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Use of *Moringa oleifera* in drinking water treatment: study of storage conditions and performance of the coagulant extract

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ABSTRACT

Access to safe drinking water is one of the main human rights. However, today, more than 700 million people live without access to safe drinking water, especially in rural areas. Simple, effective, and low-cost technical solutions are being sought to resolve this situation. *Moringa oleifera* is an alternative, since their seeds contain a natural coagulant able to effectively reduce the turbidity of the raw water. Use of the seed as coagulant in water treatment is often performed as an aqueous extract but requires daily preparation in order to keep its clarification properties. This work studies the storage of *M. oleifera* extract so it can be used for water treatment without reducing its turbidity removal capacity. *M. oleifera* extract has been stored at different temperatures (25, −4, and −18°C), and it has been stored as liquid (crude extract) or as solid precipitate for 78 weeks. The turbidity removal capacity of extracts was studied by Jar test in synthetic turbid water of 100 NTU at 1, 3, 9, 14, 27, 40, and 78 weeks. It has been found that coagulant protein present in extract can be stored at room temperature as a solid precipitated by more than 78 weeks maintaining its maximum turbidity removal capacity comparable to fresh crude extract. Liquid crude extract storage is recommended at −18°C in order to maintain turbidity removal capacity. The results obtained are very useful and would allow extending the application of the Moringa extract, its storage and it would prevent from daily fresh preparation.

Keywords: Moringa oleifera; Coagulation activity; Storage conditions; Temperature

1. Introduction

Despite the great proliferation of existing studies with respect to the implementation of the seed of *Moringa oleifera* (MO) as coagulant for drinking water treatment [1,2], just some authors specify the storage conditions of extracts or seeds and their influence on the turbidity removal capacity.

Conservation of the extracts is important not only to work with the MO extract at research level, but also when considering its possible application in continuous water treatment at small-scale or point of use level, since it would define the functionality of the system.

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Most research works recommend the preparation of extracts immediately to use, taking as a basis the implementation of the traditional system in which usually fresh extracts are prepared daily, although without making any study on the conditions of conservation [3]. Other authors, however, recommend the daily addition of preservatives to extracts such as concentrated hydrochloric acid in order to prevent possible deterioration [4].

With regard to the storage conditions of the extracts, few studies are carried out. Jahn studied, in 1988 [1], the conditions of storage of extracts obtained from *M. oleifera* seed. The main conclusion of his study was the influence of the storage temperature for the maintenance of the turbidity removal capacity of extracts. In addition, he recommended the daily preparation of extracts in areas with warmer climates, and the storage time not exceeding 3 d in sealed containers when the room temperature does not exceed 18–20˚C. To maintain the turbidity removal capacity of extracts, Jahn suggests their conservation under refrigeration at 4˚C up to a week without loss of turbidity removal capacity.

In 2005, Dörries [5] observed while working with Moringa extracts the apparent instability of them due to its immediate turbidity or the accumulation of a white precipitate, after a prolonged storage.

This observation led him to carry out a study in order to determine the storage conditions in a refrigerator (4˚C) for three weeks. The study allowed to conclude that, over three weeks of storage, the total concentration of proteins in the refrigerated aqueous extracts increased with storage time. In a first conclusion, this result was associated with the growth of bacteria in the extract and an increase in its microbiological content. However, sterile filtered extracts showed the same results. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) study revealed that, regarding to fresh extract, MO extracts stored for more than 6 d refrigerated at 4˚C, showed no coagulant protein of molecular weight close to 6.5 kDa. This observation may be related to extract degradation as a result of the conservation conditions. As a recommendation, the preparation of fresh extracts was established in each application in order to avoid its degradation, coinciding with statements done by other authors previously.

Katayon et al. [6], performed a study to determine the storage conditions of *M. oleifera* seeds and their influence on turbidity removal capacity. Influence of the container (open or closed), storage temperature (refrigerated (4˚C) or room temperature (28˚C)), and time (1, 3, and 5 months), were analyzed in order to determine their influence over turbidity removal capacity of the MO extracts prepared using stored seeds. The best performance results in terms of reduced residual turbidity were obtained with extracts prepared from Moringa seeds stored up to 1 month after collection, regardless of storage temperature and the container used.

Recently, other methods to preserve coagulant activity of *M. oleifera* extract have been studied as spray drying [7] or freeze drying [8]. Megat Mohd Noor et al. [7] studied different process conditions to obtain spray-dried MO salt extract and the influence of packaging (closed or vacuum packed) and storage conditions (room temperature and refrigerated) in a 6 weeks test. It was concluded that spray-dried extract kept coagulation activity, requiring for the optimal half dose of the nonspray salt extract. Packaging conditions were not significant when extract was stored under the same temperature, while temperature storage shown that spray-dried extract showed better coagulant activity rather than refrigerated extract. Mohamed et al. [8] studied the yield of freeze drying and coagulation efficiency of different freeze-dried extracts. Potassium chloride and potassium nitrate freeze-dried extracts increased its coagulant activity and decreased the optimum dosages required compared to nonfreeze-dried extracts. Influence of storage conditions for these extracts was not analyzed in this study.

Therefore, it seems necessary to perform a study of the storage conditions in a short and medium term in order to determine those that maintain the turbidity removal capacity of the extract. This paper analyzes for the first time the influence of the extract state, its protein concentration and storage temperature on the efficiency of Moringa coagulant. The main conclusions allow to obtain useful information for the potential application of this coagulant in drinking water systems.

2. Materials and methods

2.1. Moringa crude extract preparation

2.1.1. Liquid extract

Crude liquid extract (LE) is obtained by preparing a solution 5% w/v, using *M. oleifera* seed from Ressano Garcia (Mozambique) in surface river water. Seeds and extract preparation details are described in the methodology of previous work [9].

2.1.2. Solid precipitate extract

The solid extract is obtained by adding ethanol (PRS Absolute ethanol, Panreac) as solvent to LE from

previous section in the ratio 1:4. The mixture is left in the freezer at −18˚C overnight, and subsequently centrifuged (20 MIKRO, Hethich) at 10,000 rpm for 10 min. The supernatant is discarded, and the pellet obtained is called solid precipitate extract (PE).

The liquid and the PEs thus obtained are kept under the conditions specified below to perform the stability study.

2.2. Stability study

The study consisted of conservation at three different temperatures of the LE and PE and the monitoring of their turbidity removal capacity in comparison with the fresh extract. Storage temperatures tested were as follows: room temperature (RT = 20˚C), fridge preservation (FT = 4˚C), and freezer storage (FZT = −18˚C). The extracts were analyzed after being kept in these conditions for 1, 3, 9, 14, 27, 40, and 78 weeks. Three core samples were kept for their analysis at each week test.

Analysis of the extract stability is based on the determination of protein concentration by Bradford [10] and its turbidity removal capacity measurement based on turbidity rate reduction of a synthetic turbid water of 100 NTU. In activity test, the LE is added directly, and the solid extract is resuspended in surface river water up to the original volume prior to precipitation. Control or blank, which is the synthetic turbid water without addition of coagulant under Jar test conditions, is included in each experimental test.

The activity test consists of adding increasing amounts of extract to a fixed volume of synthetic turbid water (1 L), applying Jar test conditions [9] for agitation and sedimentation. After sedimentation, the residual turbidity of the supernatant is determined for each dose of extract added. Once the entire turbidity removal curve is obtained, the volume of extract that allows to obtain minimal residual turbidity (or equivalently the maximum turbidity removal capacity) is determined.

The volume of MO extract added is used to calculate the mass of coagulant protein added, using the measured protein concentration data, as shown in Eq. (1):

$$\text{Protein mass added (mg)} = \text{Protein concentration}\left(\frac{\text{mg}}{\text{mL}}\right) \times \text{Extract volume added (mL)} \quad (1)$$

Turbidity removal capacity is understood as turbidity reduction when a coagulant is added to a turbid water (in this case with an initial value of 100 NTU). This rate is calculated following Eq. (2), using the measurements of initial and final water turbidity after being treated with coagulant extract:

$$\text{Turbidity removal capacity } (\%) = \frac{\text{NTU}_{\text{initial}} - \text{NTU}_{\text{final}}}{\text{NTU}_{\text{initial}}} \times 100 \quad (2)$$

The values of turbidity removal capacity and the volume of MO extract added configure the coagulant curve. From this curve, it is selected the maximum which indicates the dose of protein that allows to obtain the highest turbidity removal capacity for the coagulant stored under each specific conditions.

2.3. Electrophoresis

Samples of both extract were also analyzed by SDS-PAGE using an 18% gel following the protocol of Laemmli [11]. Coomassie blue dye (Sigma–Aldrich) was used to visualize the protein bands. The molecular marker used was PageRulerTM (Fermentas).

3. Results and analysis

3.1. Extracts turbidity removal capacity

First, there are shown the main results obtained for turbidity removal capacity of LE.

Fig. 1 plots the maximum turbidity reduction capacity, obtained with a certain dosage of protein added for each extract (shown in Fig. 3).

As it has been indicated in previous section, all activity tests included a control or blank, made up of synthetic turbid water under Jar test conditions but with no coagulant added. Fig. 1 does not include values for turbidity removal capacity for the control

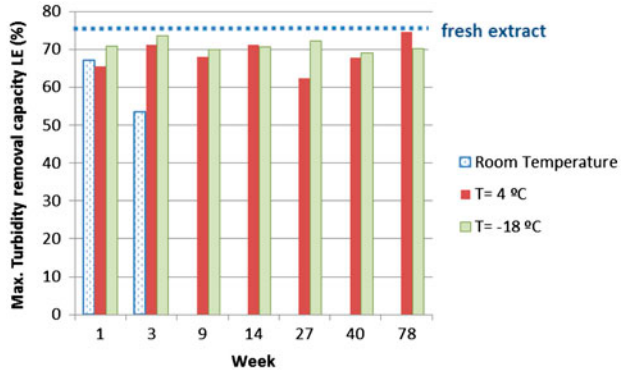

Fig. 1. LE maximum turbidity removal capacity over 78 weeks of stability test.

because is a very low value (3.49 ± 0.65% of turbidity reduction rate) due to minimum sedimentation of turbid water without coagulation. This value remains constant in all experiments because synthetic turbid water of 100 NTU is used.

Fig. 1 shows as a dotted line which represents the maximum turbidity removal capacity of 75 ± 0.06% for fresh extract. As it can be seen in the figure, maximum turbidity removal capacity for LE preserved at room temperature decreases compared to the fresh extract from the first week of testing. From week 3, the extract is degraded (bad smell and microbiological growth was observed) and no turbidity removal capacity is shown for all the coagulant dosages tested (up to 10 times those tested in weeks 1 and 3), so it is not shown in the figure after the third week.

Furthermore, the LEs preserved below room temperature maintain their maximum turbidity removal capacity, in values higher than 65% for those preserved at 4˚C, and over 70% for those stored at −18˚C, during the 78 weeks of test. Coagulant dosages need to reach these maximums will be discussed in Section 3.2. This last week, maximum turbidity removal capacity reaches values of 74.29% (4˚C) and 70.28% (−18˚C). These values are only between 0.82 and 4.71% lower than those for the fresh extract.

Fig. 2 shows the results of turbidity removal capacity for PE in the stability test.

Fig. 2 shows that for pellet resuspended extract (PE) maximum turbidity removal capacity is maintained at values above 60% for extracts stored at room temperature and above 65% for extracts stored at 4 and −18˚C from the first to the 78-week test. Coagulant dosages needed to reach these maximums will be discussed in Section 3.2. Values of turbidity removal capacity at week 78 are 69.58, 67.32, and 69.18% for room conservation temperature, 4 and −18˚C, respectively. These values represent percentages of 5.42 and 7.68% lower than those observed for fresh extract.

Therefore, it is observed that the LE maintains its maximum turbidity removal capacity refrigerated at 4˚C or frozen at −18˚C. If the extract is stored as precipitate (PE), it can be stored also at room temperature up to 78 weeks without reducing significantly its maximum turbidity removal capacity. Even though the ability of the extracts to reach maximum values of turbidity removal are maintained when extracts are stored in that way, effectiveness of the extracts (understood as a dosage of coagulant protein needed to reach these maximums) is reduced as will be discussed in next section.

3.2. Dosage of coagulant protein

Fig. 3 shows the dose of protein added for maximum turbidity removal capacity for each of extract tested. It also includes (in dashed line) the dose of protein of fresh extract that is necessary to add for maximum turbidity removal capacity (75 micrograms ±5.55%).

For LE, Fig. 3 shows that since the first week, the dose of protein that must be added to achieve the maximum percentage of turbidity removal increases. This means that the stored LE preserves its turbidity removal capacity while its effectiveness in terms of protein dosage added is reduced. In the case of storage at room temperature, Fig. 1 showed that from week 3 extract has not turbidity removal capacity independently of protein dosage added, so this series has been not plotted from that week either in Fig. 3. For extracts stored at 4 and −18˚C, protein coagulant dose required to reach the maximum turbidity removal capacity is doubled at week 14 for the extract

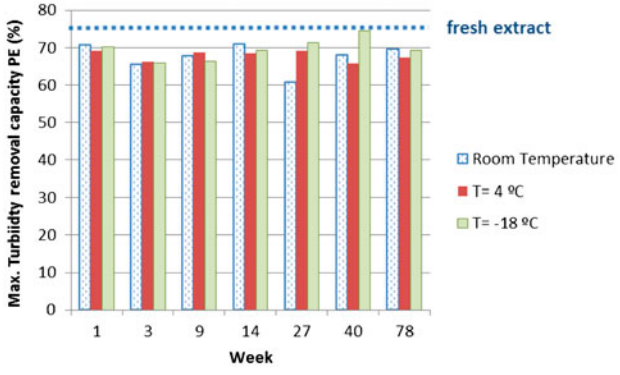

Fig. 2. PE maximum turbidity removal capacity over 78 weeks of stability test.

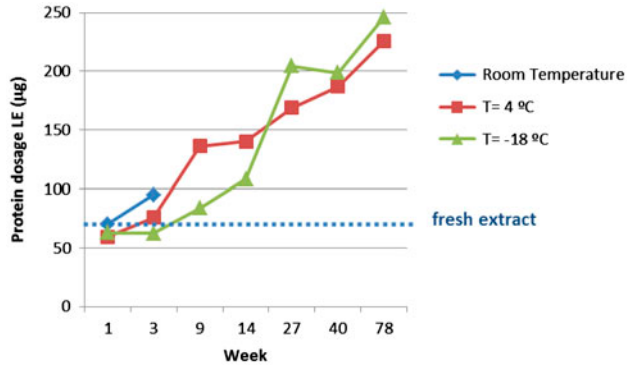

Fig. 3. Protein dosage added to maximum turbidity removal capacity of LE.

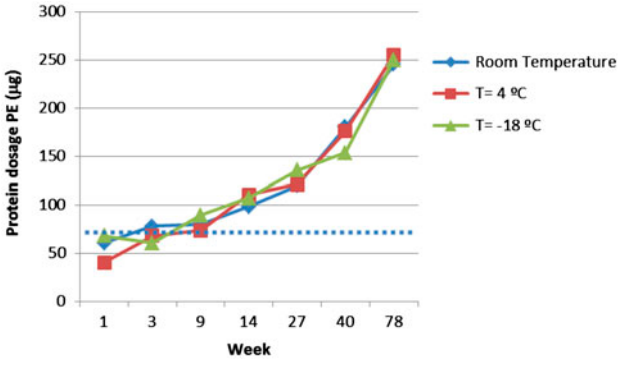

Fig. 4. Protein dosage added to maximum turbidity removal capacity of PE.

preserved at 4 °C, and between weeks 14 and 27 for the extract preserved at −18 °C. Finally, at week 78, it is necessary to add protein dose 3.3 times higher than the value for fresh LE to achieve the same turbidity reduction rate.

Fig. 4 shows the results for the re-suspended pellet extract (PE).

In this case, it is observed that for all three tested PE extracts (after storage at room temperature, 4 and −18 °C), the dose of protein added for maximum turbidity removal capacity is the same as for fresh crude extract through week 9 test. After that week, protein dosages added increase in the same rate for the three samples (room temperature, 4 and −18 °C), until needing to add more than 3 times of protein to reach similar maximum turbidity reduction rates. This means that after that week no difference between the three extracts is observed in terms of effectiveness.

Finally, Fig. 5 compares protein dosage of the extracts needed for maximum coagulant activity for LE and PE at different storage conditions.

It is noted that the results regarding to the dose of added protein of the extracts stored at 4 and −18 °C are similar to fresh extract (dashed line), during 3 weeks for conservation at 4 °C for LE and during 9 weeks for conservation at −18 °C.

In the case of room temperature, a significant improvement can be appreciated with the PE extract since effectiveness (in terms of protein added) is similar to fresh extract for 9 weeks storage, while the LE showed no coagulant activity after 3 weeks. This implies that the PE maintains its efficiency (because it is no necessary to add more protein dosage to reach similar values of maximum turbidity reduction rates) for a period of time three times higher than LE, even it is stored at room temperature or at 4 °C.

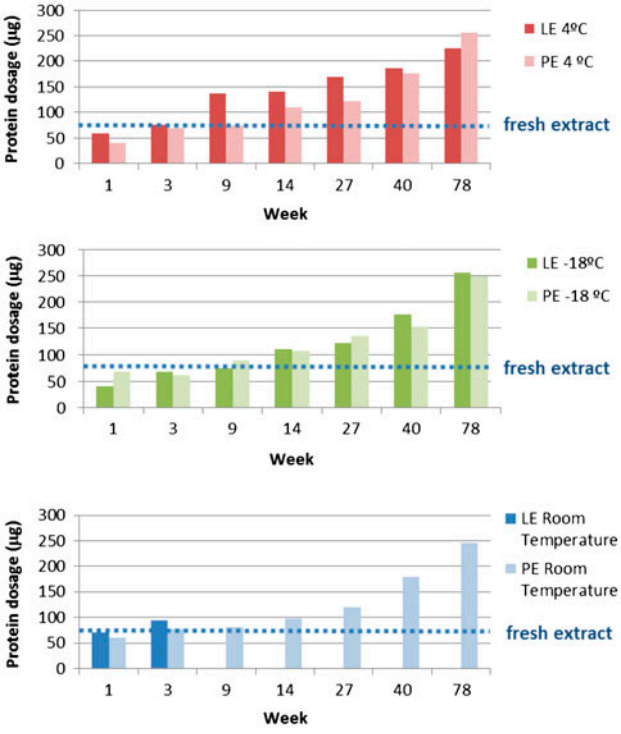

Fig. 5. Comparison between LE and PE stored at 4, −18 °C, and room temperature.

3.3. Analysis of protein present in extracts

Fig. 6 shows the electrophoresis obtained for each week of the test. It is included the protein marker, and the LE extracts stored at room temperature (RT), refrigerated (FT) and freezed (FZT) for each week.

It can be observed that in week 1 extracts show the three bands present at the same height, corresponding to a major protein band around 6.5 kDa as observed for other authors [12]. In week 3, the bands corresponding to 6.5 kDa protein coagulant remain for extracts stored at 4 and −18 °C. However, to extract sample stored at room temperature is less significant and new bands emerge between 15 and 25 kDa as it begins to degrade.

By week 9, the degradation of the sample at room temperature is much more noticeable than in previous weeks of analysis, and consequently more bands between 35 and 170 kDa appear. The appearance of these bands matches with the absence of activity of the extract in turbidity removal capacity test. Moreover, the FT and FZT extracts maintain the same bands corresponding to 6.5 kDa.

For week 14, sample at room temperature practically has disappeared and it has been divided into multiple bands, because the sample was in an advanced state of degradation. This causes a negative

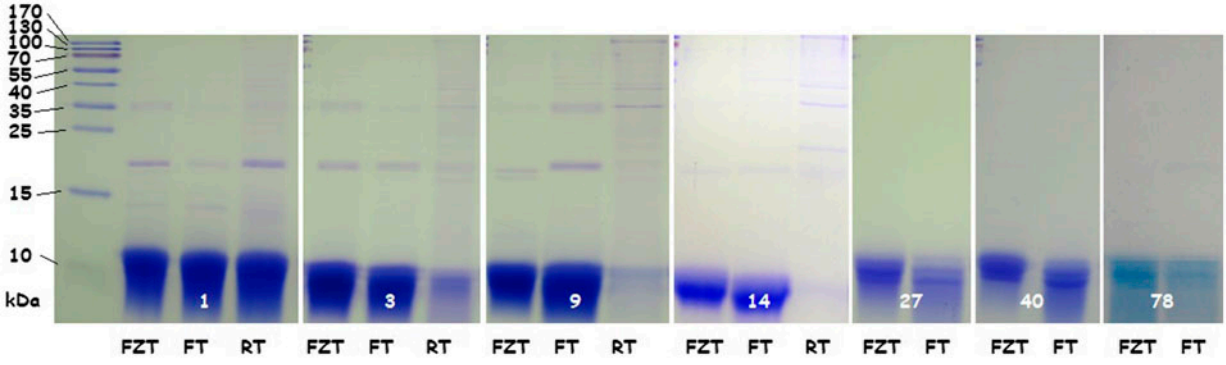

Fig. 6. Electrophoresis of LE for each week test (from left to right: week 1, 3, 9, 14, 27, 40, and 78).

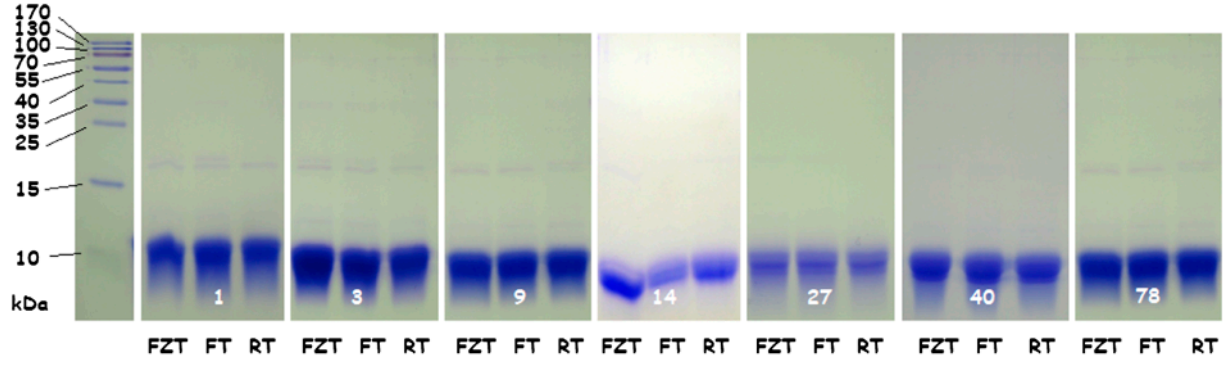

Fig. 7. Electrophoresis of PE for each week test (from left to right: week 1, 3, 9, 14, 27, 40, and 78).

effect on nearby loaded sample, which generates that FT and FZT samples do not show the same definition, although the 6.5 kDa band still can be seen. From week 14, the crude extract stored at room temperature is not included in the gel does not affect the remaining samples. Therefore, in the gel are represented the FT and FZT samples of LE.

Furthermore, in weeks 27, 40, and 78, the results are similar. The major protein band around 6.5 kDa is seen, implying the presence of *M. oleifera* protein.

Fig. 7 shows the electrophoresis obtained for each week of the test. It is included the protein marker, and then, PE extracts stored at room temperature (RT), refrigerated (FT) and freezer (FZT) for each week.

In electrophoresis gels, no difference is observed among the loaded samples over time of test, yielding the same characteristic major protein band corresponding to 6.5 kDa for the samples stored at room temperature, 4 and −18˚C. This fact is consistent with the observed turbidity removal capacity for extracts, where the maximum turbidity removal capacity is maintained during 78 weeks test, regardless of storage temperature tested.

4. Conclusions

In view of these results, we can conclude the following:

(1) Room temperature LE loses turbidity removal capacity from week 1 of storage, but when it is stored at 4 or −18˚C LE remains values of turbidity removal capacity comparable to those of the fresh extract values for 78 weeks of test.
(2) PE turbidity removal capacity is maintained at room temperature, cooled at 4˚C and at −18˚C for 78 weeks. Activity values are slightly below the ones of the fresh crude extract.
(3) LE protein dose required for maximum turbidity removal capacity increases with increasing storage time, independently of the temperature of storage since the first week of monitoring.
(4) PE dose required for maximum turbidity removal capacity remains similar to fresh extract for 9 weeks without losing significant efficiency when is stored at room temperature, 4 or −18˚C.

In conclusion, this study has shown that it is possible to preserve Moringa extract as pellet and store it at room temperature for a period of at least 78 weeks keeping its maximum turbidity removal capacity comparable to that of fresh extract. Nine weeks is the time for which efficiency of room temperature PE is comparable to fresh extract in terms of amount of protein dosage added to reach maximum turbidity removal capacity.

These results open the possibility of storing Moringa extracts for their use in short and medium term without daily preparation, by adjusting the levels of protein that must be added to attain maximum coagulant activity as a function of the storage conditions employed. These findings represent a significant advance in the application of MO extracts for continuous drinking water treatment systems to small-scale or point of use, which would increase the functionality of them under these conditions and reduce the storage cost significantly.

Acknowledgements

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