

Effect of storage of shelled *Moringa oleifera* seeds from reaping time on turbidity removal

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ABSTRACT

Moringa oleifera is an indigenous plant to Malaysia whose seeds are used for water purification. Many studies on *Moringa oleifera* have shown that it is highly effective as a natural coagulant for turbidity removal. In this study, two different methods for extraction of *Moringa*'s active ingredient were investigated. Results of sodium chloride (NaCl) and distilled water extraction of *Moringa oleifera* seeds showed that salt solution extraction was more efficient than distilled water in extracting *Moringa*'s active coagulant ingredient. The optimum dosage of shelled *Moringa oleifera* seeds extracted by the NaCl solution was comparable with that of the conventional chemical coagulant alum. Moreover, the turbidity removal efficiency was investigated for shelled *Moringa oleifera* seeds before drying in the oven under different storage conditions (i.e. open and closed containers at room temperature, 27 °C) and durations (fresh, and storage for 2, 4, 6 and 8 weeks from the time the seeds were picked from the trees). Our results indicate that there are no significant differences in coagulation efficiencies and, accordingly, turbidity removals between the examined storage conditions and periods.

Key words | extraction methods, *Moringa oleifera*, natural coagulant, sources of seeds, storage, turbidity removal

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INTRODUCTION

One of the critical factors in water treatment is the reactivity of particles and accordingly the amenability of these particles to destabilization (Newcombe & Dixon 2006). In the water treatment industry, turbidity removal can be achieved by a coagulation–flocculation process, which entails use of coagulant(s), followed by sedimentation and filtration steps. In general, coagulants may be classified as inorganic, synthetic organic polymers and natural coagulants. Aluminium salts are the coagulants most commonly used in water treatment (Ndabigengesere *et al.* 1995; Okuda *et al.* 1999; Katayon *et al.* 2006). Recent research has shown that using aluminium as a coagulant has serious imperfections. Its relationship with Alzheimer's disease (Ndabigengesere *et al.* 1995; Okuda *et al.* 1999; Katayon *et al.* 2006), production of high volumes of sludge (James & O'Melia 1982), reaction with alkalinity thus decreasing the pH of the water (Degremont 1988), and low coagulating effect in cold water (Morris & Knocke 1984; Haarhoff & Cleasby 1988)

are examples of the problems concomitant with use of aluminium salts as coagulants. Furthermore, using aluminium in some developing countries corresponds to high treatment costs and low turbidity removal efficiencies (Ndabigesere & Narasiah 1998). On the other hand, ferric salts and synthetic organic coagulants show limited coagulation effects. In light of this, there is a need for identifying and/or synthesizing coagulants that are more efficient and user-friendly than those already known and employed (Kawamura 1991; Ndabigesere & Narasiah 1998; Okuda *et al.* 1999, 2001). Within this context, it is hypothesized that natural coagulants may offer a reasonable substitute.

Natural coagulants of herbaceous and mineral origins were employed for turbidity removal even before the genesis of chemical coagulants (Ndabigesere & Narasiah 1998). They can be produced by and/or extracted from animals, plants and microorganisms (Okuda *et al.* 1999, 2001). Biodegradation and human health and safety were, and will still

be, the main driving forces behind the use of natural coagulants. Many researchers have shown that the seeds of *Moringa oleifera* are an impressive potential natural coagulant (Jahn 1986; Muyibi & Evison 1995; Ndabigesere et al. 1995; Okuda et al. 1999; Katayon et al. 2006).

Moringa oleifera is a tropical plant that belongs to the Moringaceae family, which comprises 14 different species. These species grow rapidly and can survive in bad weather for long periods of time. Moreover, they display varying coagulation potentials (Morton 1991; Ndabigesere & Narasiah 1998; Folkard et al. 2001).

Moringa oleifera has been characterized as a vegetable, medicinal plant and a source of vegetable oil. Owing to this and to the fact that most of its parts are useful for a variety of applications, it is described as a miracle tree (Ndabigesere & Narasiah 1998; Ghebremichael 2004). In Malaysia, locals, in general, and the Indian Malaysians, in particular, use the slender green pods as vegetables in their diet and consume the seeds of brown pods as peanuts.

Numerous studies have reported that the seeds of *Moringa oleifera* show effective coagulating characteristics that may be taken advantage of in the water treatment processes, especially to eliminate or reduce turbidity. Corresponding research shows that *Moringa oleifera* has many advantages: (i) it is effective in water softening (Muyibi & Evison 1995); (ii) it has appreciably good, almost perfect, effects on reducing turbidity of raw water (Jahn 1986; Ndabigesere et al. 1995; Ndabigesere & Narasiah 1998; Okuda et al. 1999, 2001); (iii) it is non-toxic (Grabow et al. 1985); (iv) it improves and amends produced sludge (Ademiluyi 1988); and (v) the coagulation efficiency of *Moringa* seeds powder is independent of the different storage conditions, container types and duration of storage (Katayon et al. 2006). Extraction of *Moringa oleifera* seeds with water releases the active ingredient dimeric protein which has a molecular weight of nearly 13 kDa and an iso-electric point between 10 and 11. Generally, the use of *Moringa oleifera* seeds as the main coagulant, after either distilled water or NaCl solution extraction, to eliminate turbidity from raw waters and synthetic turbid waters indicates that these seeds offer turbidity removal efficiencies within the range of 80–98% (Muyibi & Okuofu 1995; Ndabigesere et al. 1995; Ndabigesere & Narasiah 1998; Okuda et al. 1999; Katayon et al. 2006).

Distilled water extraction of *Moringa oleifera* seeds effects appreciable extraction of the active ingredient and, as a consequence, turbidity removal efficiency, yet performance under this scenario lags far behind that effected by salt extraction. Okuda et al. (2001) reported that salt extraction of *Moringa oleifera* seeds has better coagulation activity, and hence efficiency in removal of kaolin turbidity, than distilled water extraction. The former has optimum dosages that are on the average 7.4 times lower than those of the latter. Many researchers studied the different aspects of *Moringa oleifera* seed extraction using distilled water, in general, and the effects of the water extract on turbidity removal, in particular. However, the literature is lacking investigations of the turbidity removal potential and efficiency using the salt-extracted active ingredient of *Moringa oleifera* seeds. It is proposed for this work to address this knowledge gap. The objective of the present study is therefore to investigate the effects on turbidity removal and removal efficiency of: (i) different types of *Moringa* seed (shelled and non-shelled) under different extraction methods (distilled water and NaCl extraction); and (ii) seed storage conditions of the NaCl extracts of *Moringa oleifera* seeds.

MATERIALS AND METHODS

Preparation of shelled *Moringa oleifera* seed powder

The *Moringa oleifera* seeds used in this study were obtained from Seri Serdang, Malaysia. Good quality seeds were taken from the pods and dried in the oven for 24 hours at 50 °C. Wings and shells of the dry seeds were removed and the kernels were ground and smashed into fine powder with a mortar and pestle. The non-shelled seeds were ground and smashed into fine powder shortly after seed oven-drying.

Preparation of shelled *Moringa oleifera* seed stock solution

Stock solutions of *Moringa*'s seed powders were prepared by dissolving 1,000 mg of the powder in 40 mL of extractant (distilled water and 1 mol/L NaCl solution separately) and mixing the resultant suspension using a domestic blender for 2 min at high speed in order to extract *Moringa*'s

active ingredient. The suspensions were then filtered through muslin cloth and the filtrate collected and made up to 100 mL to produce a stock solution of 10,000 mg powder/L solution. The working solutions of *Moringa* seed extracts were prepared fresh by appropriate dilutions right before each coagulation test.

Preparation of non-shelled *Moringa oleifera* seed stock solution

Depending on seed size and degree of maturity, the different seeds of *Moringa oleifera* have different kernel and shell masses. During the early stages of this work, the researchers determined the average percentage mass of shells and kernels in seeds using 100 seeds each time. Later, the seeds with known masses were used for preparation of *Moringa* stock solutions. Twenty pods of *Moringa oleifera* were used and five seeds out of each 20 were selected at random such that large, medium and small seeds could be selected. Finally, the percentages of shells and kernels of the selected seeds were determined. The results of this part of the study indicate that the average percentages of shells and kernels are almost 25.53 and 74.47%, respectively. In light of this, for us to prepare 100 mL of a 1% stock solution of non-shelled *Moringa Oleifera* seeds, for example, we need to dissolve around 1.3428 g of non-shelled *Moringa* seeds in 100 mL of solution. If, however, we dissolve 1.0 g of the non-shelled seeds in 100 mL solution, we end up with a concentration of nearly 0.74%. The subsequent steps in the preparation of stock solutions and, later, working ones, of non-shelled *Moringa* seeds are similar to those followed in preparing the shelled seed stock solutions.

Preparation of synthetic turbid water

Laboratory grade kaolin was used for preparing turbid water samples for all experiment runs. Ten grams of kaolin were added to 1 litre of distilled water. The suspension was stirred slowly at 20 rpm for 1 hour in a jar test apparatus in order to achieve uniform dispersion of the kaolin particles. The suspension was left standing for 24 hours in order to achieve complete hydration of the kaolin. This kaolin suspension served as the stock solution and was diluted using distilled water to prepare water samples of 200 NTU.

Storage of *Moringa oleifera* seeds

After picking the dry *Moringa oleifera* pods and separating the seeds from pods without any drying, seed wings and coats were immediately removed and some of the seeds were stored in closed containers while some others were stored in open ones. The closed and open containers used in this study were made of glass and had plastic caps. Storage temperature in both cases was set and maintained at room temperature (27 °C) for all experimental runs and tests. Furthermore, and in an effort to explore the effect of storage time on the efficiency of turbidity removal, seeds in both the open and closed containers were further subjected to various storage periods corresponding to 2, 4, 6 and 8 weeks post seed harvest. At the end of each storage period, seeds were dried in the oven at 50 °C for 24 hours.

Coagulation tests

The coagulation tests were conducted following the jar test method which is the method most commonly used for simulating the coagulation–flocculation process in a water treatment plant (Ndabigengesere *et al.* 1995). A six-place jar test device was employed in the coagulation and flocculation–sedimentation experiments. Six 500-mL beakers were filled with 500 mL each of synthetic turbid water. The operating variables in this study for the jar test were stirring for 4 minutes at 100 rpm (rapid mixing) and for 25 min at 40 rpm (slow mixing) followed by 30 minutes of sedimentation (Muyibi *et al.* 2002; Katayon *et al.* 2006). At the end of the sedimentation period, a 30-mL aliquot of the sample was collected from the middle of each beaker for measurement of the residual turbidity using a turbidimeter.

Experimental runs

First, optimum dosage of shelled and non-shelled *Moringa oleifera* seeds with initial turbidity of 200 NTU with different extraction methods (DW and 1 mol/L NaCl) were obtained, using jar test (JLT 6 VELP, Scientifica Europe). Different dosages of shelled and non-shelled *Moringa oleifera* were applied until the optimum dosage for the removal of 200 NTU turbidity was found. In the next step, the experimentally identified optimum dosage was used to investigate the effects

of different storage conditions on the turbidity removal efficiency.

RESULTS AND DISCUSSION

Effects of shelled and non-shelled *Moringa oleifera* seeds on turbidity removal

Removal of turbidity by shelled and non-shelled *Moringa oleifera* seeds was investigated. Figure 1 shows the efficiency of turbidity removal of shelled and non-shelled *Moringa oleifera* seeds under different extraction methods (1 mol/L NaCl solution and distilled water). Our results indicate that the turbidity removal and the optimum dosage of each kind of seed using these two extraction methods were 7.96 NTU using 140 mg/L shelled *Moringa oleifera* seeds extracted by distilled water (SMOS-DW), 11 NTU using 220 mg/L non-shelled *Moringa oleifera* seeds extracted by distilled water (NSMOS-DW), 5.9 NTU using 40 mg/L shelled *Moringa oleifera* seeds extracted by 1 mol/L NaCl solution (SMOS-NaCl), and 10.2 NTU using 50 mg/L non-shelled *Moringa oleifera* seeds extracted by 1 mol/L NaCl solution (NSMOS-NaCl). This clarifies that the amount of turbidity removed using different kinds of seed and different methods of extraction were almost the same and that the differences between any were not significant. The main difference observed in the turbidity removal efficiency between the different types of seed and method of extraction was the optimum dosage. For example, for removal of kaolin turbidity (initial turbidity 200 NTU), the shelled *Moringa*

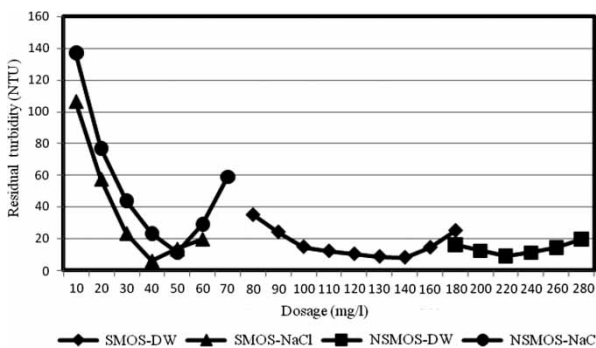


Figure 1 | Turbidity removal efficiency of shelled (SMOS) and non-shelled (NSMOS) *Moringa oleifera* seeds under different extraction methods (1 mol/L NaCl solution and distilled water).

oleifera seeds extracted by 1.0 mol/L NaCl solution showed better coagulation activity than those extracted with SMOS-DW. Dosages were 3.5 times lower for the former extracts than for the latter, and 5.5 times lower than those using NSMOS-DW. However, the optimum dosages of SMOS-NaCl and NSMOS-NaCl were almost the same. The improvement in optimum dosage of shelled and non-shelled *Moringa* seeds by NaCl solution extraction is due to the salting-in, also known as the common-ion, effect whereby protein-protein dissociations, and consequently protein solubility, increase as the solution ionic strength is increased by the added salt(s) (Okuda et al. 1999).

Figure 2 shows the effects of seed bark extracted by distilled water and NaCl solution on turbidity removal. As the figure shows, the seed bark (shell) does not exhibit any coagulation effect. The differences between dosages of shelled and non-shelled seeds may be explained by the differences in protein concentrations between the shelled and non-shelled seeds because the protein concentration in non-shelled seeds is less for the same initial seed mass than that in the shelled ones (Ndabigengesere & Narasiah 1998).

According to Muyibi & Evison (1995), overdosing results in saturation of the polymer bridge sites and leads to re-stabilization of the destabilized particles due to lack of an adequate number of particles to create more inter-particle bridges. The purpose of employing NSMOS as a coagulant in this study was to explore the possibility of saving time, effort and money through use of non-shelled seeds without compromising the turbidity removal efficiency. For use of *Moringa* seeds as coagulant in water treatment plants, shell removal is a critical step for the success of turbidity removal. Otherwise, water treatment will be somewhat awkward and a substantial waste of treatment time is anticipated. Our results show that the optimum dosages of NSMOS-NaCl were 2.8 times lower

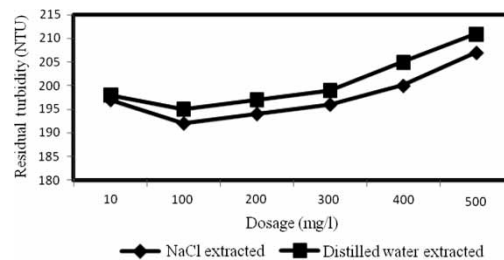


Figure 2 | Effects of seed bark extracted by distilled water and NaCl solution on turbidity removal.

than those of SMOS-DW and 4.4 times lower than those using NSMOS-DW. The optimum dosage of the *Moringa* seeds extracted by NaCl solution in 200 NTU suspensions, which was identified earlier to be 40 mg/L, was then held constant throughout for studying the optimum seed storage conditions.

Influence of seed storage on the coagulation efficiency

After reaping the pods and removing the seeds from them under ambient temperature, the seed wings and barks were removed from around the seeds. Subsequently, some of these seeds were stored in closed containers and some in open ones. Storage duration was based on picking time in successive two-week time intervals, i.e., 2, 4, 6 and 8 weeks after picking from the trees and before oven-drying of the seeds. During the storage periods, no changes were observed on the physical appearance of the seeds, neither in the open, nor in the closed containers. Figure 3 presents the moisture content of *Moringa oleifera* seeds that were stored at room temperature in the open and closed containers before oven-drying. Generally speaking, the seed moisture contents changed rather little during the storage periods and both in the open and closed containers their values fell within the range of 1.1 to 3.1%. According to the *Agronomy Guide (2005–2006)*, seeds exposed to air gain or lose water depending on the relative humidity of their immediate surroundings. Nonetheless, this study did not investigate the effect of moisture content on turbidity removal because all seeds were oven-dried by the end of the storage period prior to any subsequent use in the coagulation tests.

Results of the effect of seed storage on the residual turbidity of water are presented in Figure 4. The efficiency of

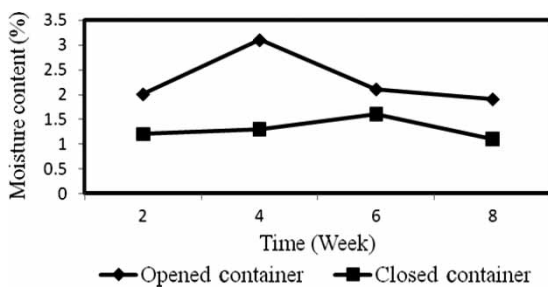


Figure 3 | Moisture content of *Moringa* seeds stored at room temperature in open and closed containers before oven-drying as a function of duration of storage.

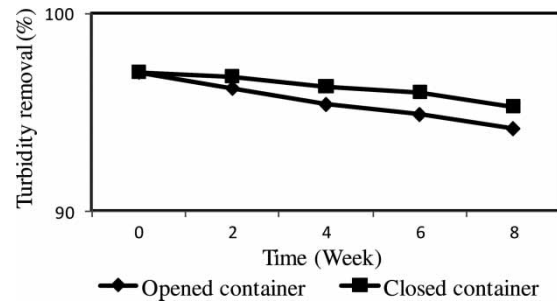


Figure 4 | Effect of seed storage on the residual turbidity of water.

Moringa oleifera seeds stored in open and closed containers did not significantly change over the storage periods from the fresh seeds (zero storage) through two months of storage. The percentage of turbidity removal remained in the range of 97% to 94.2%. *Katayon et al. (2006)* studied the effect of *Moringa oleifera* seed powder storage and reported that the coagulation efficiency of *Moringa oleifera* seeds is independent of storage in different conditions and for different durations.

CONCLUSION

Shelled and non-shelled *Moringa oleifera* seeds, both those extracted by NaCl solution and those extracted with distilled water, proved to be effective in coagulation. Turbidity removal efficiencies using different kinds of seed and different methods of extraction were comparable. The observed differences were significant. The main difference between the different examined combinations of seed types, extraction methods, and storage conditions and durations was the optimum dosages. The optimum dosages of shelled and non-shelled seeds extracted by salt solutions were far less than those of seeds extracted using distilled water. Over and above this, our results showed that the optimum dosage of non-shelled *Moringa oleifera* seeds extracted by NaCl solution is almost three times lower than that of shelled seeds extracted by distilled water. In light of this, we suggest direct (i.e. without shell removal) use of *Moringa oleifera* seeds as coagulants.

In regard to storage conditions, we found that storage for up to two months from reaping time has no significant effects on the coagulation efficiency of *Moringa oleifera* seeds.

Our findings bear comparison with the findings of comparable earlier research on the potential use of *Moringa* as a natural, particularly appealing alternative to synthetic coagulants in common use in the water and wastewater treatment plants since it is an environmentally friendly coagulant that is readily biodegradable in the aqueous environment and offers remarkable turbidity removal efficiencies.

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