ± 0.014	3.25 ± 0.042	3.13 ± 0.014

The results for phytochemical composition of maize grain are shown in Table 7 below. Of all the metabolites oxalate was found to be in the range 10.13-10.39% for Nnewi and Awka, followed by phytate 3.57-4.01%, glycoside 3.13-3.51% while other metabolites like alkaloid, tannin, phenol and flavonoid presents were at very low percentage. Statistical analysis using Anova shows no significant differences between the entire metabolites obtained from different Locations.

European Journal of Biology
ISSN 2709-5886 (Online)
Vol.5, Issue 2 No.1, pp 15-43, 2020

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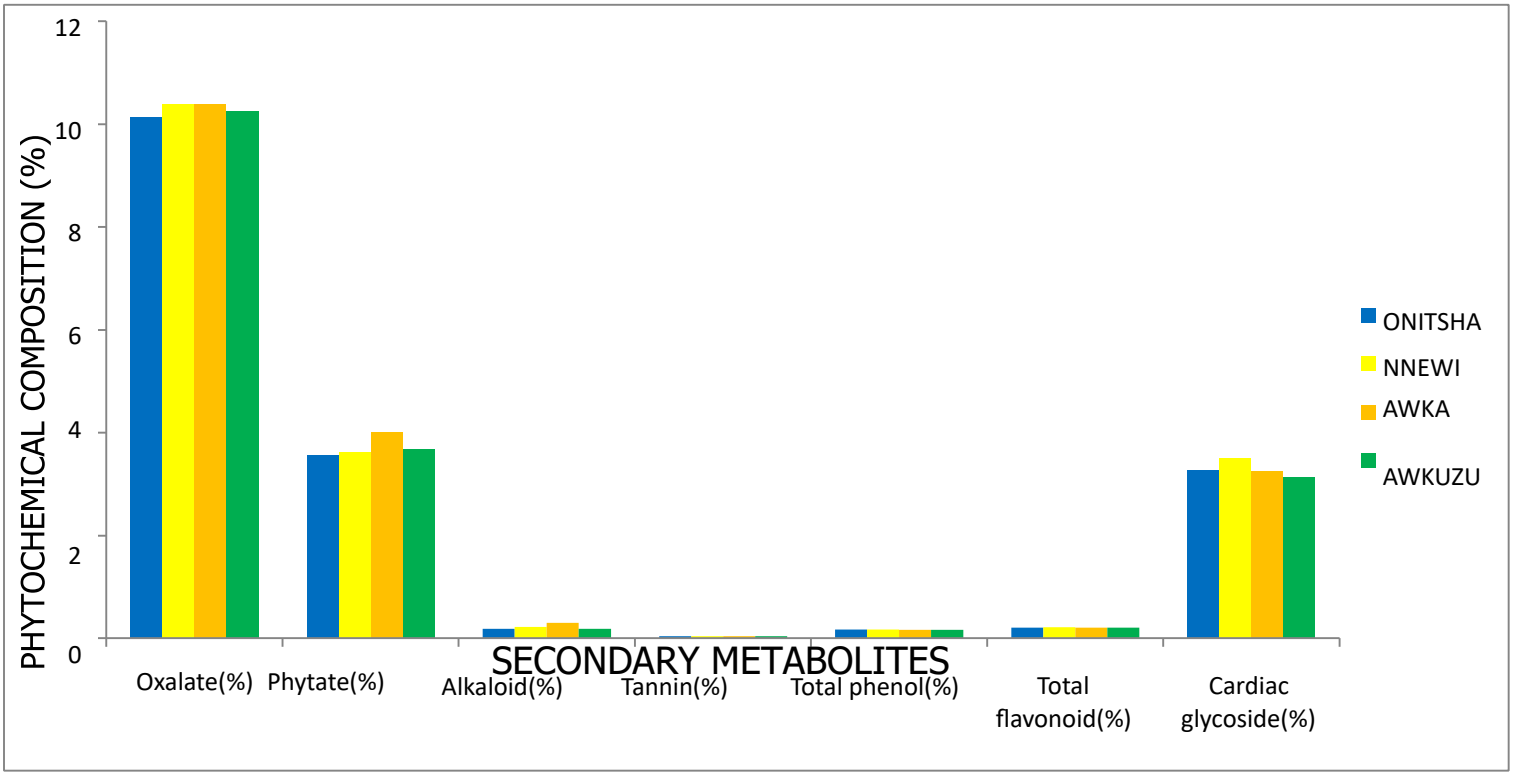

Figure 7: Anova presentation of phytochemical compositions of maize grains from different location.

European Journal of Biology
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Vol.5, Issue 2 No.1, pp 15-43, 2020

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Plate 11: The four samples and their locations

Plate 12: Direct plating of maize grains

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Vol.5, Issue 2 No.1, pp 15-43, 2020

DISCUSSION

Stored grains are considered an ecological system. Jian and Jayas (2012) described it as an approach by which grain integrated with other factors such as relative humidity and temperature to promote protection of grain and environments to deliver good quality grain at the end of storage time. Practice of storage of grain has direct effects on quality of stored grain. In maize storage ecosystem the most important factors that influence molds and insects infestations are water activity, air and temperature (Montross *et al*., 1999). The storage conditions can influence the presence or absence of spoilage organisms where they multiply and cause undesired effects.

Fungal isolates were identified by their cultural and morphological characterises as presented in Table 4. The isolates include *Rhizopus* spp, *Aspergillus niger*, *A. flavus*, *Fusarium* spp and *Curvularia* spp. In all analyzed samples, the most prevalent genera was *Aspergillus* spp, its dominance could have been that the maize was not properly dried. It was shown that predominating fungi genera in analyzed samples were *Aspergillus niger* (31.5%), followed by *Aspergillusflavus* (31%), *Rhizopus* spp (15.25%), *Fusarium* spp (14.5%) and the least is *curvularia* spp according to Table 2.

However, the rate and degree of spoilage has been shown to be higher under moist or high humidity conditions. All the fungal organisms identified, characterized and isolated in this study are capable of causing death to man and animals resulting from mycotoxins they are capable of producing. Also, the result of this study show that the conditions to which maize grains are exposed in the field or store as well as storage method used to preserve it have effects on the type, rate and extent of infection of the maize grains by fungi.

Determination of nutritional (proximate) and phytochemical (metabolites) compositions of maize will go a long way in providing substantive nutritional information on maize. Spoilt maize grain nutritional analysis was conducted after the forth month of purchase. The major component of maize is carbohydrate which provides up to 68-72% of the maize grain weight for the good ones and between 55-63% for spoilt maize grains. Maize grains contain simple sugars present as glucose, sucrose and fructose in amounts that vary from 1-3% of the grains. The findings of Ujabadenyi & Adebolu (2005) reported similar value 65.63-70.25% carbohydrate content of maize grown and stored in Nigeria. The composition of maize starch maybe genetically controlled. The moisture content of the maize grains in Location 3 with 8.55% , Samir *et al*. (1998) reported that the moisture content of maize grains is between the range of 5-9 %. This report supports the findings of this work as seen in Table 5. The slight variation among the Locations could be attributed to the environmental factors such as the type of soil used during cultivation, storage facilities and conduction or other agronomic practices used. The low moisture content is important as it enables long storage duration by minimizing fungal contamination and spoilage of the maize grains stored. In this context, Location 2 has the least moisture content of 5.45% to compare to others. Duxton *et al*. (2000) reported ash content of maize grains in the range of 1.3-3.3%, the percentage of ash obtained from this work falls within the proposed range as seen in Table 5 where location 2 has the highest content with 1.52%, followed by Location4 with 1.34 %, Location 1 with 1.35 and the least is Location 3 with 1.25%.

After carbohydrate the next largest chemical component of the grain is protein. The percentage of protein of maize in this work was consistent to those reported on different maize varieties in Nigeria with a range of 10.0-11.25%. Location 4 has the highest content of 11.16% while Location 3 has the least protein content of 10.28%. This variations maybe because of the agronomic practices (soil type, duration before harvesting, the type of fertilizer or manure used) and also the climatic factors from the different Locations, the type of maize seedlings (hybrid) and also the proper exposure of the farmer to the modern way agriculture. The oil content of maize grains comes mainly from the germ with varieties ranging from 3-5%. Maize oil has a low level of saturated fatty acid, on the other hand it contains relatively high level of polysaturated fatty acid mainly linoleic acid. Maize oil is highly regarded because of its fatty acid distribution mainly oleic and linoleic acid (Matilda *et al*., 1993). Ikenie *et al*. 2002 also reported that the range of stored maize grains in Nigeria for lipids is between 3.0-4.7%, these findings aligned with the results obtained in Table 5 of this work where Location 1 has 4.65%as the highest among the four samples and Location 4 were the least crude lipid content with 3.5%. The Crude fibre was found to be the third largest composition after carbohydrate and protein. The fibre content was found highest in Location 1 with 9.05% followed by Location 3 with 6.82%, then Location 4 with 6.5% and Location 2 has the least fibre content of 5.42%. All the variations in the nutritional contents of these four samples may have been linked to storage facilities and factors or other agronomic practices.

Nutritional (proximate) composition for spoilt maize grains in this work which was done after four months of purchase cannot be over emphasised, there were a huge reduction of the basic nutrient starting from carbohydrate, protein and others. Protein is about 4% in the whole sample which I believed was due to actions of some microorganisms (insects and weevils) that feed on them and used them for their growth, the spoilage of this maize grains could also be attributed to the climatic conditions and storage system. There was noticeable increase in the moisture contents of the grains and it could be of the reasons of major causes of the spoilage because low moisture content enables the long storage duration by reducing insects and fungal contaminations which causes spoilage of stored maize grains. It were observed that Location 3 has the highest moisture content of 16.16%, followed by Location 4 which is 15.48%, Location 2 which is 13.99% and Location 1 has the lowest of 13.47%. The percentage for total lipids falls slightly the report of Ikenie *et al*. (2002) in the range of 3.0-4.7% but with difference range of 2.5-3.5% which is as a result of spoilage of the maize grains as observed in Table 6 above. Location 4 has the least lipid content of 2.5% while Location 2 has the highest lipid content of 3.5%. The fibre content of the spoilt maize grains in the four different Locations where almost little to none , as Location 3 has the highest fibre content of 2.08% followed by Location 4 which is 1.8%, Location 1 by 0.85% and the least is Location 2 by 0.6%. Spoilt maize grains are more of carbohydrate (starch) and little other nutrients when compared to the good maize grains. There were huge difference in the spoilt maize grains from the findings of Wilson *et al*. (1999) who gave a report in his findings to be in the range of 72-73%. Table 6 above shows great difference to his findings which was due to spoilage of the maize grains caused by fungi, insects and weevils growth on the grains. It was observed that Location 3 has the least carbohydrate content of 55% while Location 2 has the highest carbohydrate content of 63%. From my work I think its advised to stay out of spoilt maize or long stored maize grains for human

consumption as it's only starch contents will be available while other nutrients components maybe little or absent.

The phytochemical (metabolites) of maize grains in oxalate showed higher percentage followed by phytate in all samples. However, it was observed that the alkaloid content of Location 3 was higher with 0.30% and same level in Location 1 andLocation 4with 0.18%. The result for tannin was more predominant in Location 1 and Location 3 with 0.047% respectively and slight difference in Location 4 with 0.044%. There was higher percentage of phenol in Location 1 with 0.167% and slightly difference in Location 4 with 0.161%. Flavonoid composition in Location 2 was higher with 0.210% and slightly different in Location 1 with 0.204%. The cardiac glycoside composition was more prevalent in Location 2 with 3.51% than other Locations while Location 4 is the least with 3.13%in these contexts. The data indicated that maize is high in carbohydrate and protein. Chapter 4 shows the Tabled results and statistical analysis among the four cardinal Locations used in this project.

CONCLUSION

Having looked at the problems of food losses in maize production in its entire ramification, it is therefore obvious that food losses in maize production are as products of many variables, some of which are inter-woven. This could be seen as maize has the gradient of infestation from the field (pre-harvest) to the store (post-harvest). Therefore, losses due to it are difficult to assess precisely because of the stages involved. The outcome of this thesis showed that heaping of maize in one place or loading them in a sack for a long time causes mould to form. Also poor storage structures (bulk storage facilities and warehouses), improper handling during harvesting resulting in mechanical injuries cause losses in maize production with improper drying before storage causes loss in maize through deterioration. The methods of transporting maize from the farms to the store and the reabsorption of moisture from humid air during storage through the use of inappropriately designed and crack storage materials causes deterioration of the stored maize thereby reducing the quality and the market value of the grains. A good agricultural management practices through the combination of both preventive and control measures such as the appropriate techniques of harvesting of grains, transportation, treatment of grains, provision of good storage structure and favourable environment devoid of pests infestation and microbial activities will minimize the losses in maize production.

RECOMMENDATIONS

Based on the findings, the following recommendations are made:

Periodical training workshop on storage of produce should be organized for maize farmers. There is need for international information network.

A key factor influencing the magnitude of post-harvest losses is severity of mechanical damage to the crop during harvest and subsequent handling because it provides pathways for invasion by fungi and bacteria.

The users of post-harvest chemicals must ensure that the dosages and residues conform to internationally accepted maximum levels; for example, the FAO/WHO codex alimentarius commission.

The Nigerian Stored Products Research Institute (NSPRI) should be given sufficient funds to execute its research programmes on effective crop storage in Nigeria. This will help in reducing pre and post-harvest food losses.

Proper monitoring of temperature and relative humidity of maize grain and surrounding atmosphere on storage especially in the initial stage of storage to maintain the highest possible quality of stored grain; in general, the lower the temperature and moisture content the longer it can be stored without being infected by mold and insects.

To avoid deterioration of maize in tropical and subtropical regions, maize should be dried to moisture contents below 14% immediately after harvest.

Hygiene and sanitation from harvest to storage are key factors in eliminating sources of infection and reducing levels of contamination.

Sorting or separating foreign materials and broken corn kernels produced during harvesting from clean maize; those promote development of grains pest and molds.

Maize should be stored in a sealed, airtight container or structure, to reduce oxygen concentration, which will limit the presence of aerobic organisms.

Clean, fumigate, or separate maize grain immediately after discovery of insects and molds.

Remove or separate old grain from new grain, and maize should be placed on pallets above the floor to avoid cold conditions that may lead to mold contamination.

According to the Irish ambassador to Nigeria, Kyle O Sulliva, on the 8th October, 2008 at the International Institute for Tropical Agriculture (IITA), Ibadan, said that the funding of research in food production and security was one way to tackle the global problem of rising food prices.

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