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EFFECT OF STORAGE ON THE PROXIMATE COMPOSITION OF *MORINGA OLEIFERA* SEED USED IN WATER PURIFICATION

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ABSTRACT

This study was conducted to determine the effect of storage time and mode of storage on the proximate composition of Moringa oleifera (MO) seeds used in water purification. MO seeds were stored for a period of 150days in various forms, namely Winged seed in bottles (WS_{BO}), Winged seed in cellophane bags (WS_{CB}), Seed pod in baskets (SP_{BA}), Shelled seed in bottles(SS_{BO}), Shelled seed in baskets(SS_{BA}) and Winged seed in baskets(WS_{BA}).Proximate analyses were carried out at 30-day intervals to monitor the bio-composition and hence the health status of the stored seed as storage time increased. Results show that the storage time for *MO seeds had significant effects on the proximate properties, unlike the mode of storage. The increase in moisture content was attributed to ambient moisture content due to the rainy season. Carbohydrates and Fibre contents generally increased as storage time progressed, while minimal reductions in lipid content, 0.43%-2.25%, were observed. The ash content persistently decreased until 120days of storage but increased afterwards. This study has shown that storage time and not storage mode has a significant effect on MO seed stored for a period of 150days.*

Keywords: proximate, *Moringa oleifera*, purification, storage

INTRODUCTION

An emerging method for water purification is the use of natural coagulants which are obtained from plant materials. Some of the plants commonly used include *Moringa oleifera*(Megersa *et al*, 2014), *Hibiscus esculentus* (Okro) (Thakur and Choubey, 2014),*Genus citrullus* (Water Melon)(Idris, *et al*, 2016) and *Carica papaya* (Pawpaw)(Verma *et al*, 2015). These plants contain some natural compounds called phytochemicals which are reported to have antifungal, antibacterial and antioxidant properties (Braide *et al* ,2012; Nwankwo et al ,2014; Fowoyo and Oladoja,2015). The proximate properties, especially protein, have also been found to be responsible for the effectiveness of *MO* seed extracts in water purification (Nwaiwu et al, 2021).

Proximate analysis conventionally includes determinations of the amount of water, protein, fat (ether extract), ash and fiber. Quantification of proximate content of *Moringa oleifera* seed has been carried out by Fowoyo and Oladoja (2015) and Dalen *et al (*2009).

The aim of this work is to determine the effects of time and mode of storage on the proximate

properties of the *Moringa Oleifera* seeds whose extracts are used for water purification purposes. This is important because it will indicate the potential of stored *MO* seeds to still be effective in water purification.

MATERIALS AND METHODS

Materials

Collection of *Moringa oleifera* seeds

Moringa oleifera seeds were obtained from local growers located in Agulu, Anambra state, Nigeria. The seeds were thoroughly mixed together, and air-dried for fourteen days before being segregated for storage. During this period of drying the seed, a few seed kernels were selected daily and used for moisture content determination using the gravimetric method.

Collection of water sample

Turbid water samples were collected near a 35-metre-long bridge at Amansea, on the Enugu-Onitsha Express Road. The water was transported to the laboratory immediately for determination of the microbial load. Ice packs were used to keep the water cool while being transported.

Seed Storage

The air-dried seeds of *Moringa oleifera* were stored at room temperature of about 30ºC in different forms and containers for a period of 150days. Winged seeds were stored in covered baskets (WS_{BA}), corked glass bottles (WS_{BO}) and cellophane bags (WS_{CB}). Shelled seeds were stored in covered baskets (SS_{BA}) and corked glass bottles(SS_{BO}). Seeds not removed from the pods were stored in covered baskets (SP_{BA}). Winged seeds refer to seeds removed from pods exposing the outer brown husk exhibiting a wing-like shape. Shelled seeds refer to the husked seeds (inner white kernel).

Proximate Analyses

Proximate analyses were carried out at 30-day intervals to monitor the proximate composition and hence the status of the stored seed.

(a) Determination of Crude protein

The Kjeldahl method was used. A Nitrogen standard curve was prepared with varying concentrations of ammonium sulphate. The ammonium sulphate was mixed with 30% sodium hydroxide (NaOH) which converts the ammonium to ammonia and the steam was distilled into Boric acid solution. The distillate was titrated with 0.001N hydrochloric acid (HCl). A graph of nitrogen concentration against the volume of acid used was plotted.

The concentration of nitrogen in the sample was then extrapolated from the standard curve. The milligram of nitrogen obtained from the kjeldahl method was multiplied by a conversion factor (6.25) to give the milligram of protein in the sample.

(b) Fiber Content

The sample was first defatted with petroleum ether. The defatted sample was boiled in 0.255N sulphuric acid (H_2SO_4) for 40mins; later in 0.313N sodium hydroxide(NaOH) for 40mins and finally in water. The sample was then washed with acetone and allowed to dry in a pre-weighed crucible. The sample was dried to a constant weight and the weight was recorded. The sample was then ashed and weighed.

$$Weight\ of\ fibre = weight\ of\ dry\ sample - weight\ of\ ash \quad 3$$

(c)Moisture content

Half gram(0.5g) of the sample was placed in a pre-weighed crucible. The sample was dried in an oven at 90-110ºC until a constant weight was obtained. The weight of the crucible with the dry sample was subtracted from the weight of the crucible with the fresh sample to obtain the weight of moisture in the sample.

$$\%\text{Moisture} = \left(\frac{\text{weight of Moisture}}{0.5}\right) x\ 1004$$

(d)Lipid content

Two solvents were used, namely solvent A = acetone:hexane (25:10) and solvent B = hexane: diethylether (9:1).10g of the sample was blended and homogenized in 35ml of

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solvent A. It was centrifuged and the supernatant was poured into a 50ml solution of 0.1M phosphoric acid in normal saline. The residue was re-extracted 2 more times with 25ml of solvent B, centrifuged and pooled with the first extract. The extract was inverted several times and poured into a separating funnel. The water part was discarded and the lipid fraction was collected into a pre-weighed crucible. The crucible was dried and weighed again to determine the mass of the lipid.

$$\%Lipid = \left(\frac{B-A}{10}\right) x\ 1005$$

A = Weight of Crucible

B = Weight of Crucible and lipid

(e) Ash content

0.5g of the sample was weighed in a crucible. The sample was subjected to heat at 500-550^{o}C until it turned to ash. The crucible and ash were weighed and the ash content was calculated.

$$\text{Ash content} = \left(\frac{\text{A}-\text{B}}{0.5}\right) \text{x } 1006$$

A = weight of crucible and fresh seed

B = weight of crucible and ash

Statistical Analyses

Correlation and Regression

The multiple regression analysis tool from the Excel worksheet(MS Excel 2010 version) was used to determine relationships between the proximate composition of the stored seed, storage time and storage mode. The storage periods used were 0, 30, 60, 90, 120 and 150 days while the storage modes had categorical data assigned. The categorical data were numbers 1-6 for shelled seeds stored in corked glass bottles(SS_{BO}),shelled seeds in covered baskets(SS_{BA}), winged seeds in corked glass bottles (WS_{BO}), winged seeds stored in covered baskets (WS_{BA}), winged seeds in cellophane/plastic bags(WS_{CB}) and seeds not removed from the pods were stored in covered baskets(SP_{BA}) respectively.

Statistical Distribution of Collected Proximate Content Data

The normality of the collected data of stored seed proximate content was validated to justify use in statistical analyses. The monthly values of seed proximate content for various storage containers were used to obtain the values for the mean and standard deviation. The normal statistical distribution was carried out using Microsoft Excel Worksheet.

RESULTS AND DISCUSSION

Moisture Content

The initial moisture content of the freshly harvested dried seeds was 7.8%. On air drying, there were fluctuations in the following days(Figure 1), but the air drying was terminated after the values of moisture content had stabilized at 6.33% and there was no appreciable reduction in the moisture content of seed. During air drying, room temperature varied from 28.0˚C to 31.6˚C.

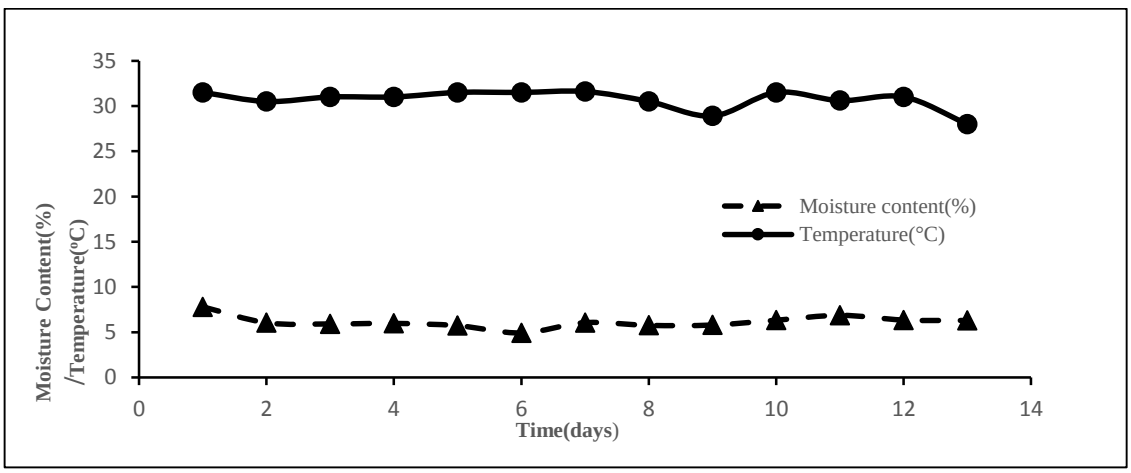

Figure 1 Seed moisture content and temperature against drying time(before storage)

The maximum experimental storage period for the *Moringa oleifera* seeds was 150 days during which moisture content values were recorded for the seeds at 30day intervals (Figure 2).

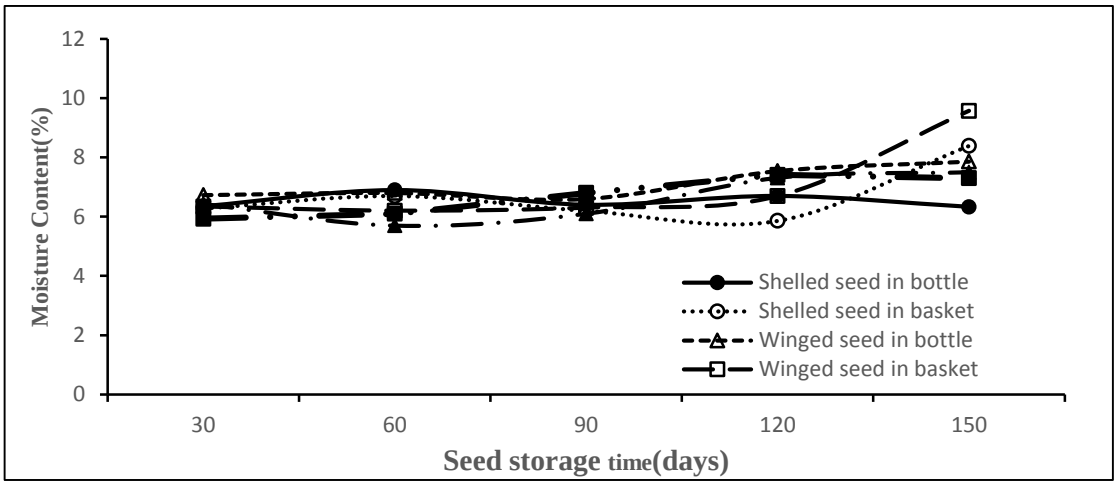

Figure 2 Moisture contents of stored *Moringa oleifera* seeds

For all the storage containers, except the shelled seed kernels in bottle, there were increments in the moisture content of the stored seeds. This can be explained by the fact that moisture content percentage of dried seeds like *Moringa oleifera* is proportional to the ambient environmental humidity as the storage period was within the raining season.

Proximate Composition

Lipids

The percentage of lipid throughout the period of 150 days and in all the modes of storage was between 31.24% and 34.08%. The shelled seeds stored in bottles had a lipid percentage decrease of 1.26%. Values of lipid reduction observed for other storage modes including shelled and winged seeds in basket, pod in basket and winged seeds in polythene bags were 0.43%, 2.25%, 1.88% and 0.71% respectively. Reduction in lipid content is a known characteristic of the ageing process in seeds (Lin and Pearce, 1990) and this is supposedly caused by lipid degrading enzymes (Devaiah et al, 2007). Phospholipase D(PLD) accelerates lipid degradation (Lee et al, 2012) and lipid peroxidation is one of the main causes of the deterioration of oil in seeds during storage (Stewart and Bewley, 1980; Wiebach et al, 2019; Lopez-Fernandez et al,2018)

From the study, the activity of PLD in the stored *Moringa oleifera* seeds appears naturally suppressed during the 150 days of storage evidenced by minimal reduction of 0.43%-2.25% thereby eliminating evidence of membrane phospholipids(Lee et al,2012).The lipid content observed before storage (34.08%) corresponds with the values of 30%-35% reported by Muthuraman and Sasikala (2014).

Fibre Content

The fibre content in the seed freshly harvested and just before storage were 2.27 and 2.34% respectively. The values of fibre increased under all the storage modes in the first 30 days in the range of 2.88%for the winged seed in basket to 3.85% for the seeds in pod stored in basket. The value of fibre content is at variance with the results of Madubuike et al (2015), Sodamade et al (2017), Chatepa and Mbewe (2018) and Auremia et al (2019)who obtained 9.94, 17.60, 4.8 and 25.59 respectively. These differences occurring in the above results may be due to geography, soil composition, meteorology, harvest time, and extraction method (Compaore et al, 2011; Abiodun et al, 2012).The fibre contents range between the 60 and 90 as well as 120 and 150 days storage periods were 2.08% to 2.8% and 1.24% to 1.7% respectively. This shows that the fibre content of the stored *Moringa oleifera* seeds

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decreased with an increase in storage duration. The reduction of fibre content in the seed with storage agrees with the finding of Killiand Ornek (2016) on fibre reduction in cotton seed with storage time.

Ash Content

The occurrence of minerals in the *Moringa oleifera* seed is evidenced by the ash content. The freshly harvested dry seeds had the highest ash content of 4.69%. The typical mineral composition of *Moringa oleifera* seeds includes Ca, Mg, Fe in large quantities, Cu, Cr, Pb and Cd in smaller quantities (Chapeta and Mbewe, 2018).There was a noticeable reduction in the ash content within the first 120days of storage in all containers. After this period, the content increased in all vessels of storage except winged seeds stored in plastic bags. The reduction may be a result of the loss of some mineral composition during storage while the increment may be attributed to the reintroduction of the minerals due to biochemical interaction in the seeds.

The ash content of the freshly harvested dry seeds is within the range of value of 4.1% obtained by Abiodun et al (2012) for *Moringa* flour and 4.6% by Chatepa and Mbewe(2018). On the other hand, the obtained value is lower than 7.9% ash content from the work of Madubuike et al (2015). Sodamade etal (2017) and Auremia et al(2019)also reported lower values of ash content for *Moringa oleifera* seed

Carbohydrate and Protein Contents

The carbohydrate content of the stored seeds in all containers increased steadily as the storage period increased. The seed carbohydrate content increased from 30.56% just before storage to 50.63% at the end of the 150 day storage period for the winged seeds in plastic bags. Protein on the other hand reduced in all the storage vessels with increments in storage time from a value of 23.65% just before storage to 4.5% in the winged seed stored in plastic bags. The carbohydrates and protein contents are respectively higher and lower than the results obtained by Abiodun et al, (2012) from *Moringa* flour which were 10.59% and 28.04%.Conditions of cultivation may be responsible for these variations. The storage compounds of seeds which are carbohydrates, proteins and lipids are interconvertible in metabolism(Luttge, 2013). This may explain the reasons for the respective increment and reduction in the carbohydrate and protein contents of the stored seeds. Furthermore, this phenomenon has been explained by Janney (1915) that glucose is an intermediary product of protein metabolism, hence an increase in carbohydrates with a descent in the protein seed content. Coolbear(1995)and McDonald(1999) agree that seed quality deteriorates gradually during storage which includes protein degradation.

Correlation of proximate constituents of stored Moringa oleifera seeds

Table 1 shows the correlation of the proximate composition of the stored *Moringa oleifera* seeds under the stated storage conditions. There is a high inverse correlation between carbohydrates and protein in all the containers. This is in the range of -0.983 to - 0.99. Fibre and lipids content also correlated significantly with carbohydrates and proteins. The negative correlation between proteins and carbohydrates shows that a reduction in protein content had a resultant increase in carbohydrate content. This observation is in line with the proposal of Hoekstra(n.d.) that there is the metabolic interrelationship between carbohydrates, fats and proteins.

Regression relationships of seed proximate constituents with storage conditions

a) Seed proximate constituents with storage time and storage mode

Linear multiple regression relationships were established between the proximate constituents, storage time and modes of storage. Table 2 shows the derived equations for proximate content as well as the coefficient of determination(R^2), t-test values and f-model validation values. Storage time exhibited significant effects on lipid, fibre, carbohydrate and protein content of the stored *Moringa*

oleifera seeds having respective t-test values of – 3.112, 5.95, 12.844 and – 15.432 at 5% levels of significance. The storage mode did not show any influence on all the proximate constituents. This is in agreement with Stefanello et al (2015) that storage period and not storage conditions had a significant impact on some chemical parameters of stored land race maize seeds. Lee et al (2012) also stated that the critical factors that affect physical, physiological and chemical changes in seeds during storage are temperature, moisture and storage time. The regression equations show a low coefficient of determination for lipid (0.26), fibre (0.518) and ash (0.11) contents in relation to storage time and mode although the respective model validation F value for lipid and fibre are significant (F_{lipid} = 5.866; 0.0066<p<0.05; F_{fibre} =17.74,6E-06<p<0.05). This scenario means that although the values of R^2 are low, the models relating seed lipid and fibre contents with storage conditions are valid but not suitable for prediction purposes. More conditions affecting the parameters during storage should be explored to improve the models. On the other hand, the equations for predicting seed carbohydrate and protein contents with respect to storage time and mode respectively have high coefficients of determination values as 0.834 and 0.879. This means that reliable prediction can be achieved using both models. In addition, the obtained models are stable having F=82.96(1.39E-13<p<0.05) and F = 119.87(7.34E-16<p<0.05) respectively.

Table 2: Regression of proximate contents of stored *Moringa oleifera* seed with storage time(X_1) and storage mode(X_2)

Proximate contents of stored *Moringa oleifera* seed(%)	Regression equations	Coefficient of Determination (R^2), t-test	F -statistic
Lipid	Seed lipid content = -0.009 X_1 - 0.123X_2	R^2 =0.262; t_{x1} = -3.112; p= 0.0038 t_{x2}= -1.43; p= 0.162	5.866; 0.0066 <p<0.05
Fibre	Seed fibre content = - 0.0093X_1 + 0.0117X_2	R^2 =0.518; t_{x1} = -5.95; p= 1.12E-06 t_{x2} = 0.249; p= 0.8043	17.74; 6E - 06<p<0.05
Ash	Seed ash content = - 0.0028X_1 + 0.064X_2	R^2 =0.11 t_{x1} = -1.632; p= 0.112 t_{x2} = 1.24; p= 0.223	2.11; 0.138>p>0.05
Carbohydrate	Seed carbohydrate content = 0.134X_1 + 0.231X_2	R^2 =0.834 t_{x1} = -12.844; p= 2.18E-14 t_{x2} = 0.738; p= 0.4656	82.76; 1.39E - 13<p<0.05
Protein	Seed protein content = - 0.1213X_1 – 0.2967X_2	R^2 =0.879 t_{x1} = -15.432; p= 1.2E – 16 t_{x2} = -1.259; p= 0.217	119.87; 7.34E - 16<p<0.05

Distribution Fitting of Proximate Data

The mean and standard deviation for fibre, ash, lipid, carbohydrate and protein contents were 0,1,8.71E-16, -4.1E – 15, 1.95E – 15 and 8.71E -16 respectively(see Table 4). The normal distribution was used to fit in the proximate data collected from various storage containers and modes throughout 150days. The Dcalculated values for all the parameters were less than the Dmax values of 0.2172

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from the Kolmogorov-Smirnov table. This means that the collected proximate analysis data are normally distributed and therefore very suitable for statistical analysis.

Table 4 Kolmogorov -Smirnov normality test for stored *MO* seed proximate content

Proximate contents of stored *Moringa oleifera* seed	**Statistical parameters**			**Inference (Dmax=0.2172)**	
	Mean	**Standard deviation**	**D calculated**		
Fibre	0	1	0.125	Dcalculated < Dmax	Normal distribution
Carbohydrate	1.95E-15	1	0.097	Dcalculated < Dmax	Normal distribution
Ash	8.71E-16	1	0.145	Dcalculated < Dmax	Normal distribution
Proteins	8.71E-16	1	0.059	Dcalculated < Dmax	Normal distribution
Lipid	-4.1E-15	1	0.090	Dcalculated < Dmax	Normal distribution

CONCLUSION

The investigations carried out show that the storage time for *Moringa seeds* had significant effects on the proximate properties, unlike the mode of storage. There were also significant correlations between the proximate contents, especially carbohydrates, proteins, lipids and fibre. The Kolmogorov-Smirnov test shows that the normal distribution can be used to describe all the collected seed proximate content data.

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