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Effects of Storage on Nutritional, Mineral Composition and Mycoflora of Stored Sundried *Citrullus lanatus* Thunberg (Melon) Seeds

Emmanuel Dayo Fagbohun[1*] and Ayodeji Segun Ogundahunsi[1]

[1]Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author EDF designed the study, author ASO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ASO and EDF managed the analyses of the study. Author ASO managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The effect of storage on the nutritive values, proximate composition and mycoflora of *Citrullus lanatus* was investigated during a 24 weeks of storage. The mycoflora were isolated using direct plating and washing methods. Eight fungi were isolated namely *Fusarium* sp., *Rhizopus* sp., *Mucor* sp., *Aspergillus niger, A. fumigatus, A. flavus, Penicillium chrysogenum* and *Penicillium* sp. The fungal count was found to increase as the storage time increased. The proximate analysis showed a decrease and an increase on some parameters investigated. The mineral composition was found to decrease as the storage time increased. The following nutrients (g/100 g) were found to have reduced: ash content 4.29 - 3.19, fibre content 2.06 - 1.51, fat content 57.02 - 50.90, while the following increased: Moisture content 7.21-12.22, crude protein 29.24-30.90, carbohydrate content 0.20-2.01. The results for minerals analysis (mg/100 g) showed a decrease in all parameters investigated: sodium 2.01-1.49, potassium 65.52-56.21, calcium 13.10-10.19, magnesium 46.34-

**Corresponding author: E-mail: drfagbohun08@gmail.com;*

37.61, Iron 8.12-3.31, zinc 4.76-1.24, copper 2.84-2.11 and phosphorus 3.46-1.60 while lead and cadmium were not detected. However, storage was found to cause deterioration and reduction in the nutritive value and mineral content of *C. lanatus* seeds. It is therefore recommended that these seeds must be stored under controlled conditions that will prevent the growth of fungi and reduction of nutrients.

Keywords: Mycoflora; shelled melon; proximate; minerals; Citrullus lanatus.

1. INTRODUCTION

Citrullus lanatus(Thunberg) melon is a drought leguminous plant that grows best in warm temperate and tropical areas of the world [1]. It belongs to the family Cucurbitaceae and the genus Critullus [2]. *Citrullus lanatus* is rich in minerals, protein, vitamins, carbohydrate and fibre [3]. It has a life span of about 120 days of warm or hot weather from planting to harvesting [4]. *Citrullus lanatus* is the biological ancestor of the watermelon now found all over the world, but originated from West Africa. Melon is a member of the Cucurbitaceae family. Unlike the common watermelon, whose flesh is sweet and red, the melon's juicy flesh is pale yellow or green, and also tastes bitter. A creeping annual herb, the melon has hairy stems, forked tendrils and three-lobed hairy leaves [5]. The seeds of melon are harvested, cleaned, sun dried and stored in a cool dry condition [4]. However, storage conditions have effect on the proximate and chemical composition of the stored melon seeds because of the growth of some spoilage fungi that strive in that conditions [6]. 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 [7]. The environ-mental factors that aid the development of fungi in stored products include moisture content [8], temperature [6], aeration [9], pH [10], relative humidity [11]. However, the effects of these storage fungi on stored products include deterioration and spoilage of stored products [6] [12], reduction of market value [13] and production of chemical substances that are toxic to human health [14]. There have been several reports of depletion of nutrients of stored farm products during storage [15, 16]. However, there is no report of effects of storage on *Citrullus lanatus*, therefore, this investigation was to study the effects of storage on the nutrient composition and the mycoflora of sundried melon seeds during a 24 weeks of storage.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The seeds of Citrullus *lanatus* were collected from Oja Oba market in Ado-Ekiti, Ekiti State, Nigeria. The seeds were identified as *Citrullus lanatus* at the Department of Plant Science, Faculty of Science, Ekiti State University, Ado Ekiti, Ekit State, Nigeria. The seeds were shelled and sun dried for three weeks. The samples were stored for six months in an insect free container, labeled and kept in the laboratory. The seeds were examined for the changes in the mycoflora and nutrients composition on monthly basis during storage.

2.2 Isolation of Fungi from Stored Sun Dried *Citrullus lanatus* Seeds

2.2.1 Direct plating method

The sundried melon seeds were examined randomly for the presence of moulds according to the method of Amusa [17]. The surfaces of five randomly selected seeds were sterilized separately with ethanol and washed in two changes of sterile distilled water. Using sterile dissecting forceps, a portion of the *C. lanatus* seeds was randomly picked and scraped aseptically on potato dextrose agar and malt extract agar plates. The plates were incubated at room temperature for 3-5 days [17,18]. The fungal growth on the plate was sub cultured and further subcultures were made until pure colonies were obtained by successive hypha tip transfer [19]. The cultures were examined under the microscope to determine the common fungi present.

2.2.2 Washing method

One gram of *C. lanatus* seeds was weighed using a weighing balance and dispensed into 9ml of sterile distilled water. This was shaken vigorously and 1ml of the solution (10^{-1} stock dilution) was added to 9ml of sterile distilled

water. This was shaken thoroughly and further dilutions were made up to $10^{-4.}$ One ml of 10^{-3} and 10^{-4} each was added to molten potato dextrose agar and malt extract agar respectively [20]. The plates were swirled gently to obtain thorough mixing. The plates were allowed to solidify and incubated at 28ºC for 3-5 days. The fungal colonies were observed daily.

2.3 Identification of Mycoflora

Slides of pure cultures of each fungus isolated from *C. lanatus* were prepared for microscopic observation and identification. The cultural and morphological characteristics of each isolate was observed and noted and formed part of the criteria used for identification [21,22]. Detailed morphological characteristics of the fungi such as hyphae (septation), reproductive structure (sporangia/conidia) in chain or single; the type of spore, etc were observed and recorded. The following references were consulted to check the identification of fungi [23,24]. The isolates were examined under bright daylight for the colour of the culture and further examinations were carried out using the following methods.

2.3.1 Needle mount preparation method

The method of Fsgbohun [19] 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.

2.3.2 Slide culture technique

From a plate approximately 2 mm deep, 1 cm^2 PDA was cut and placed on a sterile glass slide. Each isolated fungus was inoculated 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 28ºC until adequate growth was observed. After removing the medium with scalpel, the fungus adhering to both coverslip and slide was examined [25]. 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.

2.4 Proximate Analysis

Proximate analysis involving the moisture content, protein, fat, carbohydrate, and crude fibre were determined according to the procedure of AOAC [26]. All determination was performed in duplicates.

2.5 Mineral Analysis

Standard method obtained of AOAC [26] was used. Atomic absorption spectrophotometer was used for the analysis of the following elements; Na, K, Ca, Mg, Fe, Zn, Cu, Pb, Cd and P. The standard for each element using suitable salt of each of the element were prepared. The instrument was switched on and the emission lamp for each element was fixed. All elements that were analysed used hallow cathode lamps and air acetylene flame. The standard for each element were aspirated into the flame as well as the samples and their respective concentration in mg/l were read for each sample while the absorbance of the standard were noted.

2.6 Statistical Analysis

Statistical analysis was carried out to determine the overall mean, overall standard deviation and standard error of mean of each sample [27].

3. RESULTS AND DISCUSSION

Eight fungi comprising five genera were isolated from the sun-dried melon seeds (*Citrullus lanatus*) stored for 24 weeks. They were identified based on cultural and morphological characteristics. The isolated fungi were *Rhizopus* sp*., Aspergillus niger, A. fumigatus, A. flavus, Penicillium chrysogenum, Penicillium* sp*., Mucor* sp*., and Fusarium* sp*.* The fungi isolated from stored sundried *Citrullus lanatus* during 24 weeks of storage using direct plating and washing methods are shown on Tables 1 and 2 respectively. Majority of these fungi are known to be surface contaminants of most agricultural products [28]. These fungi cause decay of the agricultural produce and cause reduction in their market and nutritional value [8].

There was an increase in the number of fungi isolated from the melon seeds as the study time progressed. Using direct plating method, for instance; *Aspergillus flavus, A. fumigatus*, *A. niger, Rhizopus*spwere isolated in the first week of study while others were isolated as the study progressed. Fungi of the genera *Penicillium* and

Aspergillus were frequently isolated. Fungi of the genus *Aspergillus* and *Penicillum* are widely distributed as storage fungi of melon seeds [29]. They cause seed discolorations, decreased nutritive values, increase in free fatty acid and peroxide values, decrease seed germination and producing a number of toxic metabolite including aflatoxin [30,29]. The result of this work is similar to the findings of Fagbohun [19] who reported the isolation of seven fungi from *Citrullus vulgaris* namely *Fusarium* sp*., Rhizopus* sp*., Penicillium sp., Mucor* sp*., Aspergillus niger , Aspergillus tamarii, Penicillium* sp. Similarly, this result is also agreement with the findings of Amadi and Adebola [31,30] who reported the isolation of six mould species namely *Aspergillus flavus, Aspergillus niger, Aspergillus fumigates, Penicillium* sp*.* and *Rhizopus* sp. from garri after eight weeks of storage. Fagbohun [7] also reported the nutritional and mycoflora changes during the storage of plantain chips that was stored for sixteen weeks. Species of *Aspergillus, Mucor, Penicillium* and *Rhizopus* have been detected as contaminants of the soil. Some of the field fungi die gradually, depending on the moisture content, temperature and competition by other fungi [32,33]. These fungi are associated with severe degradation of some stored products both in field and in storage [28]. Mould contamination of *Citrullus lanatus* may occur from the farm during harvesting, drying, and transportation or at the market; which is the point of sale. These aforementioned potential causes of contamination of *Citrullus lanatus* (egusi melon) showed that the two categories of fungi (field and storage fungi) were responsible. Also, during the shelling, it could have been exposed to spores of fungi present in the air [33]. Mycotoxins are toxic metabolites produced by fungi. They constitute an heterogenous group of secondary metabolites with diverse potent pharmacological and toxic effects in humans and animals. Moulds belonging to *Aspergillus*, *Penicillium* and *Fusarium* genera which were isolated from this work has been reported to produce mycotoxins [34].

The results of the proximate analysis (g/100 g) of *Citrullus lanatus* Thunb are shown on Table 3. Freshly shelled melon seed had ash content of 4.29, moisture content (mc) of 7.21, crude protein (CP) of 29.24, fat of 57.02, fibre content (FC) of 2.06 and carbohydrate (CHO) of 1.80. However, after six months of storage ash, fat and fibre decreased to 3.19, 50.90 and 1.51 respectively. These findings agreed with the report of Fagbohun [7] who reported a decrease in the fat and fibre content of sun dried

Table 1. Summary of fungi isolated from *Citrullus lanatus* after 24 weeks of storage using direct plating method

Week of storage	Isolates
0	A,B,C,D
4	A,B,C,D,E
8	A,B,C,D,E,F
12	A, B, C, D, E, F,G
16	A,B, C, D, E, F, G
20	A, B, C, D, E, F, G, H
24	A, B, C, D, E, F, G, H

Legend: A-Aspergillus flavus, B- Aspergillus fumigates, C- Aspergillus niger, D- Rhizopus sp, E- Penicillium sp, F- Mucor sp, G- Penicilium chrysogenum, H- Fusarium sp

Table 2. Summary of fungi isolated from *Citrullus lanatus* after 24 weeks of storage using washing method

Week of storage	Isolates
0	C & D
4	C,D,E
8	A,C,D,E,F
12	A, B, C, D, E, F,G
16	A,B, C, D, E, F, G
20	A, B, C, D, E, F, G, H
24	A, B, C, D, E, F, G, H

Legend: A-Aspergillus flavus, B- Aspergillus fumigates, C- Aspergillus niger, D- Rhizopus sp, E- Penicillium sp, F- Mucor sp, G-. Penicilium chrysogenum, H- Fusarium sp.

Table 3. Summary of the result of proximate analysis of *Citrullus lanatus* during a 24 weeks of storage (g/100 g)

Weeks of storage	Ash	Moisture content	Crude protein	Fat	Fibre	CHO
0	4.29	7.21	29.24	57.02	2.06	1.80
4	4.31	7.24	29.32	55.05	2.08	2.02
8	4.34	7.35	29.41	56.77	2.09	2.07
12	4.24	8.24	29.66	55.38	1.98	2.12
16	3.46	10.49	30.90	53.24	1.87	2.20
20	3.23	11.87	29.67	51.21	1.62	2.38
24	3.19	12.22	29.69	50.90	1.51	2.41
Mean	3.87	9.23	29.70	54.22	1.89	2.14
S.D	0.54	2.07	0.51	2.31	0.21	0.21
C.V (%)	13.95	22.43	1.71	4.26	11.11	9.81

Key: (CHO= Carbohydrate) (S.D = Standard Deviation) (C.V (%) = Coefficient of Variation)

Table 4. Summary of mineral analysis of *Citrullus lanatus* during a 24 weeks of storage (mg/100 g)

Weeks of storage	Na	K	Ca	Mg	Fe	Zn	Cu	Pb	Cd	P
0	2.01	65.52	13.10	46.34	8.12	4.76	2.84	Nd	Nd	3.46
4	2.13	65.87	13.21	46.40	8.23	4.82	2.89	Nd	Nd	3.53
8	2.04	65.94	13.12	45.53	7.53	4.09	2.64	Nd	Nd	2.95
12	1.95	61.38	12.47	43.73	5.33	3.21	2.45	Nd	Nd	2.54
16	1.84	60.37	11.74	41.33	5.30	2.94	2.34	Nd	Nd	2.28
20	1.60	58.22	10.22	37.64	3.33	1.29	2.23	Nd	Nd	1.69
24	1.49	56.21	10.19	37.61	3.31	1.24	2.11	Nd	Nd	1.60
Mean	1.87	61.93	11.82	42.65	5.87	3.19	2.5	-	-	2.58
S.D	0.24	3.95	1.33	3.85	2.12	1.49	0.30	-	-	0.78
C.V (%)	12.83	6.40	11.30	9.02	36.11	46.70	12.00	-	-	30.23

Key: Na = Sodium, Cd = Cadmium, Nd = Not detected, K = Potassium, Mg = Magnesium
S.D = Standard Deviation, Cu = Copper, Zn = Zinc, C.V (%) = Coefficient of Variation
Ca = Calcium, Pb = Lead, Fe = Iron, P = Phosphorus

Plantain chips stored for sixteen weeks. Meanwhile, the CP and CHO increased to 29.69 and 2.41 respectively. This is in contrast to the findings of Fagbohun [7] 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 12.22, this may be due to the degrading activity of the fungi as reported by Fagbohun and Onifade [19]; [35]. The proximate analysis of stored shelled melon seeds showed 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 [6]. The mineral analysis of the melon seeds during storage in mg/100g are shown on Table 4. The result revealed the following minerals Na (2.01), K (65.52), Ca (13.10), Mg (46.34), Zn (4.76), Fe (8.12), P (3.46) and Cu (2.84) 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 [12] who reported a decrease in the minerals content of melon seeds after two weeks of storage. This is due to the activities of storage fungi which metabolized the minerals for their physiological activities such as growth and enzymatic activities [28].

4. CONCLUSION

Melon seeds are economically important to man because it is abundant and an essential source of nutrients for metabolic activities. In order to maintain the economic and food value of melon seeds, it should be stored under controlled environment that will prevent the growth of the fungal flora thus preventing deterioration of stored melon seeds and reducing its chemical composition. The preventive measures that can be employed against the growth of the storage

fungi include biological control [10], chemical control [36] and physical control [37]. During harvesting, there is glut in production; therefore farmers/producers usually store these melon seeds for off-season. For the facts that the mould species were isolated from the stored *Citrullus lanatus* seeds and are capable of reducing the nutritional quality, it is most desirable that their presence should be greatly reduced. To reduce quality loss in stored egusi (melon) seeds, rapid drying to low moisture is often emphasized, because all scenarios leading to mould contamination and subsequent damage relate to non-maintenance of stored products at safe moisture content [38].

This can be achieved by taking greater care of melon seeds during processing and storage. Drying should also be thorough and the seeds should be subsequently packed in polythene bags before they are displayed for sale in the market. Hence, effective and adequate storage conditions should be designed to prevent and control the activities of the spoilage mycoflora.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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