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Effect of Storage Conditions on Coagulation Properties of *Moringa Oleifera* Seed

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Abstract
The effects of shelf life, storage form and container on coagulation properties of Moringa oleifera seeds used for clarification of turbid water were investigated. Turbid water was collected from Ezu River in Amansea, Anambra State, Nigeria. Turbidity ranged between 65 NTU and 322 NTU, depending on the season the water sample was procured. The seeds were stored for150days in three different forms- seed inside pod, winged and shelled. Three storage vessels were used-bottle with plastic cork, locally made baskets with cover and black cellophane bag. The percentage content of the coagulant active ingredient (protein) was monitored through proximate analyses. The seeds of Moringa oleifera were ground and defatted with n-hexane using the Soxhlet apparatus. This was followed by aqueous extraction of the coagulant. The coagulant active ingredient (seed protein) was isolated through acetone precipitation and used in the purification of the water. Jar test apparatus was used to determine optimum coagulant dosage. Coagulant dosages of 30 mg/l, 40 mg/l and 50 mg/l were used. There were fluctuations in the efficiencies exhibited in the different modes of storage with time. Efficiency of the shelled in bottle, winged in bottle and Pod seed in basket decreased from 98%, 86% and 96% to 60%, 62% and 90% respectively. Shelled seed in basket and winged seed in cellophane bag started and ended with 95% and 92% respectively, while the efficiency of winged seed in basket rose from 71% to 94%. Shelled seeds stored in basket showed better consistency in coagulation property with time than all the other modes of storage. The percentage protein content of the seeds in all the containers decreased drastically with storage, which resulted in reduced yield in the extracted protein in gram per gram. This means that although stored seeds could be used effectively for water purification, higher quantities of seed will be required to obtain same amount of coagulant.

Keywords: *Shelf life, Water disinfection, Moringa oleifera, Seed protein, Coagulation*

1. Introduction

In order to make clean water available, water from almost all sources must undergo some treatment. Conventional water-treatment processes make use of various chemical coagulants depending on the characteristics of the water. Aluminum and iron salts are the most commonly used chemical coagulants. These two have been indicted by various studies as having a number of problems such as reduced efficiency in coagulation in cold water, high sludge volume production, linkage with Alzheimer's disease and reduction of pH (Rajput, *et al* 2012).
These deficiencies of the chemical coagulants, in addition to their inappropriateness for application in some developing countries because of cost and low availability, make imperative the search for an alternative approach. The new approach should pose no danger to human and environmental health, be less expensive, easy to obtain and readily available whenever needed.

Research is now directed towards the use of natural coagulants produced or extracted from plants for the treatment of turbid water (Shukla 2016; Abidin *et al* 2011; Ali *et al* 2010; Camachoa *et al* 2015; Nwaiwu *et al*

2012a,b).However, most of the plants from which the coagulants are extracted are seasonal or may not be within the reach of users at all times. This means that either the extracted coagulant or the material from which the extraction is made will undergo some storage to be available when needed. Humans, of course, need water every day, hence water treatment is a semi-continuous activity that will require availability of the treatment material. The chemical coagulants normally used in the conventional methods of water treatment are stored over long periods without loss of effectiveness.

Studies have shown that one of the main coagulating agents in plant materials is the protein content (Vijayaraghavan *et al* 2011; Bodlund 2013). However, protein has been shown to be unsuitable for storage over even a relatively short period (Warrier *et al* 2014). Protein is especially vulnerable in aqueous form, which is the form in which the extracts are usually prepared. The available option is the storage of the plant material itself. The effectiveness of the extracts from the stored plant material needs to be investigated.

Few researchers have worked on the effect of storage of either stock solutions or seeds of natural coagulants. Katayon *et al* (2004) studied the effects of storage duration and temperature on coagulation properties of *Moringa oleifera* stock solution. The study showed that the highest turbidity removals were observed for *Moringa oleifera* stock solutions which were kept at room temperature for one day. For *Moringa oleifera* stock solutions, which were stored longer up to 3 days, the turbidity removal efficiencies drastically decreased. In the case of *Moringa oleifera* stock solutions which were stored at 3°C, a maximum storage period of up to 5 days was reported.

Warrier *et al* (2014) studied the effects of storage duration and temperature on the coagulation efficiency of *Strychnos potatorum* stock solution. It was reported that *S. potatorum* stock solutions, which were kept at room temperature (28 °C), had a shelf life of only five days. The highest turbidity removals were observed for stock solutions, which were kept for three days. Storage in a refrigerator extended the shelf life to seven days.

Valverde*et al* (2014) studied the changes in the coagulation efficiency of *Moringa oleifera* powder with respect to length of time of storage in the refrigerator. The researchers observed that coagulant properties decreased with the storage time and the product could be stored for a maximum period of one week.
Katayon *et al* (2006a) studied the coagulation efficiency of ground *Moringa oleifera* seed kept in different storage conditions and durations. It was reported that coagulation efficiency of ground *Moringa oleifera* is independent of storage temperature and container, but however decreased as storage duration increased.
Katayon *et al* (2006b) investigated the effects of storage temperature, packaging methods, and freeze-drying on the preservation of *M. oleifera* seeds powder. Closed container and vacuum packing were found to be more appropriate for the preservation of non-freeze-dried *M. oleifera*, compared to open container. Freeze-dried *Moringa oleifera* was reported to retain its high coagulation efficiency regardless of the storage temperature and packaging method for up to 11 months.

These researchers investigated the storage of either stock solutions or ground seed. Although the use of freeze-drying by Katayon *et al* (2006b) achieved a significant storage period of 11months, the method is cumbersome and expensive. Hence it is still necessary to go further and investigate the storage of the seed material itself.

Díaz*et al* 2018 prepared a coagulant extract, labeled "A", with *Moringa oleifera* seeds stored and preserved for 6 months, and coagulant extract labeled "B", with seeds stored for 4.5 years. It was reported that although coagulant extracts' physical appearances were different, coagulant activity between the extracts showed no statistically significant differences. Based on the results obtained, the researchers suggested that it is possible to assert that seeds stored in dry containers and at room temperature can be used as a coagulant in a time period less than or equal to 4.5 years. However, the researchers did not make available sufficient information on which comparison between the various storage periods could be based, as the two classes of stored seeds were not said to be obtained from the same parent stock.

This work presents a study on the effect of storage period, storage form, and container on the coagulation property of *Moringa oleifera* whole seed. Previous studies on storage of *Moringa oleifera seeds* were mostly based either on ground seed or on the liquid extract (Katayon, *et al* 2004; Katayon *et al* 2006 a,b; Valverde, Coldebella, Nishi, *et al* 2014). Although Díaz *et al*, 2018 studied the effect of whole-seed stored for 6 months and 4.5 years respectively, it does not seem that the two samples were taken from the same parent stock as to enable comparison, neither were baseline values made available. It is important to obtain a baseline coagulation property of the seed and monitor possible changes or absence of it with time. The present work seeks to achieve this. In addition, different storage

forms were adopted and storage containers were employed that reflect simple materials which are easily and locally available.

2.0 Material and methods

2.1 Location of Source of Turbid Water.

Water samples were collected from Ezu River in Amansea, located at Longitude 6° 15′ and Latitude 7° 08′, in Awka North Local Government Area, Anambra State. A section of the river obtained from Google Earth is shown below, indicating the sampling point.



Plate 1: Section of Ezu River (Source:Google Earth)

2.2 Collection of Water Sample

The raw water samples were collected near the 35-metre long bridge located at Amansea, on the Enugu-Onitsha Express Way. The water was collected from the river side each time by immersing a plastic container until it was full and cap was inserted while the container was still underwater. The water was immediately transported to the laboratory.

Water sampling was always done early in the day when the sun had not risen up. The purpose of sampling early in the day was to avoid the heat of the sun during the short period of transporting the water from the river to the laboratory. In addition to the early morning sampling period, ice-packs were used to keep the water cool while transporting it to the laboratory.

2.3 Sourcing the Seed Materials

Moringa oleifera seeds were mainly obtained from local growers located in Agulu, Anambra State. Some were obtained from *Moringa oleifera* trees planted near the fence bounding St. Patrick Catholic Church, Awka Town. A few seeds were harvested from Science village, Nnamdi Azikiwe University, Awka. Matured seeds with dried pods were freshly harvested from these locations by the researcher at the time of commencement of the laboratory work. All the seeds were thoroughly mixed together.

2.4 Identification of Seed

The identity of the seeds of *Moringa oleifera* were authenticated by a Herbarium Curator, Dr. Mrs. Aziagba of the department of Botany, Nnamdi Azikiwe University, Awka, Nigeria. A sample of the seed in the pod was retained in Cabinet Number 02, Shelf Number 29 of the Herbarium of Botany Department.

2.5 Storage of *Moringa oleifera* Seed

Matured dry seeds of *Moringa oleifera* were harvested directly from the trees. The seeds were air-dried in a laboratory while natural moisture content was determined daily until a constant value of about 6.03% was obtained. The seeds were there after stored in the following ways. Some seeds removed from the pod but retained in the wing-like shells (Winged seeds) were stored in covered baskets (Wba), some in corked glass bottles (Wbo) and some in Cellophane bags (Wcb). Some shelled seeds (exposing the inner white kernel) were stored in covered baskets (Sba) and some in corked glass bottles (Sbo). Seeds not removed from the Pods were stored in baskets (Pba). A total of six storage variances were used.

2.6 Proximate Analysis

Proximate analyses of the seeds from the various storage modes were performed every 30days. Proximate parameters determined were moisture, fibre, lipid, ash, protein and carbohydrate contents.

2.7 Extraction and Isolation of Coagulants:

2.7.1 *Defatting of Moringa oleifera seed*

Good quality seeds of *Moringaoleifera*, as confirmed by the Curator, Botany Department, Nnamdi Azikiwe University, Awka, were selected. The seed kernels were ground to fine powder, using an ordinary food processor. *Moringa oleifera* seed is reported to contain between 30% and 35% (w/w) of vegetable oil known as Behen (or) Ben oil (Muthuraman and Sasikala, 2014; Ali *et al*, 2010). This oil was extracted from the ground seeds, using a modified form of the method adopted by Nwaiwu *et al* (2012b). The procedure involved Soxhlet extraction with n-hexane. The extracted oil was recovered by letting off the hexane through mild heating of the flat bottom flask. The resulting cake after extracting the oil was spread on a flat disc and air-dried for 24hours in order to remove all residual n-hexane.

2.7.2 *Aqueous extraction of coagulant active ingredients.*

The method of Nwaiwu *et al* (2012b) was adopted. The debris of *Moringa oleifera* seedremaining after oil extraction was blended with water for about 2-3 minutes, using a domestic food blender. The sample was filtered through a muslin cloth. The filtrate was the crude aqueous extract.

2.7.3 *Isolation of protein*

The crude extract was further processed to obtain the relatively pure protein and also reduce it to a dry-powdered form for easy transportation to different laboratories for different aspects of the work without the protein content of the extract being denatured. Cold Acetone precipitation was adopted. The precipitated protein can be stored for a long period of time (Garcia-Fayos, *et al*, 2015).

2.8 Physicochemical Analysis

Physicochemical analyses were carried out before and after treatment of the raw water samples. These were temperature, pH, turbidity; total solids, total suspended solids (TSS); total dissolved solids (TDS), total hardness, phosphates and conductivity. The tests were carried out in accordance with Standard Methods for Examination of Water and Wastewater (Greenberg, 1992).

2.9 Determination of Coagulant Dosage:

Coagulant dosage was determined using the Jar test. The standard jar test procedure included rapid mixing for 1minute at 120rpm, agitation (slow mixing) for 30 minutes at 30rpm and 30 minutes settling time (Judith*et al*, 2014). In this work, however, the settling time was extended to 60minutes to allow for more coagulants contact time. Magnetic stirrers were used to effect the rapid and slow mixing.

Three different dosages,30 mg/l, 40 mg/l and 50 mg/l, of each protein precipitate were added to three of the beakers respectively, while one beaker was left to stand blank as the control. At the end of the settling period, clarified samples were collected from the supernatant in each beaker for determination of all the parameters previously determined before treatment of the water

3.0 RESULTS AND DISCUSSIONS

3.1 Proximate Analysis

(a) Moisture Content

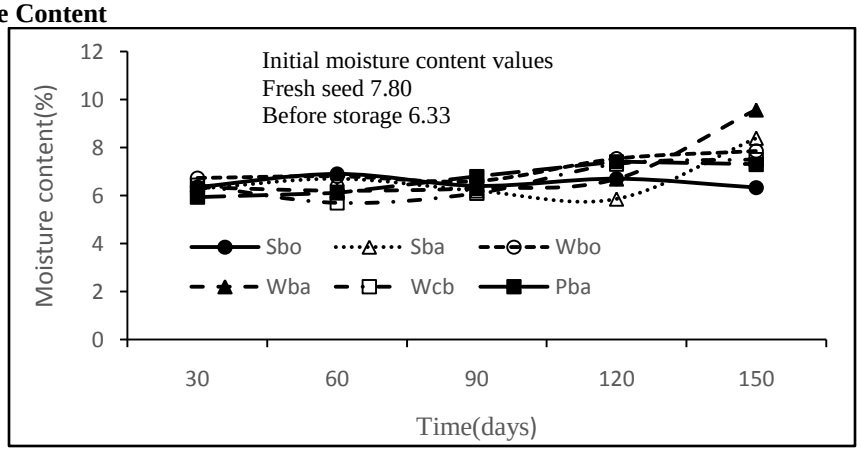


Figure 1. Moisture content against storage time

Sbo = Shelled seed stored in bottle
Sba = Shelled seed stored in basket
Wbo = Winged seed stored in bottle
Wba = Winged seed stored in basket
Wcb = Winged seed stored in cellophane bag
Pba = Pod seed stored in basket

Moisture content of the seeds did not show any specific trend throughout the period of storage. It did not also show any correlation with the coagulation efficiency of the seed.

(b) Lipid Content

Figure 2 shows monthly results of Lipid contents determination. The result shows that the percentage of lipid throughout the period of storage and in all the modes and containers of storage remained at a value between 31.24 % and 34.08%. This range of lipid content observed corresponds with the range of 30% to 35% reported in literature (Muthuraman and Sasikala, 2014; Ali *et al*, 2010). There was no observed specific trend of increase or decrease in lipid content as storage duration increased and no evidence of a link between lipid content and coagulation activity of the seed.

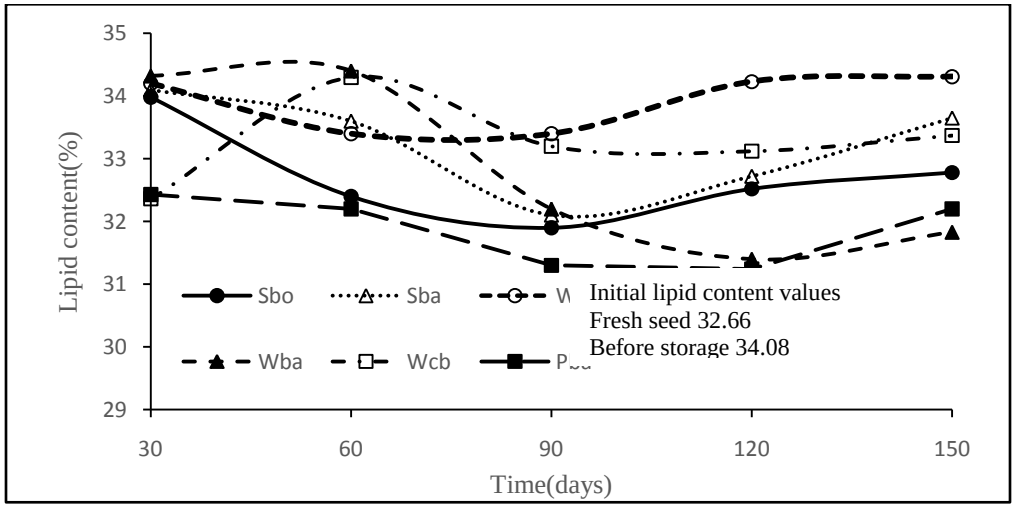


Figure 2. Lipid content against storage time

(c) Fibre Content

Results of fibre content determination are presented in Figure 3. The fibre content in the fresh seed and just before storage commenced was 2.2% and 2.34% respectively.The values of fibre content increased in all the modes and containers of storage to an average of approximately 3% after 30days of storage. They were below 3% after 60days and 90days. The values reduced further to less than 2% after 120 and 150days of storage. In general, fibre content decreased as storage duration increased. It did not show any trend that could be linked with the coagulation efficiency of the seed.

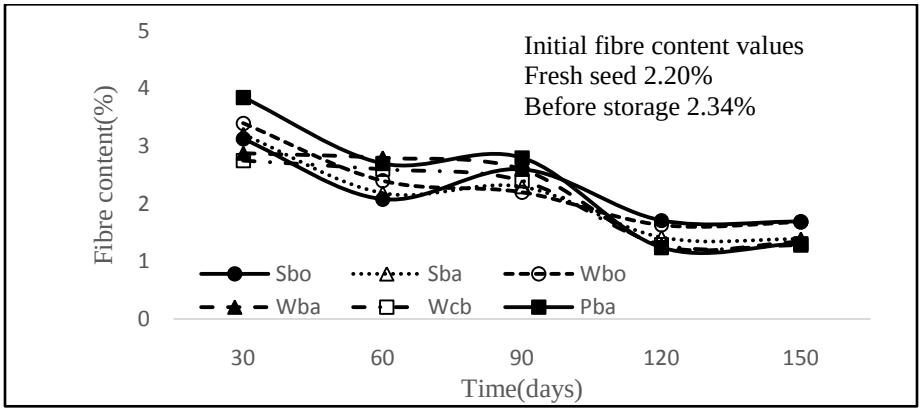


Figure 3. Fibre content against storage time

(d) Ash Content

Results of ash content determination are presented in figure 4. The Ash content was highest in the freshly harvested seed (4.69%).Ash content reduced from 4.69% for the freshly harvested seed to 3.04% after drying before separation into the various modes of storage. A range of 3% to 4.27% was observed after 30daysof storage. After 60days of storage and through the remaining storage period, the values fluctuated between 2.52% and 4.22%. Ash content did not show any trend that could correlate with coagulation efficiency of the seed.

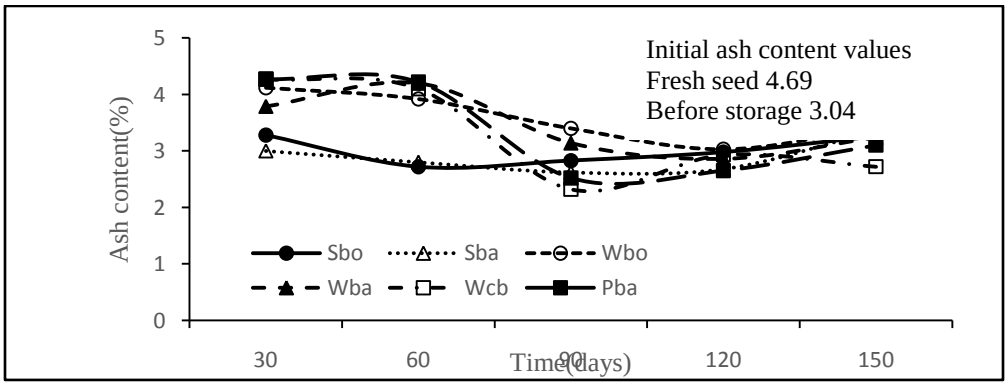


Figure 4. Ash content against storage time

(e) Carbohydrate Content

Results of carbohydrate contents are presented in Figure 5. The carbohydrate content steadily increased in all the modes and containers of storage as the storage period increased. After 120 and 150 days of storage, carbohydrate content was above 40% in all the modes and containers of storage. Carbohydrate content rose above 50% for shelled

seed stored in basket after120days and also for winged seed stored in cellophane bag after 150days of storage. It did not show correlation with the coagulation efficiency of the seed.

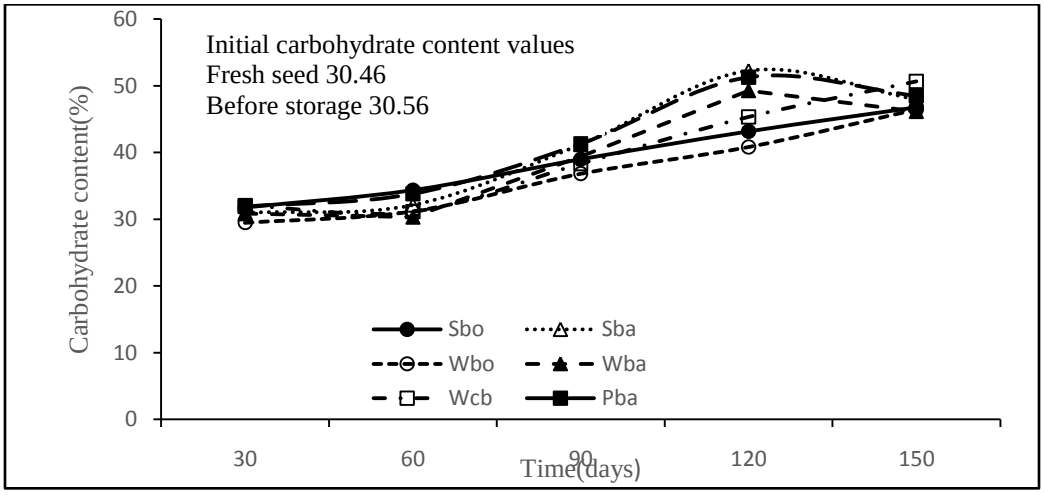


Figure 5. Carbohydrate content against storage time

(f) Protein Content

Proximate analysis of the seeds showed that seed protein content, which is the main coagulating component of the seeds, was significantly decreasing in all the storage forms and conditions as storage duration increased. Results of protein content are presented in Figure 6. It was observed that the protein content declined in all the modes of storage as the storage time increased. Result of analysis of variance test on the percentage protein content showed that there were significant differences between the monthly values of protein content (P-value = 2.9E-13< 0.05 and $F_{observed}$ = 108.5332 >$F_{critical}$=2.866081) as storage period increased. The result also showed that there is no significant difference between the percentage protein contents of the different storage modes (P-value = 0.109244 > 0.05 and $F_{observed}$ = 2.089157 <$F_{critical}$ = 2.71089). It shows that percentage protein content in *Moringa oleifera* seed is affected by storage duration but is not affected by the mode of storage.

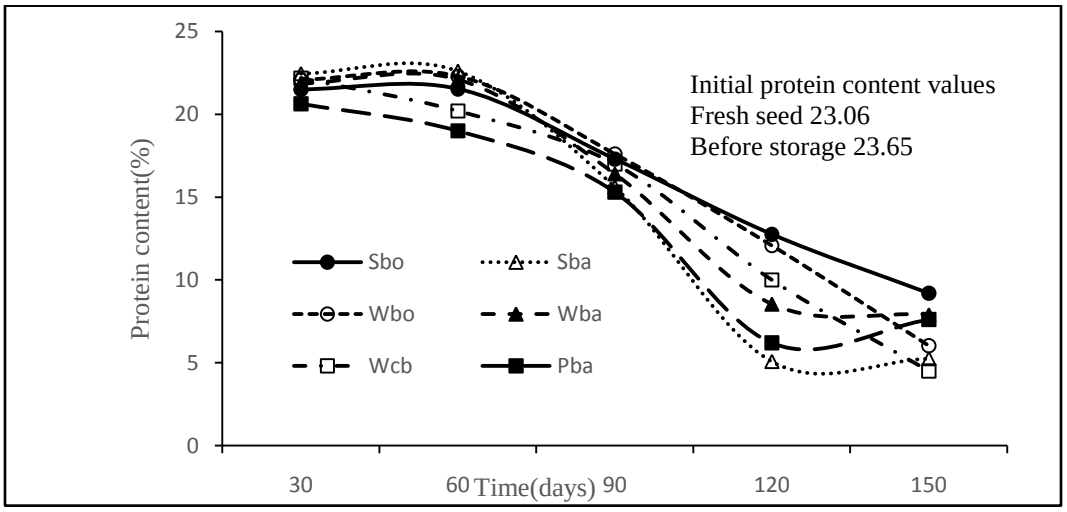


Figure 6. Protein content against storage time

(g) Protein yield

The effect of the decrease in protein content with storage time was a decreasing yield of the isolated protein in gram per gram of the seed as storage time increased. This is shown in Figure 7. Two-way analysis of variance result shows that there were significant differences with time in the values of protein yield (P-value = 2.92E-13<0.05 and $F_{observed}$ = 108.4575 >$F_{critical}$= 2.866081). The result also showed that the protein yield in the different storage modes were not significantly different (P-value = 0.329601 > 0.05 and $F_{observed}$ = 1.235366 <$F_{critical}$ of 2.7089). This means that protein yield from *Moringa oleifera* seed is affected by storage period but is not affected by the mode of storage

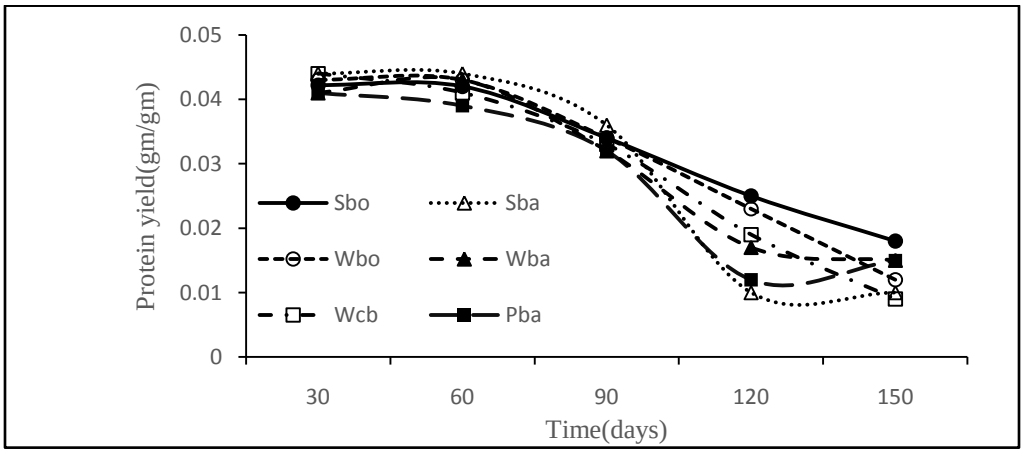


Figure 7. Protein yield versus storage time

3.2 Turbidity Removals and Dosages

Figures 8 to 13 show the turbidity removals at the various 30-day test intervals and the coagulant dosages that were applied according to the modes of storage. It was observed that percentage turbidity removals for all the modes of storage decreased with increase in storage period, although not equally in all the modes of storage and some were actually highly fluctuating in absolute values. However, the shelled seed stored in basket was consistent and more effective in turbidity removal than all the other modes of storage. This agrees with Golestnbagh *et al* (2011). Its turbidity removal fluctuated between 92 and 95percent.

Initial turbidity of the raw water sample used at the 120days of storage was very low. Contrary to previous reports in literature that *Moringa oleifera* seed extract is less efficient in turbidity removal in low turbidity water (Muyibi and Evison, 1995), the turbidity removal efficiency was not affected by the low level of initial turbidity. This was probably because this research made use of protein that was isolated from the crude extract whereas the crude extract of *Moringa oleifera* seed is commonly used in water treatment and purification (Idris *et al*, 2016).

It was observed that a dosage of 50mg/l was more prevalent in obtaining maximum percentage turbidity removals. At 120days of storage, however, when the initial turbidity of the water sample was low, the dosage of 30mg/l was prevalent.

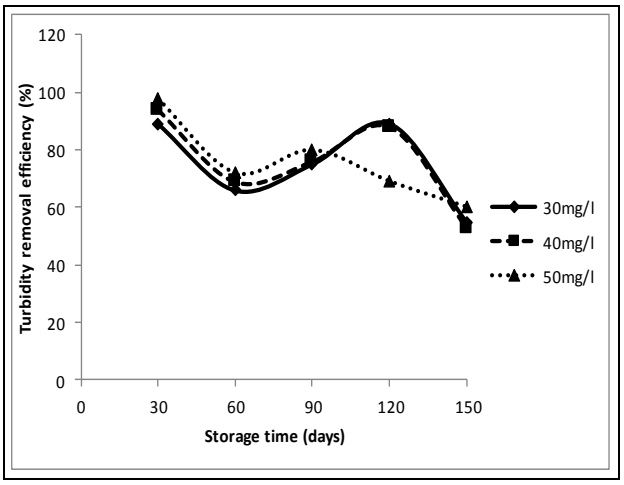


Figure8. Turbidity removal against storage time for shelled seed stored in bottle

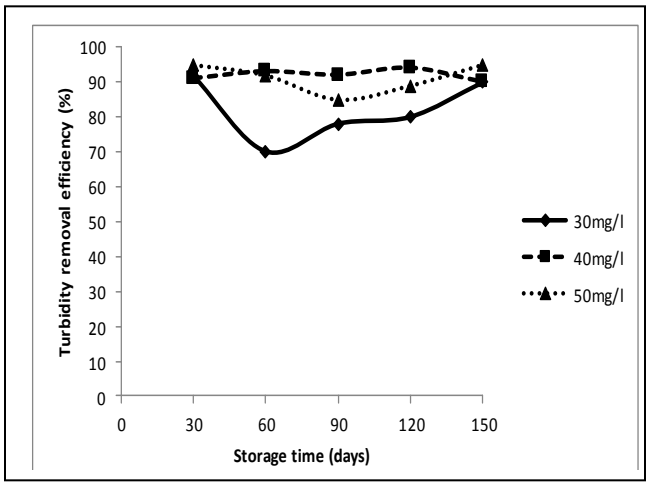


Figure 9. Turbidity removal against storage time for shelled seed stored in basket

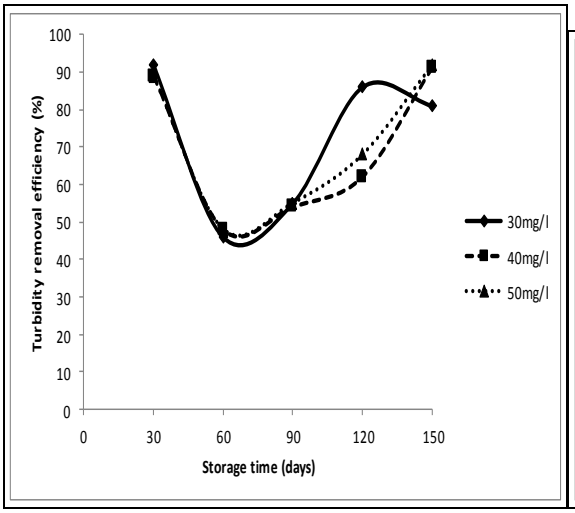


Figure 10. Turbidity removal against storage time for winged seed stored in bottle

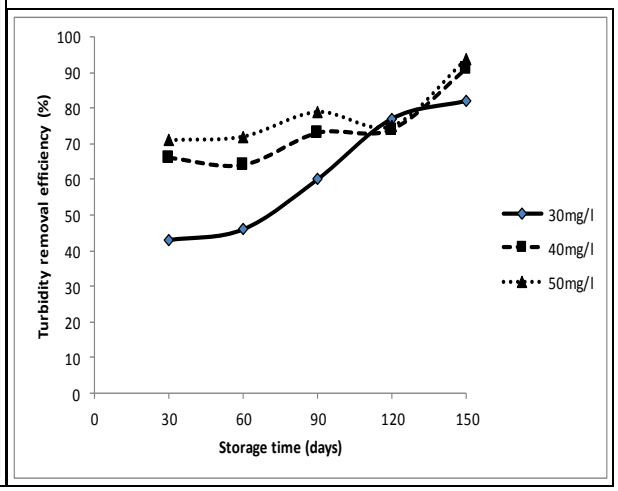


Figure 11. Turbidity removal against storage time for winged seed stored in basket

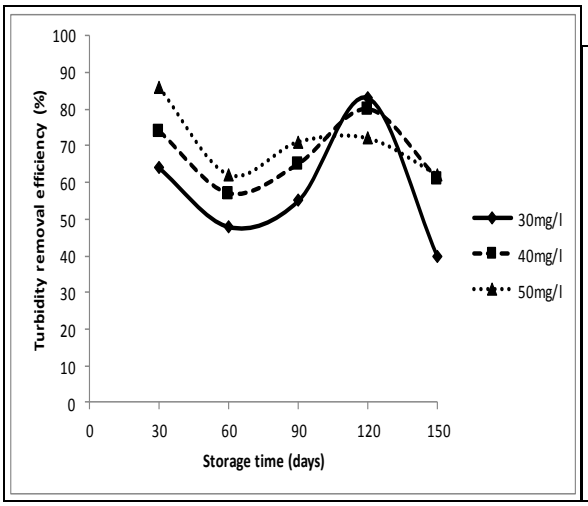


Figure 12. Turbidity removal against storage time for winged seed stored in cellophane bag

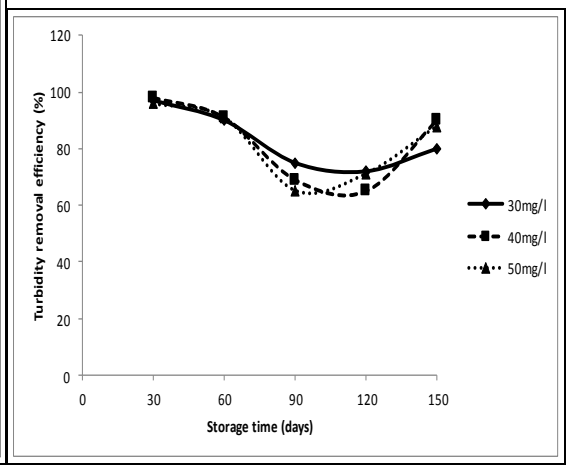


Figure 13. Turbidity removal against storage time for seed pod stored in basket

4.0 Conclusion

Water collected from Ezu river in Amansea, located at Longitude 6° 15′ and Latitude 7° 08′, in Awka North Local Government Area, Anambra State was purified using *Moringa oleifera* seed protein isolated from the stored seed using chilled acetone precipitation. Turbidity of the raw water ranged between 65NTU and 322 NTU, depending on the season the water sample was procured.

Moringa oleifera seeds were mainly obtained from local growers located in Agulu, Anambra State, some were obtained from *Moringa oleifera* trees planted near the fence bounding St. Patrick Catholic Church, Awka Town and a few seeds were harvested from Science village, Nnamdi Azikiwe University, Awka. The seeds of *Moringa oleifera* were authenticated by the Herbarium Curator, Dr. Mrs. Aziagba of the department of Botany, Nnamdi Azikiwe University, Awka.

The *Moringa oleifera* seeds were air-dried for 14days and stored at room temperature of about 30°C in different forms and different containers for a period of 150days. Winged seeds were stored in covered baskets, corked glass bottles and cellophane bags respectively. Some shelled seeds were stored in covered baskets and some in corked glass bottles. Seeds not removed from the pods were stored in baskets.

Proximate analysis of the seeds showed decreasing percentage protein content as storage duration increased. This had the effect of decreasing the protein yield of the seeds in gram per gram but did not affect the coagulation properties of the seeds. Efficiency of the shelled seed stored in bottle, winged seed in bottle and pod seed in basket decreased from 98%, 86% and 96% to 60%, 62% and 90% respectively. Shelled seed in basket and winged seed in cellophane bag started and ended with 95% and 92% respectively, while the efficiency of winged seed in basket rose from 71% to 94%. Shelled seeds stored in basket showed better consistency in coagulation property with time than all the other modes of storage.

The results showed that stored seeds could be used effectively for water purification, although higher quantities of seed will be required to obtain a given amount of coagulant. The shelled seed stored in basket was more consistent and more effective in turbidity removal than all the other modes of storage. Its turbidity removal fluctuated between 92 and 95percent.

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