

## Full Length Research Paper

# The chemical composition and mycoflora of sundried shelled melon seeds (*Citrullus vulgaris*) during storage.

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The chemical composition and the mycoflora of shelled melon seeds were investigated during twenty weeks storage. Seven fungi were isolated namely *Fusarium sp.*, *Rhizopus sp.*, *Mucor sp.*, *Aspergillus niger*, *Aspergillus tamari* and two species of *Penicillium*. The fungal count was found to increase as the storage time increased. The proximate and the mineral composition were found to decrease as the storage time increased. The fat content was found to decrease from 45.95% in the freshly shelled melon seeds to 39.49% in the stored seeds. The ash content decreased from 4.05% to 3.46% in the stored melon seeds while the fibre content decreased from 7.18% to 5.75% in the stored seeds. The mineral composition in mg/100g also decreased during the storage. The sodium content decreased from 2.71 to 2.47, calcium from 0.59 to 0.24, magnesium from 5.91 to 5.75, zinc from 0.75 to 0.63, iron from 1.11 to 0.63, copper from 0.26 to 0.19 and manganese was not detected.

**Keywords:** Mycoflora, Shelled melon, Proximate, Minerals, Chemical composition

## INTRODUCTION

Melon is a drought leguminous plant that grows best in warm temperate and tropical areas of the world (Huxley, 1992). It belongs to the family *Cucurbitaceae* and the genus *Citrullus* (Pearson, 2002). It has a life span of about 120 days of warm or hot weather from planting to harvesting (Wolford *et al.*, 2005). The seeds of melon are harvested, cleaned, sun dried and stored in a cool dry condition (Wolford *et al.*, 2005). Melon has numerous importance. It can be roasted or ground into powder for soap making and for soup condiment (Rossengarten, 1984; Facciola, 1990). They can also be used for the production of tempeh (Amadi *et al.*, 2003). Moreover, they are used as a domestic remedy for urinary tract infection, hepatic congestion, intestinal catarrh, worm remedy, abnormal blood pressure (Moerman, 1998).

Melon is rich in minerals, protein, vitamins, carbohydrate and fibre (Duke and Ayensu, 1985). However, storage conditions have effect on the proximate and chemical composition of the stored melon because of the growth of some spoilage fungi that thrive in such

conditions (Abaka and Norman, 2000). The fungi that invade stored product are generally grouped into two categories namely field fungi which attack developing and matured seeds in the field and storage fungi which are predominantly species of *Aspergillus* and *Penicillium* which attack the stored products (Fagbohun *et al.*, 2010).

The conditions of the stored product determine the extent of invasion of the stored product. The environmental factors that aid the development of fungi in stored products include moisture content (Amusa *et al.*, 2002), temperature (Abaka and Norman, 2000), aeration (Burrell, 1974), pH (Aderiye, 2004), relative humidity (Kuku, 1979). However, the effects of these storage fungi on stored products include deterioration and spoilage of stored products (Abaka and Norman, 2000; Ekundayo and Idzi, 2005), reduction of market value (Muller, 1991) and production of chemical substances that are toxic to human health (Richard and Wallace, 2001). The preventive measures that can be employed for the growth of the storage fungi are biological control (Aderiye, 2004), chemical control (Rice, 2002) and physical control (FAO, 2002).

However, the aims and objectives of this study was to study the effect of storage on the chemical composition and the mycoflora of sundried melon seeds.

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## MATERIALS AND METHODS

**Collection of Samples:** The seeds of *Citrullus vulgaris* were collected from Bodija market in Ibadan, Oyo State, Nigeria. The seeds were shelled and sun dried for one week. The samples were stored for six months in an insect free container, labeled and kept in the laboratory. The samples were examined for the changes in the mycoflora and nutrients composition after each month of storage.

### Isolation of fungi from the stored sun dried melon

**Direct Plating:** From the sun dried melon seeds, 10 seeds 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 melon seeds were scrapped and was plated aseptically on Potato Dextrose Agar (PDA) plate and incubated at room temperature for 5 - 7 days as described by Amusa (2001) and Arotupin and Akinyosoye (2001). The fungi cultures were subcultured until pure colonies were obtained by successive hypha tip transfer (Egbebi *et al.*, 2011). The cultures were examined under the microscope for fruiting bodies, hyphae to determine the common fungi present.

**Dilution Plate Method:** This method was used to determine the type of fungi present in the stored sun dried melon seeds. About one gram of the sample was sterilized with ethanol and grinded with 10ml of sterile distilled water. This was shaken thoroughly and 1ml of suspension was pipetted into a sterile test tube containing 9ml of distilled water. This was thoroughly mixed together. The sample was serially diluted and 1ml 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 - 7 days. The fungal colonies were counted every 24 h. Successive hyphae tip were transferred until pure cultures of each of fungus was obtained.

**Washing Method:** This was carried out by weighing 1 g of the sample into 10 ml 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 room temperature for 5 - 7 days and were observed for visible fungi growth.

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

**Needle mount preparation method:** The method was

carried out according to Tuite (1961), Crowley (1969) and Egbebi *et al.*, (2011) 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<sup>2</sup> PDA was cut and placed on a sterile glass slide. Fungus was innoculated into the four vertical sides using a sterile needle. A sterile coverslip 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 coverslip 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 (2002) the percentage Nitrogen was converted to crude protein by multiplying 6.25. Carbohydrate was determined by difference. 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), using NaCl and KCl to prepare the standards. Phosphorus was determined colometrically using Spectronic 20 (Gallenkap, UK) as described by Pearson (2002) with KH<sub>2</sub>PO<sub>4</sub> as standard. All other metals were determined by atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk CT, USA). All determinations were done in triplicates. All chemicals used were analytical grade (BDH, London). Earlier, the detection limit of the metals has been determined according to Tuite (1961). The optimum analytical range was 0.1 - 0.5 absorbance unit with a coefficient of variation of 0.87 - 2.20%. All the proximate values were reported as percentage while the minerals were reported as milligram/100g.

## RESULTS AND DISCUSSIONS

A total of seven fungi were isolated from stored sundried melon seeds and were identified based on their cultural and morphological characteristics. The fungi include

**Table 1:** the fungi isolated from stored shelled melon using direct plating method

Weeks Of Storage	Fungal Isolated
Freshly Shelled	B, F, G
4 Weeks	B, D, E, F, G
8 Weeks	B, C, D, E, F, G
12 Weeks	B, C, D, E, F, G
16 Weeks	A, B, C, D, E, F, G
20 Weeks	A, B, C, D, E, F, G

**Table 2:** the fungi isolated from stored shelled melon using washing method

Weeks of storage	Fungal isolated
Freshly Shelled	B, F, G
4 Weeks	B, D, E, F, G
8 Weeks	B, C, D, E, F, G
12 Weeks	B, C, D, E, F, G
16 Weeks	A, B, C, D, E, F, G
20 Weeks	A, B, C, D, E, F, G

**Table 3:** the fungi isolated from stored shelled melon using dilution plating method

Weeks of storage	Fungal isolated
Freshly Shelled	B, F, G
4 Weeks	B, D, E, F, G
8 Weeks	B, C, D, E, F, G
12 Weeks	B, C, D, E, F, G
16 Weeks	A, B, C, D, E, F, G
20 Weeks	A, B, C, D, E, F, G

*Fusarium* sp. *Rhizopus* sp. *Penicillium* sp., *Mucor* sp., *Aspergillus niger*, *Aspergillus tamari* and *Penicillium* sp. The fungi isolated from sun dried melon seed using different methods are shown on Tables 1 to 3 and the summary of the fungi isolated from stored sundried melon seed using various methods are shown on Table 4. In addition, results of the proximate and mineral analysis are shown on Tables 5 and 6 respectively.

The results showed that *Fusarium* sp. *Rhizopus* sp. two species *Penicillium* sp., *Mucor* sp., *Aspergillus niger*, *Aspergillus tamari* were found to be associated with the stored sun dried melon seeds most of which are known to be surface contaminants of most agricultural products. They cause decay of agricultural produce thereby reducing their market and nutritional value (Amusa *et al.*, 2002). The fungi isolated using washing method are those capable of growing in the seed. The fungi isolated by any of the three methods used could therefore be field or storage fungi.

In this study, there was an increase in the number of

fungi isolated as the study progressed. Four fungi *Rhizopus* sp., *Aspergillus niger*, *Aspergillus tamari* and *Penicillium* sp. were isolated at the first week of the study while others joined the number as the study progressed. This result is in agreement with the findings of Broadbent *et al.*, (1969) who reported the isolation of a total number of eighty eight fungi from stored palm kernel. Similarly, this result also agreed with the findings of Amadi and Adebola (2008) who reported the isolation of six mould species (*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigates*, *Penicillium* sp. and *Rhizopus* sp.) from garri for eight weeks. Fagbohun *et al.*, (2010) also reported the nutritional and mycoflora changes during storage of plantain chips that was stored for sixteen weeks. However, this result is in contrast to the findings of Ogundana *et al.*, (1990) who reported a decrease in fungi quantity in stored products.

The fungi isolated in this study could be from the air, soil, storage house and or improper handling of the products. The penicillia are as common and as

**Table 4:** a summary of the fungi isolated from stored shelled melon using various methods of isolation

Weeks of storage	Fungal isolated
Freshly Shelled	B, E, F, G
4 Weeks	B, D, E, F, G
8 Weeks	B, C, D, E, F, G
12 Weeks	B, C, D, E, F, G
16 Weeks	A, B, C, D, E, F, G
20 Weeks	A, B, C, D, E, F, G

Legend

A = *Fusarium* sp.  
 B = *Rhizopus* sp.  
 C = *Penicillium* sp.  
 D = *Mucor* sp.  
 E = *Aspergillus niger*  
 F = *Aspergillus tamarii*  
 G = *Penicillium* sp.

**Table 5:** the summary of the results of the proximate analysis of shelled melon seeds (*Citrullus vulgaris*) during storage

Weeks of storage	%ASH	%MC	%CP	%FAT	%FIBRE	%CHO
Freshly shelled	4.05	8.03	34.24	45.95	7.18	0.56
4 Weeks	6.21	8.29	34.66	42.02	7.78	1.06
8 Weeks	5.69	9.45	34.86	42.34	6.34	1.35
12 Weeks	3.54	10.23	34.86	38.84	5.84	6.71
16 Weeks	3.49	10.93	37.63	37.51	5.83	4.56
20 Weeks	3.46	11.01	38.71	39.49	5.75	1.56

Legend

MC = Moisture Content  
 CP = Crude Protein  
 CHO = Carbohydrate

**Table 6:** the summary of the results of the mineral analysis of melon seeds (*Citrullus vulgaris*) in mg/100g during storage

Weeks of storage	Na	K	Ca	Mg	Zn	Fe	Cu	Mn
Freshly shelled	2.71	2.15	0.59	5.91	0.75	1.11	0.26	-
4 Weeks	2.92	2.48	0.60	6.33	0.83	1.23	0.26	-
8 Weeks	2.77	2.31	0.47	6.01	0.78	1.18	0.25	-
12 Weeks	2.64	2.24	0.43	5.90	0.74	1.15	0.22	-
16 Weeks	2.57	2.15	0.29	5.74	0.71	1.11	0.20	-
20 Weeks	2.47	2.25	0.24	5.75	0.53	1.10	0.19	-

cosmopolitan as the aspergilla. They are so called green molds and blue molds which so frequently find on *Citrus* and other fruits, on jellies and preserves and on other foodstuffs that have become contaminated with their spores (FAO, 2002).

Some of the fungi associated with stored products are capable of producing toxin metabolites or chemicals that are detrimental to the health of consumers. However, consumption of exercise amount of these chemicals in the stored products can cause illness or death (Anon,

1993; Mirocha *et al.*, 2003). Based on the concern of the hazard to livestock and man, concerted effort is now being directed at finding every cheap and reliable methods of minimizing aflatoxin formation in stored products (Bankole and Adebajo, 2003).

The result of the proximate analysis are shown on Table 5. This result revealed that the freshly shelled melon seed had ash content of 4.05%, moisture content (mc) of 8.03%, crude protein (CP) of 34.24%, fat of 45.95%, fibre content (FC) of 7.18% and carbohydrate

(CHO) OF 0.56%. However, after six months of storage the % ash, fat and fibre decreased to 3.46%, 39.49 and 5.75% respectively. This agreed with the findings of Fagbohun *et al.*, (2010) who reported the decrease in the percentage fat and fibre content of sun dried plantain chips stored for sixteen weeks.

Meanwhile, the %CP and CHO increased to 38.71 and 1.56% respectively, this is in contrast to the findings of Fagbohun *et al.*, (2010) who reported a decrease in the percentage of CP and CHO of sun dried plantain chips stored for sixteen weeks. The moisture content also increased to 11.01%, this may be due to the degrading activity of the fungi as reported by Egbebi *et al.*, (2011).

The proximate analysis of stored shelled melon seeds revealed that there was a decrease in the nutritive value of the stored melon seeds compared to the freshly shelled melon seeds. This is due to fungal activity that caused changes during storage of the product. Nutrients are lost because of changes in carbohydrate, protein, lipids and vitamins (Abaka and Norman, 2000).

The mineral analysis of the melon seeds during storage in mg/100m are shown on Table 6. The result revealed the following minerals Na (2.71), K (2.15), Ca (0.59), Mg (5.91), Zn (0.75), Fe (1.11) and Cu (0.26) in the freshly shelled melon seeds. This result showed that the mineral composition of the stored melon seeds decreased during storage. This is in agreement with the findings of Ekundayo and Idzi (2005) who reported the decrease in the minerals content melon seeds after two weeks of storage.

## CONCLUSION

Melon seeds are of great economic importance and in order to maintain the quality, they should be stored under controlled environment that would not be favourable for the growth of fungal flora thereby preventing deterioration of the stored melon seeds and reduction in the chemical composition. This present study has revealed the effect of storage on the chemical composition and mycoflora of melon. However, apart from good hygiene, proper handling and processing practice should be employed to reduce the contamination of stored sun dried melon seeds. The isolated fungi can degrade the melon seeds as substrate thereby making consumers especially the immunocompromised individual vulnerable to microbial infections. There is an urgent need to design a good means of reducing this contamination so as to meet the international standards of good manufacturing practice.

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