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Effect of Storage Methods on the Germination and Proximate Composition of *Treculia africana* Seeds

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Abstract

The seeds of *Treculia africana* (African breadfruit) are widely consumed in West African, especially among rural dwellers, thus playing important role in food security, economic empowerment and rural employment. The effect of storage methods on the germination and proximate composition of *T. africana* seeds were investigated. Seeds were stored for eight weeks and were subjected to germination trials and proximate composition at the end of each week. Some seeds were sown immediately after extraction, which served as control. Generally, germination commenced ten days after sowing. Both storage methods and storage duration had significant effect ($p < 0.05$) on the germination. For storage methods that did not kill the seeds, there was significant and progressive decrease in germination as storage duration increased. Storage in deep freezer, room condition (for longer than one week) and drying under the sun resulted in the death of *T. africana* seeds. Better germination results were obtained from seeds stored in refrigerator for up to four weeks, beyond which further storage killed the seeds. Except for one to two weeks refrigerator storage, germination of control seeds was significantly higher than that of those under all storage treatments. Storing of *T. africana* seeds in the investigated methods will lead to poor or no germination, with germination becoming poorer as storage duration increases. Thus, until appropriate storage method is discovered, the seeds will have to be sown immediately after extraction. Fresh *T. africana* seeds have carbohydrate, crude protein, moisture, crude fibre, ash and ether extract (fat) contents of 38.26, 17.67, 3.82, 15.85, 3.97 and 15.85%, respectively. The proximate compositions of fresh seeds were generally higher than those of seeds subjected to storage treatments. Both storage methods and duration significantly affected ($p < 0.05$) proximate values. The decrease in some proximate compositions of *T. africana* seeds subjected to different storage treatments implies a decrease in the nutritive values of the seeds, thus adversely affecting its importance as food supplement for humans and animals.

Introduction

Treculia africana Decne (African Breadfruit) is a large evergreen tropical food tree species belonging to Moraceae family. Three varieties (*africana, inversa* and *mollis*) have been distinguished (Okafor, 1981; Keay, 1989). Their taxonomic differences are mainly based on fruithead size, hairiness of branchlets and leaves. Var. *africana* produces fewer but larger fruitheads and superior seed weight than var. *inverse,* while var. *inverse* produces twice as many branches as var. *africana* (Okafor, 1981; WAC, 2005). The species is distributed in tropical Africa, within the approximate latitude range of 15°N to 20°S (Keay, 1989; WAC, 2005). Due to its domestic and economic importance, the tree has been heavily exploited resulting to its current highly endangered status.

The seeds are widely consumed, thus playing important role in food security, especially among rural dwellers in West Africa. It enhances economic empowerment and provides rural employment. Many rural dwellers in Nigeria and Cameroon are engaged in collection, processing and sale of *T. africana* seeds as a means of livelihood. The seeds can be boiled, pounded and eaten with soups and stew or cooked into porridge. The seeds can also be roasted and eaten the same way as peanuts. The seed kernel is used in preparing pudding, as a thickener in traditional soups and in the manufacture of food products such as flour for bread, beverages and weaning food for children.

Seed storage is important for several reasons. Seed ripening and collection often does not correspond with the time for seedling production and tree planting. The location of seed source may be far from intended planting sites while some species do not produce seed every year. Stored seeds are important back-up for plant species that are threatened with extinction. The main purpose of seed storage is to secure the supply of good quality and quantity seeds for planting programme whenever needed. If sowing time follows immediately after seed harvesting and

processing, seeds can go directly to the nursery but this is rarely the case. Every year, large volumes of high quality seeds are lost for planting because of inadequate drying and storage. If seeds are not properly dried, they stand the risk of loosing viability due to high moisture content, which is one of the factors that determine whether or not seed can be stored safely (Siddique and Wright, 2003). Also, the length of time seed will remain viable varies greatly by species and storage conditions. This study investigates the effect of storage method on the germination and proximate composition of *T. africana* seeds.

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Methodology

The field trial (germination) and proximate analyses were conducted at nursery site and the laboratory of the Department of Forestry and Wood Technology, Federal University of Technology, Akure, Nigeria. Akure is located on latitude 7^o 17' N and longitude 5^o 10' N, with an altitude of 350 m above sea level. Rainfall pattern in the study area is bimodal, with annual mean rainfall of about 1700 mm. Mean annual humidity is about 80% while mean daily temperature is about 26^oC. The soil is alfisol, classified as ferruginous tropical soil on crystalline rock of basement complex. The soil is slightly acidic, with a predominantly sandy loam texture.

Prior to storage treatments, the seeds were subjected to viability test by soaking in cold water. Seeds that still floated after 24 hours were discarded. Seeds were subjected to three storage treatments (deep freezer (about -4^oC), refrigerator (about 5^oC) and room storage (about 27^oC)), a drying (sun-drying) and control treatment. The length of each storage/drying treatment varied from one to twelve weeks. A batch of 150 seeds was subjected to each storage, drying and control treatment. At the end of each treatment duration, the seeds were removed and sown in already prepared germination beds. Each treatment was replicated three times, implying that 50 seeds were sown on each bed. Watering was done twice daily (morning and evening). Germination count was taken daily (mornings) beginning from the first day of germination and monitored till the 65^{th}. As the germinated seeds were counted, they were pricked out to avoid error in counting. Cumulative germination data were recorded per treatment.

Proximate compositions of fresh and stored seeds were determined according to AOAC methods (1990). Moisture content (MC) was determined by oven-dry method. Moisture content (%) was computed by (1):

$$Mc\ (\%) = \frac{w_2 - w_3}{w_2 - w_1} \times 100 \quad \text{.....(1)}$$

W_1 = weight of empty Petri dish; W_2 = weight of Petri dish & fresh sample; W_3 = weight of Petri dish & dried sample. Percentage nitrogen (N) was determined following the micro Kjeldahl method (Bremner, 1965). Percentage nitrogen was calculated using equation (2) while Crude protein was obtained by multiplying the corresponding total nitrogen content by a conventional factor of 6.25.

$$\%\ Nitrogen = \frac{T \times 0.014 \times D}{w} \times 100 \quad \text{.....(2)}$$

Where D =Dilution factor; T =Titre value; W = weight of sample; 0.014 = Constant value

Fat content was determined by the soxhlet extraction procedure on 2 grams of the samples with petroleum ether as the solvent at the boiling point range of 60 – 80^0C. Percentage fat content was calculated using equation (3):

$$\%\ \text{fat} = \frac{weight\ \text{of fat extracted}}{Initial\ \text{sample weight}} \times 100 \quad \text{.....(3)}$$

Ash content was determined as the residue left after ashing about 2g of the samples in a muffle furnace. Ashing was continued until a light grey ash was obtained. Crude fibre (CF) was determined using the method described by Indrayan (2005) while Carbohydrate contents (CHO) was determined by the difference method, which was accomplished by following the calculation (CHO = 100 - %MC – CP – CF - fat –ash).

Before undertaking Analysis of Variance (ANOVA), germination data (in %) were transformed to arc sine value to ensure conformation to assumptions of ANOVA. One-way ANOVA was performed to test for significant differences in different storage and drying methods on germination of *T. africana* seeds. Proximate composition data were analysed using two-way ANOVA. Mean separation was undertaken using Duncan multiple Range Test.

Results and Discussion

Germination of seeds under control treatment as well as under the various storage treatments started 10 days after sowing and continued till the 35^{th} for control about the 50^{th} day for stored seeds. Seeds under control germinated within a shorter period of time than those under the various storage treatments. For example about 75% of seeds under control germinated between the 10^{th} and 20^{th} day compared to only 40% and 10% for seeds stored in refrigerator and room condition, respectively. Both storage methods and duration had significant effect on seed germination. Storage in deep freezer resulted in the death of seeds. Only 28.9% of seeds stored under room condition for one week germinated. Storage under room conditions beyond one week led to the death of seeds (Tab. 1). Better germination results were obtained from seeds stored in the refrigerator for up to four weeks, beyond which further storage killed the seeds. There was a significant and progressive decrease in seed germination with increase in storage duration in the refrigerator. About 77.3% of seeds stored in the refrigerator for one week germinated, which decreased to 45.8% after four weeks storage (Tab. 1). Germination of seeds stored in refrigerator for one and two

weeks were not significantly different (P>0.05) but storage beyond two weeks led to significantly lower germination. Seeds stored in the refrigerator for up two week gave similar germination results as the control. However, beyond the second week, control treatment produced significantly higher (P<0.05) germination result than seeds stored in the refrigerator. Germination of seeds stored under room conditions for one to eight weeks were significantly lower (P<0.05) than that of the under control as well as those stored in the refrigerator. The effect of sun-drying on *T. africana* seeds was drastic, resulting in no germination of the seeds (Tab. 1).

T. africana seed is classified in the group of tropical recalcitrant seeds. The failure of seeds dried under the sun to germinate is characteristics of recalcitrant seeds. Recalcitrant seeds cannot be dried without damage to the endospermic unit of the seed, thus germination of seeds in this group dried prior to sowing should not be expected. Campell (1980) reported that seeds belonging to moraceae family should not be dried prior to sowing. When freshly harvested seeds are dried, viability is at first slightly reduced as moisture is lost but then begins to reduce considerably at a certain moisture content termed critical moisture content or lowest safe moisture content and if drying continues further, viability is eventually reduced to zero (King and Roberts, 1979). This is true with respect to *T. africana* as our results demonstrated. For example, when the seeds were dried in the room for one week, germination was 28.9%, which was reduced to 9.8% by the second week and by the third week, germination was reduced to zero. The failure of the seeds stored in deep freezer to germinate indicates that the seeds cannot be stored at sub zero temperatures, probably due to freezing injury resulting from ice formation. Farrant *et al.,* (1988) had noted that recalcitrant seeds are normally sensitive to low temperature and thus, they cannot be stored at zero temperatures. Results indicate that seeds stored in the refrigerator remained viable till the fourth week of storage (Tab. 1), indicating that longevity *T. africana* seeds can be slightly increased by refrigerator storage. Longevity of recalcitrant seeds is short, varying from a few weeks to a few months for species adapted to tropical environment (King and Robert, 1979). Satisfactory methods of maintaining the viability of recalcitrant seeds over medium and long term has not been found since they cannot be dried neither can they be stored for a long time. Our results suggest that seeds of *T. africana* should not be dried before storage or sowing and that storage where necessary should be in the refrigerator for a short time. Drying and storage, depending on the duration and method will lead to considerable reduction in germination or to eventual death of the seeds. This probably explains the poor natural regeneration of the species. Consequently, until appropriate long term storage method is developed, germination of seeds of the species should be undertaken immediately after extraction, or after short storage in the refrigerator.

Table 1: Summary of the results of analysis of variance for the effect of storage and drying methods on the germination of *Treculia africana* seeds

Storage duration	Seed germination (%)				
(weeks)	Control	Refrigerator (4^{o}C)	Room Storage (27^{o}C)	Deep Freezer (-5^{o}C)	Sun-drying
1	72.3^{a}	77.3^{a}	28.9^{b}	0.0^{c}	0.0c
2	72.3^{a}	66.2^{a}	0.0^{c}	0.0^{c}	0.0c
3	72.3^{a}	54.8^{b}	0.0^{c}	0.0^{c}	0.0c
4	72.3^{a}	45.8^{b}	0.0^{c}	0.0^{c}	0.0c
5	72.3^{a}	0.5^{c}	0.0^{c}	0.0^{c}	0.0c
6	72.3^{a}	0.0^{c}	0.0^{c}	0.0^{c}	0.0c
7	72.3^{a}	0.0^{c}	0.0^{c}	0.0^{c}	0.0c
8	72.3^{a}	0.0^{c}	0.0^{c}	0.0^{c}	0.0c

Mean and standard deviation calculated from 3 replications. Values followed by the same letters are not significant different at 0.05 level of significance.

Fresh *T. africana* seed has carbohydrate, crude protein, moisture, crude fibre, ash and ether extract (fat) contents of 38.3, 17.7, 3.8, 15.9, 4.0 and 15.9%, respectively (Tab. 2). Except for crude protein of seeds stored under room condition, the proximate composition of the fresh seeds was generally higher than those of the seeds subjected to storage and drying treatments (Tab.2). Storage duration had significant effect on proximate compositions, with proximate compositions significantly decreasing as storage duration increased, except for crude fibre and ash content which followed an opposite trend. Similarly, storage methods significantly affected seed proximate composition. The effect of storage methods was much more profound on crude fibre, moisture content, ash content, and ether extract, all of which were generally higher in seeds stored under room condition and those sun-dried than those stored in refrigerator and deep freezer (except moisture content). Except for seed stored in the refrigerator, crude protein was virtually the same for seeds stored under the various storage methods.

T. africana seeds are source of edible oil and are rich in protein and fat. They serve as good protein supplement for humans and animals (Giami *et al*., 2000). The proximate composition values obtained in this study are similar to those obtained by Adindu and Williams (2003) and Giami *et al*., (2000). However, the protein content of 20.1% for *T. africana* seeds reported by Giami *et al.,* (2000) is higher than the crude protein obtained in this study. The different storage and drying treatments had only little effect on the nutritive value of the seeds. Bankole *et al.*, (2005) found no significant different in the proximate compositions of melon seeds (*Colocynthis citrullus* L.) subjected to different drying treatments. The result of this study revealed that the longer the seeds of *T. africana* are

stored/dried, the higher the rate of reduction in nutritive value of the seeds. The crude protein of *T. africana* is higher than those of some important indigenous food tree species such as *Vitex doniana* (0.82%), *Invingia gobonensis* (8.4%), etc (e.g. Okoye *et al*., 1999). The changes in the nutritive values of the species thus affect the importance of the species as a good protein supplement. Since the seeds are commonly roasted before consumption, it is advisable not to roast them under intense heat on the one hand and not to roast them for a long time on the other as this might lead to significant reduction in the nutritive values of the seeds.

Table 2: Summary of the results of analysis of variance for the effect of different storage and drying methods on the proximate Composition of *Treculia africana seed*

Storage/ Dying method	Duration (week)	Carbohydrate	Crude protein	Crude fibre	Moisture Content	Ash content	Ether extract
Fresh (control)	-	38.4 ±126	17.7±0.53	3.8± 0.29	15.9± 0.88	4.0±1.26	15.8±5.0
Room storage/ Drying	1	36.0±0.04^{a}	18.5±0.69^{a}	3.1±0.04^{a}	15.4±0.14^{a}	2.7±0.05^{a}	10.1±0.05^{a}
	2	33.9±0.06^{b}	18.0±0.12^{a}	3.4± 0.06^{b}	14.1±0.04^{b}	3.5±0.02^{b}	10.0±0.02^{a}
	3	29.4±0.06^{c}	17.1±0.09^{b}	3.6±0.08^{b}	12.2±0.07^{c}	3.7±0.11^{c}	9.8±0.04^{ab}
	4	26.4 ±0.08^{d}	16.2±0.22^{b}	3.9±0.11^{c}	7.0±0.02^{d}	4.8±0.08^{d}	9.7±0.15^{b}
Refrigerator	1	35.7±0.24^{a}	17.2±0.06^{a}	2.4±0.08^{a}	18.1±0.06^{a}	2.6 ±0.33^{a}	9.7± 0.48^{a}
	2	34.2±.07^{a}	14.3±0.25^{b}	2.5±0.10^{a}	15.8±0.08^{b}	3.1±0.08^{ab}	9.4± 0.11^{a}
	3	27.8±0.28^{b}	14.0±0.03^{b}	2.5 ±0.07^{a}	15.1±0.08^{c}	3.1±0.15^{ab}	8.7 ±0.04^{b}
	4	18.7±0.32^{c}	13.2±0.13^{c}	2.6±0.07^{a}	14.7±0.11^{d}	3.2± 0.11^{b}	7.1± 0.10^{c}
Deep freezer	1	32.5±0.9^{a}	17.4±1.2^{a}	2.6 ±0.06^{a}	18.3±0.6^{a}	2.9±0.11^{a}	9.3± 0.1^{a}
	2	32.5 ±0.83^{a}	16.3±0.08^{b}	2.6±0.16^{a}	18.3±0.09^{a}	3.1±0.05^{ab}	9.1±007^{ab}
	3	31.3± 0.14^{a}	16.2±0.11^{bc}	2.6±0.22^{a}	18.1±0.05^{a}	3.1±0.06^{ab}	9.0±0.05^{b}
	4	28.4±0.15^{b}	16.0±0.03^{c}	2.6±0.20^{a}	18.0±0.02^{a}	3.1±0.08^{b}	8.9±0.08^{b}
Sun-drying	1	34.3± 0.31^{a}	17.1±0.19^{a}	3.2±0.06^{a}	12.6 ±0.12a	3.7±0.35^{a}	11.5±0.11^{a}
	2	30.6±0.65^{b}	16.5±0.03^{b}	3.6±0.07^{a}	10.1 ±0.06^{b}	4.3±0.01^{b}	11.5±0.08^{a}
	3	28.4±0.06^{c}	16.5 ±0.03^{b}	3.5±0.07^{b}	8.9±0.11^{c}	4.4 ±0.06^{b}	11.5±0.01^{a}
	4	26.2±0.17^{d}	16.3±0.11^{b}	3.3±0.10^{b}	7.1±0.05^{d}	4.8±0.04^{b}	11.5±015^{a}

Mean and standard deviation calculated from 3 replications. Values followed by the same letters are not significant different at 0.05 level of significance.

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