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Effect of storage on chemical composition and mycoflora of okra (*Abelmoschus esculentus*)

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Abstract

The mycoflora of sundried okra (*Abelmoschus esculentus*) during twenty weeks of storage and the effects of their presence on the nutrient composition were investigated. Six fungi were isolated namely: *Rhizopus* sp., *Mucor* sp., *Aspergillus niger, Aspergillus flavus* and *Neurospora crassa*. The fungi were found to increase as the storage time increases though constancy exists. The results of proximate composition in g/100g showed that ash decreased from 8.06 to 7.56, crude protein 14.54 – 12.68, fat content 9.75 - 6.94, Fibre 36.66-35.75 while the following parameters were found to increase viz moisture 9.83-14.53 and carbohydrate 21.17-22.99. The results of mineral analysis in mg/100g revealed that all minerals decreased with storage period viz sodium (Na) 55.30 - 54.68, calcium (Ca) (81.65-80.75), iron (Fe) 0.68 – 0-50, magnesium (Mg) 75.60 – 72.43, zinc (Zn) 38.10 – 31.82, manganese (Mn) 1.75 – 0.75 and phosphorus (P) 63.5 – 61.49 but potassium (K) increased from 60.50 – 60.84. Copper (Cu) and Lead (Pb) were not detected in the samples. The nutritional composition of okra was depleted during storage. Therefore, storage should be done under controlled environment that will not favour the growth of spoilage microorganisms.

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Introduction

Okra (*Abelmoschus esculentus*) is a flowering plant in the mallow family. It is an annual or perennial plant with origin from Africa and it is cultivated in the tropical, subtropical and warm temperate regions (NRC, 2006). It is very common in West Africa most especially Nigeria. Its fruits are commonly used for making soup for consumption. The seeds are enclosed in capsules which can split open when properly matured and dried. The leaves are about 10-20cm long and broad, palmate lobed with 5-7 lobes and their flowers are about 4-8cm in diameter with five white to yellow petals, often with red or purple spot at the base of each petal (Sheila *et al*., 1996). Okra is among the most heat and drought tolerant vegetable species in the world but severe frost can damage the pods. The plant can tolerate poor soil with heavy clay and intermittent moisture (Franklin, 1982).

It is affected by diseases such as mosaic, damping off and powdery mildew which are caused by virus, *Phytum sphytophlitora* (fungus) and *Erysipha* sp respectively. They have been reported to be either soil or air borne pathogens (Allan, 1976; Pareek *et al*., 1987). It possesses great medicinal and edible uses. For instance, its leaves can be cooked and used as a powerful soup thickener while the immature pods may also be pickled (BBC food, 2011). Greenish yellow edible okra oil extracted from the seed is rich in unsaturated fats such as oleic acid and linoleic acid with the content of about 40%. Its fibre has been reported to stabilize blood sugar, curbs rate of absorption of sugar and prevent constipation (Junji, 1968; NRC, 2006).

Due to increasing world population, there arises a need to increase food production and subsequent storage. A major problem of agricultural production is the loss of farm products during and post harvest. Microorganisms, insects and rodents contribute greatly to these post harvest losses. Fungi are reported to be a major cause of spoilage in stored products in technologically advanced countries because insects and rodents are effectively controlled (Christensen and Kaufmann, 1974). Storage fungi are the fungi that invade grains or seeds during storage. They are usually not present to any serious extent before harvest. Small quantities of their spores may be present on grains going into storage or may be present on spilled grain present in harvesting, handling and storage equipments or structures. Under improper storage conditions, these small amounts of inocula could proliferate leading to a significant problem (Bankole and Joda, 2003).

The mycoflora of fresh okra has been reported to include *Botryodiplodia theobromae*, *Rhizopus* spp., *Trichoderma harziamae*, *Mucor mucedo* and *Fusarium oxysporum* and the ones associated with dried okra were *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Rhizopus* spp., *Mucor mucedo* and *Trichoderma harziamae* (Adebanjo and Shopeju, 2002). There has been no report on mycoflora and nutrient changes of dried okra during storage.

Therefore, this study is aimed at investigating the effect of storage on chemical composition and mycoflora of Okra (*Abelmoschus esculentus*).

Materials and methods

Collection of samples

The fruits of *Abelmoshus esculentus* were collected from Iworoko market in Ekiti State, Nigeria. The fruits were sliced into smaller pieces and then sundried for eight days. The sundried fruits were stored for six month in an insect free container, labeled and kept in the laboratory.

Isolation of fungi from the stored sun dried okra (*Abelmoshus esculentus*)

Direct plating: From the sundried okra pieces, 20 pieces were examined randomly for external mouldness. They were surface sterilized with ethanol and later washed with sterile distilled water. Using a sterile dissecting forceps, the surface of the stored dried groundnut were scrapped and was plated aseptically on potato dextrose agar (PDA) plate and incubated at 28°C for 5 to 7 days as

described by Arotupin and Akinyosoye (2001). The fungi cultures were subcultured until pure colonies were obtained by successive hypha tip transfer (Fagbohun *et al.*, 2011). The cultures were examined under the microscope for fruiting bodies, hyphae to determine the common fungi present.

Table 1. The fungi isolated from stored sundried okra using direct plating method.

Weeks of storage	Fungal isolates
Fresh Okra	A, B, F
4 weeks	A, B, E, F
8 weeks	A, B, E, F
12 weeks	A, B, D, E, F
16 weeks	A, B, D, E, F
20 weeks	A, B, C, D, E, F

A – *Rhizopus* sp.; B – *Mucor* sp.; C – *Aspergillus niger;* D – *Aspergillus flavus*; E – *Absidia* sp.; F – *Neurospora* crassa.

Table 2. The fungi isolated from stored sundried okra using dilution plate method.

Weeks of storage	Fungal isolates
Fresh Okra	A, B, F
4 weeks	A, B, E, F
8 weeks	A, B, E, F
12 weeks	A, B, D, E, F
16 weeks	A, B, D, E, F
20 weeks	A, B, C, D, E, F

A – *Rhizopus* sp.; B – *Mucor* sp.; C – *Aspergillus niger;* D – *Aspergillus flavus*; E – *Absidia* sp.; F – *Neurospora* crassa.

Dilution plate method: This method was used to determine the type of fungi present in the stored sun dried okra pieces. About one gram of the sample was sterilized with ethanol and grinded with 10 ml of sterile distilled water. This was shaken thoroughly and 1 ml of suspension was pipetted into a sterile test tube containing 9 ml of distilled water. This was thoroughly mixed together. The sample was serially diluted and 1 ml each of aliquots of 10-5 and 10-6 were added to molten PDA plates. The plates were swirled gently to obtain thorough mixing and were allowed to solidify and incubated at room temperature for 5 to 7 days. The fungal colonies were counted every 24 h. Successive hyphae tip were transferred until pure cultures of each of fungus was obtained.

Table 3. The fungi isolated from stored sundried okra using Washing method.

Weeks of storage	Fungal isolates
Fresh Okra	A, B, F
4 weeks	A, B, E, F
8 weeks	A, B, E, F
12 weeks	A, B, C, E, F
16 weeks	A, B, C, D, E, F
20 weeks	A, B, C, D, E, F

A – *Rhizopus* sp.; B – *Mucor* sp.; C – *Aspergillus niger;* D – *Aspergillus flavus*; E – *Absidia* sp.; F – *Neurospora* crassa.

Table 4. The summary of the fungi isolated from stored sundried okra using various methods.

Weeks of storage	Fungal isolates
Fresh Okra	A, B, F
4 weeks	A, B, E, F
8 weeks	A, B, C, E, F
12 weeks	A, B, C, D, E, F
16 weeks	A, B, C, D, E, F
20 weeks	A, B, C, D, E, F

A – *Rhizopus* sp.; B – *Mucor* sp.; C – *Aspergillus niger;* D – *Aspergillus flavus*; E – *Absidia* sp.; F – *Neurospora* crassa.

Washing method: This was carried out by weighing one gram of the dried okra pieces into 10ml of sterile distilled water in a beaker. This was shaken thoroughly and drops of suspension of contaminated water were introduced into Petri dishes containing potato dextrose agar. This was

evenly spread on the agar plate with aid of a sterile glass spreader. The plates were incubated at 28°C for 5 to 7 days and were observe for visible fungal growth (Ahmad *et al*., 2006).

Identification of mycoflora: The fungi were identified by their cultural and morphological features (Alexopoulous *et al*., 1996). The isolates were examined under bright daylight for the colour of the culture and further examinations were carried out.

Needle mount preparation method: The method of Fagbohun *et al*. (2011) was used whereby fragments of the sporing surface of the initial culture was taken midway or between the centre and the edge of the colony. This was teased out in drop of alcohol on a sterilized glass slide using a botany needle. The fragments were stained by adding a drop of lactophenol blue. A cover slip was applied and the preparation was examined under X10 and X40 objective lens of the microscope.

Slide culture technique: From a plate approximately 2 mm deep, 1 cm^2 PDA was cut and placed on a sterile glass slide. Fungus was inoculated into the four vertical sides using a sterile needle. A sterile cover slip was placed on it so that it over lapped the medium on all sides. The preparation was placed on a suitable support in a Petri dish containing blotting paper soaked in 20% glycerol in water. The preparation was kept moist at 28°C until adequate growth was observed. After removing the medium with scalpel, the fungus adhering to both cover slip and slide was examined (Crowley *et al*., 1969). A drop of alcohol was added followed by a drop of lactophenol blue and the preparation was covered and examined under the low power objective of microscope.

Proximate analysis: The proximate analysis of the samples for moisture, ash, fibre and fat were done by the method of AOAC (2005). The nitrogen was determined by micro-Kjeldahl method as described by Pearson (1976) and the percentage nitrogen was converted to crude protein by multiplying with 6.25. All determinations were performed in triplicates.

Mineral analysis: The mineral was analyzed by dry ashing the samples at 550°C to constant weight and dissolving the ash in volumetric flask using distilled water, deionized water with a few drop of concentrated HCl. Sodium and potassium were determined by using a flame photometer (Model 405 Corning, UK) with NaCl and KCl standards. Phosphorus was determined colometrically using Spectronic 20 (Gallenkap, UK) as described by Pearson (1976) with KH_2PO_4 as standard. All other metals were determined by atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk CT, USA). The detection limits had previously been determined using the methods of Varian Techtron (1975) as Mn 0.01, Cu 0.005, Co 0.05, Zn 0.005, Fe 0.02, Mg 0.002, Ca 0.004, Na 0.001ppm (all for aqueous solution). The optimum analytical range was 0.5 to 10 absorbance units with coefficient of variation of 0.05-0.04% phosphovanado-molybdate method using a Spectronic 20 colorimeter (Galenkamp, London,UK) (AOAC, 2005). All the proximate values were reported in g/100g while the minerals were reported as mg/100 g. All determinations were done in triplicates. All chemicals used were analytical grade (BDH, London).

Statistical analysis

Statistical analysis (Oloyo, 2001) was carried out to determine mean and standard deviation.

Results and discussion

The results of fungal isolation using direct plate, dilution plate and washing method are shown on Tables 1, 2 and 3 respectively while the summary of the isolated fungi within the period of storage are shown on Table 4. Six fungi were isolated namely *Rhizopus* sp., *Mucor* sp., *Aspergillus niger*, *Aspergillus flavus*, *Absidia* sp and *Neurospora crassa*. This result is in agreement with findings of Abdel-Sater and Erasky (2001) who reported the isolation of *Aspergillus* spp., *Penicillium* spp.,

Fusarium sp. and *Rhizopus* spp from stored onion bulbs. Similarly, Youssef and Palmateer, (2008) also reported the isolation o f *A. niger*, *A. flavus*, *A. fumigatus*, *Fusarium* sp., *Rhizopus oryzae* and *Penicillium* sp. from sundried jew's mallow leaves and Okra fruit. Some of the fungi associated with stored products have been reported to release chemicals that are hazardous to man and animals (Richard and Wallace, 2001). Consumption of excessive amount of these chemicals can cause illness or fatality. Many of these toxic chemicals have been reported by Youssef and Palmateer (2008) in Okra fruits to include aflatoxin B1, B2, G1 and G2, zearalenone and diacetoxyscirpenol. These pathogens possess the ability to produce extracellular hydrolytic enzymes that are capable of breaking down these stored products (Amadioha, 1998). They are also associated with diseases such as keratitis, endocarditis, endophtalmitis, otomycosis, infarction, neuroperia and hepatocellular carcinoma (Lueg *et al.*, 1996; Mitchell *et al.*, 1996; Crawford and Kumor, 2005).

The results of proximate analysis of sundried okra fruit in g/100g during twenty weeks of storage are shown in Table 5. The results showed that fresh okra has ash content of 8.06, crude protein 14.54, fat 9.75, fibre content 36.66 which were depleted within the period of storage to 7.56, 12.68, 6.49 and 35.75 respectively. This is in agreement with the findings of Fagbohun and Lawal (2011) who reported a decrease in the fibre and fat content of sundried soybean (*Glycine max*) from 5.72 -5.35 and 19.5 -18.37 respectively during the period of storage. Similarly, Amadioha (1998) reported the quantities of protein and fats to decrease appreciably during storage and infection of potato tubers. The reduction suggested that the fungi isolated utilized these nutrients for their successful establishment, cellular growth, reproduction and survival within the tissues of groundnut (Amadioha, 1998).

Also, an increase was observed in the moisture (9.83-14.53) and carbohydrate (21.17-22.99) content. This result is similar to the findings of Fagbohun *et al.*, (2011) who reported a significant increase in moisture and carbohydrate content of sundried melon seeds during storage. The increase in the moisture content with storage time might be due to the metabolic water (Ladele *et al.*, 1984).

Table 5. A summary of results of proximate analysis of sundried okra during storage (g/100g). All determinations were done in triplicates.

Weeks of storage	Ash	MC	CP	Fat	Fibre	CHO
Fresh Okra	8.06	9.83	14.54	9.75	36.66	21.17
4	8.12	10.21	14.69	9.69	36.63	20.67
8	8.24	11.36	14.97	8.64	36.51	19.99
12	7.81	12.12	15.16	7.42	36.51	19.99
16	7.66	14.10	13.71	6.79	35.83	21.97
20	7.56	14.53	12.68	6.49	35.75	22.99
Mean	7.91	12.03	14.29	8.13	36.32	21.13
S.D	0.27	1.96	0.94	1.34	0.41	1.18

MC – Moisture Content; CP – Crude Protein
CHO – Carbohydrate; S.D – Standard Deviation

The summary of the mineral analysis (mg/100g) of sundried okra is shown in Table 6. There was a decrease in value of all the minerals with storage time viz: Na (55.30-54.68), Ca (1.75-0.75), Mg (75.60-72.43), Zn (38.10-31.82), P (63.50-61.49), Fe (0.68-0.50), Mn (1.75-0.75) except Potassium that slightly increased from 60.50 to 60.84. This result is similar to the findings of Fagbohun and Lawal (2011) who reported a decrease in Na, Ca, , Fe, Mn, Cd and an increase in K, Mg and P in sundried soybean (*Glycine max*) during storage. The amount of mineral elements present in the fresh okra is important in different ways. For instance, both Ca and Mg in the sample are chiefly found in the skeleton. In addition to its structural role, Mg also activates enzymatic processes. Na and K control water equilibrium levels in both tissues and are also involved in the transport of some non-electrolytes (Welch and Graham, 2004).

Table 6. A summary of results of mineral analysis of stored sundried okra during storage (mg/100g). All determinations were done in triplicates.

Weeks of Storage	Na	K	Ca	Mg	Zn	Fe	Mn	Cu	P	Pb
Fresh Okra	55.30	60.50	81.65	75.60	38.10	0.68	1.78	ND	63.50	ND
4	55.51	60.80	82.20	75.66	39.00	0.75	1.78	ND	63.80	ND
8	56.06	65.33	86.87	75.37	38.38	0.74	0.89	ND	65.47	ND
12	56.02	62.48	84.38	75.35	36.84	0.66	0.85	ND	65.38	ND
16	54.97	62.31	81.42	74.76	35.84	0.53	0.83	ND	61.76	ND
20	54.68	60.84	80.75	72.43	31.82	0.50	0.75	ND	61.49	ND
Mean	55.42	62.04	82.88	74.86	36.66	0.64	1.15	-	63.57	-
S.D	0.56	1.81	2.32	1.23	2.63	0.11	0.49	-	1.71	-

ND – Not Detected; S.D – Standard Deviation

Conclusion

This study revealed the rate of fungal invasion and deterioration of the nutrient composition in the sun dried okra fruits. To maintain the economic use of stored products, storage should be done under controlled environment that will not favour the growth of spoilage microorganisms.

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