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Influence of extraction and freeze-drying durations on the effectiveness of *Moringa oleifera* seeds powder as a natural coagulant

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ABSTRACT

Moringa oleifera has been proven to be an effective natural coagulant, a suitable alternative to aluminum sulfate, in water treatment. Several studies have been carried out to obtain the efficiency of *M. oleifera* as a coagulant using different extraction techniques in obtaining the active ingredient, such as, water and salt (different types of salts and varying concentrations). Some studies included adding further processes to convert the extracted *M. oleifera* into powder form, such as spray-drying and freeze-drying. In this study, the yield and coagulation efficiency of freeze-dried *M. oleifera* seeds extracted under three different extraction methods (distilled water, potassium chloride and potassium nitrate) at different extraction duration (2 and 15 min); and different freeze-drying durations (24 and 65 h), were investigated. The results showed that the yield of freeze-dried distilled water extracted *M. oleifera* was neither affected by the duration of extraction or by the duration of freeze-drying. However, the freeze-dried salt extracted *M. oleifera* showed that the amount recovered through the process increased by 0.85, 60.5, and 40.4% for distilled water, potassium chloride, and potassium nitrate, respectively. When the extraction duration was increased from 2 to 15 min and the freeze-drying duration was increased from 24 to 65 h. In terms of coagulation efficiency, the results showed that for the freeze-dried distilled water extracted *M. oleifera*, the increase in duration of extraction time and freeze-drying did not improve the coagulation activity. On the contrary, the increase in the extraction and freeze-drying durations increased the optimal dosage from 132 to 163 mg/l and decreased the coagulation efficiency by 2.64%. As for the extraction using potassium chloride or potassium nitrate; the increase of extraction and freeze-drying durations caused a decrease in the optimal dosage from 97 to 70 mg/l for potassium chloride and from 148 to 121 mg/l for potassium nitrate. The coagulation activity of both freeze-dried salt extracted *M. oleifera* increased by 2.9 and 0.67% for potassium chloride and potassium nitrate respectively. As a conclusion, the salt extraction *M. oleifera* optimum dosage decreased by 28 and 18% due to the increase of

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extraction and freeze-drying durations using potassium chloride and potassium nitrate in the extraction process, respectively.

Keywords: Extraction; Freeze-drying; Duration; *Moringa oleifera*; Effectiveness; Coagulation

1. Introduction

Water is undoubtedly one of the most precious natural resource in the world, which covers over 70% of the earth. Although large quantity of water is present over the earth's surface, only 0.4% is available for use. Without the presence of water, life on earth will be non-existent [1]. According to a survey conducted by the United Nations Environment Program, 20% of the world's population lacks access to safe drinking water and 50% of the world's population lacks access to safe sanitation. Polluted water is estimated to affect the health of about 1,200 million people and contribute to the death of 15 million children under the age of five, every year [2].

Water treatment technologies have been developed since the last century in order to provide better quality of water for human consumption. Safe drinking water is essential to health and welfare for a community and water from all sources must have some form of purification before drinking. Coagulation is one of the ineluctable processes of accepted drinking water treatment practice in which coagulation, flocculation, and sedimentation processes are combined in series to remove solid or colloidal particles in water. The wide use of alum as a primary coagulant in turbidity removal by water supply authorities has been found in many countries including developing countries. A significant economic factor is that many developing countries can hardly afford the high cost of imported chemicals for water and wastewater treatment [3,4]. Since the cost of procuring these chemicals is increasing rapidly, many water supply companies tend to under dose the coagulant to cope with this situation. The consequences resulted in production of low quality of drinking water [5]. Researchers also found that the residual aluminum salts from alum in treated water is the major cause of Alzheimer's disease and other health problems [6–8].

Alternatives have been searched to replace this conventional treatment chemical and people found that, *Moringa oleifera* has a great potential in water treatment as a coagulant [9,10]. Its uses and effectiveness as a natural coagulant in water or wastewater treatment, softening agent, and bactericidal agent have reported as well [11–15]. The active agents of coagulation are dimeric cationic proteins of molecular weight approximately 13 kilodaltons (kDa) having an isoelectric point between 10 and 11 [4]. Several studies have been done to extract this active component in *M. oleifera* using distilled water extraction method [5,11,16–20]. Muyibi and Evison [13] indicated that the method has increased the coagulation activity, but it is effective only in high turbid water. Another disadvantage of this method in water treatment is to increase dissolved organic carbon. Okuda [18] used salt solution (sodium chloride) to extract active component in *M. oleifera* seeds and found that it was more efficient than conventional methods. Other salt solutions are potentially being carried out for its effectiveness in extracting the active agent in *M. oleifera* seeds.

No study has been done yet using other techniques like spray-drying to determine the extracted seeds powder shelf life.

Current study investigates the effects of extraction duration (2 and 15 min) and freeze-drying duration (24 and 65 h) under different types of extraction methods (distilled water extraction, potassium chloride (KCl) extraction, and potassium nitrate (KNO_3) extraction method.

2. Material and methods

2.1. Collection of M. oleifera seeds

In this study, the *M. oleifera* seeds were used as a coagulant and had been collected from the local neighborhood and from the farm of the University Putra Malaysia. Only dry pods which are in brownish color were collected. Good quality seeds were selected from that which was not rotten, old, or infected with diseases. In this study, only shelled seeds were selected to be used.

2.2. Preparation of M. oleifera seeds powder

The collected *M. oleifera* seeds with husks and wings were dried in the oven at 50˚C for more than 24 h. After that, the husks and wings were removed in order to get the kernel. Kernels were grounded into a fine powder by using domestic blender.

2.3. Storing of M. oleifera seeds powder

After the kernels were grounded into a fine powder, it was stored in small containers and measured the net weight using an analytical balance. The containers were then sealed with the parafilm seal, and covered with aluminum foil before storing in the refrigerator at 4˚C. The purpose of doing this is to minimize the degradation of the powder which may be caused by ultra violet sunlight and the atmospheric moisture content.

2.4. Preparation of salt solution

The chemical extract agent used in this study was 1.0 M potassium chloride (KCl) and 1.0 M potassium nitrate (KNO_3) salt solution. 74.55 g of potassium chloride (KCl) and 101 g of potassium nitrate (KNO_3) were prepared by using an analytical balance. Each salt was then dissolved in approximately 600 ml of distilled water a baker with a capacity of 1.0 L. To ensure all salt was completely dissolved, agitating process was applied. The solutions were transferred to a 1.0 L standard flask and distilled water was added up to 1.0 L. These solutions were stored overnight at room temperature before use.

2.5. Salt solution extraction method

15 g of raw fine powder was added into 75 ml of salt solution in order to prepare a saturated stock solution of 20% solid content for freeze-drying. This solution was then being blended (using domestic blender) to allow the extraction of active ingredient. In this study, 2 and 15 min of extraction duration were carried out for 2 different batches of freeze-drying processes. The effects of applying different extraction durations will be discussed in terms of yield of the freeze-dried powder and coagulation efficiency. The resulting suspension was filtered through muslin cloths and the filtrates were then used as saturated stock solution. The filtrates were filled in COD vials with 2 ml for each vial and sent for freeze-drying immediately. The weights of each vial before and after filling, before and after freeze-drying were recorded.

2.6. Distilled water extraction method

In this method, the extraction agent used was distilled water. 15 g of raw fine powder was added into 75 ml of distilled water in order to prepare a saturated stock solution of 20% solid content for freeze-drying. This solution was then being blended (using domestic blender) to allow the extraction of active ingredient. As similar to the above method, 2 and 15 min of extraction duration were carried out for 2 different batches of freeze-drying processes. The resulting suspension was filtered through muslin cloths and the filtrates will be used as a saturated stock solution. The filtrates were filled in COD vials with 2 ml for each vial and sent for freeze-drying immediately. The weights were recorded for each vial before and after filling and also before and after freeze-drying.

2.7. Preparation of freeze-dried M. oleifera seeds powder

The freeze-dryer used in this study was model Virtis BENCHTOP available in Putra Infoport, Institute Bio Science Laboratory, University Putra Malaysia. Before freeze-drying process, all samples were pre-freezed in a freezer at a temperature of –30˚C for overnight. Samples were placed in the freeze-dryer flask where the bigger flask can contain 8 COD vials, but small flask can only contain 1 COD vial. Hence, the maximum COD vials for each batch of the freeze-drying process were 54. This freeze-dryer was operated at fixed parameters where the temperature was –52.6˚C and pressure of 100 μ Bar. The surrounding temperature was maintained at 25˚C by an air conditioning system. After freeze-drying for 24 h (Batch 1) and 65 h (Batch 2), the vials were collected and the freeze-dried powder was stored in containers. The containers were sealed and stored in refrigerator at 4˚C before use to prepare a stock solution.

2.8. Preparation of M. oleifera stock solution

In order to prepare the desired concentration while running the jar test, three types of coagulant where produced from salts solutions extraction and distilled water extraction. Raw *M. oleifera* powder were used to prepare stock solutions. A 2.5 g from each coagulant powder was added into 250 ml distilled water to prepare a stock solution of 10,000 mg/l and stirred for one min by using the blender to make sure all the coagulant powders are fully dissolved. The desired concentration and volume of distilled water to be added are determined from Equation (1).

$$C_1 \times V_1 = C_0 \times V_0 \quad (1)$$

where C_0 = Stock solution concentration, V_0 = Stock solution volume, C_1 = Required concentration, V_1= Required volume.

2.9. Coagulation run/jar test

Jar test is the most widely used method for coagulation process. In this study, the jar test apparatus used was (Stuart Science, Flocculator SW1), in each coagulation run. This jar test apparatus allowed 6 beakers to be agitated simultaneously. 500 ml of synthetic and natural waste water samples were filled in 1 liter beaker and then placed in the stirring machine. The stirring machine can operate at the required intensity of rapid and slow mixing. The mixing parameters used in this study are shown in the Table 1. After rapid mixing, the slow mixing takes place and duration of all mixing was controlled by stop watch. Settling time of 30 min was allowed before measuring the residual turbidity of the samples. All tests were conducted at room temperature without vibration in order to get the reading as accurate as possible. Every experiment was repeated at least twice in order to obtain less than 5% deviation in terms of residual turbidity.

Table 1 shows the operating variables used to run the jar test [21].

2.10. Optimizing dosage of freeze-dried M. oleifera seeds powder

Jar tests were conducted using freeze-dried distilled water extraction powder and freeze-dried salt extraction powder as coagulant. The preliminary coagulation test was first conducted from coagulant dosage 50 to 500 mg/l to find the range of optimum dosage, where, the optimum dosage is the dosage which results in a lowest residual turbidity. Relationships between residual turbidity and dosage were plotted to determine the optimum dosages for each run. The range was widened if the optimum dosage was not found in that range. Refinement range of dosing with 10 mg/l interval was selected in the second run in order to get the most accurate result. For comparison purpose the optimum dosages were found through conversion to the equivalent raw *M. oleifera* dosage.

2.11. Preparation of synthetic and natural waste water

Synthetic waste water for coagulation experiment was prepared by adding kaolin powder (R & M Chemicals) to distilled water. About 10 g of kaolin was added to 1 L of distilled water. It was then stirred at 20 rpm for 1 h using jar test apparatus (Stuart Scientific, Flocculator SW1) for uniform dispersion of the kaolin particles. After that, it was placed in a safe place for 24 h, to ensure the complete hydration of the kaolin suspension has been taken place. Then, it was stored in the container as a stock solution for the preparation of waste water samples for the coagulation test.

Table 1
Operating variables used to run the jar test

Mixing type	Speed (rpm)	Time (min)
Rapid mix	100	4
Slow mix	40	25

3. Results and discussion

3.1. Productivity of different extraction method under different durations

The input weight of raw *M. oleifera* seeds powder to produce the freeze-dried *M. oleifera seeds* powder for different methods of extraction was the same (15 g), but the amount of freeze-dried *M. oleifera* powder collected for each extraction method was different. Fig. 1 shows the quantity of pure freeze-dried *M. oleifera* seeds powder with different extraction methods. The results show that using 2 min distilled water extraction method with 24 h freeze-drying, the weights were 4.55 g while 4.59 g for 15 min extraction with 65 h freeze-drying. The observed increment in yields was 0.85%. For potassium chloride extraction method, the weights for 2 min extraction with 24 h, freeze-drying (Batch 1) and 15 min extraction with 65 h freeze-drying (Batch 2) were 5.76 and 9.25 g respectively. The production of Batch 2 was 60.5% more than the quantity collected from Batch 1. The amount of pure freeze-dried *M. oleifera* seeds powder for 2 min potassium nitrate extraction with 24 h freeze-drying was 5.55 g while for 15 min extraction with 65 h freeze-drying, it increased to 7.80 g. It achieved 40.4% increment in production. When comparing between batches for same extraction method, distilled water extraction method does not improve much in production (0.85%) while potassium chloride and potassium nitrate extraction method have a lot of increment (60.5 and 40.4%, respectively). This might be caused by the presence of salts for longer extraction duration and freeze-drying duration and are actually improving the extraction of active component from the raw *M. oleifera* seeds powder. Similarly, the comparison among extraction methods also show that the yields are more for salt extraction method compared with distilled water extraction.

3.2. Coagulation performance of freeze-dried M. oleifera for different extraction solution with different extraction and freeze-drying durations

Figs. 2–4 show the results for each mode under different extraction and freeze-drying durations. Fig. 2

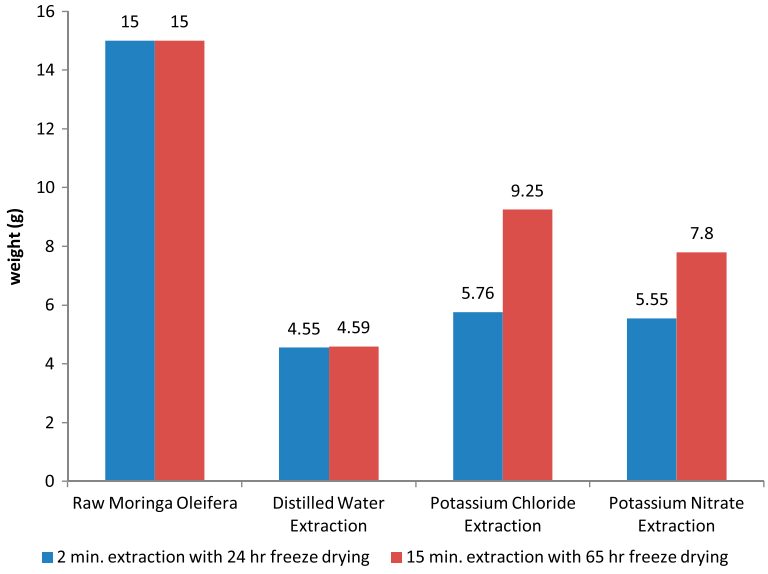

Fig. 1. The yields of pure freeze-dried *M. oleifera* seeds powder for different extraction methods.

shows the coagulation performance for freeze-dried *M. oleifera* distilled water extraction with different extraction and freeze-drying durations. For 2 min extraction with 24 h freeze-drying, the optimum dosage was 40 mg/l equivalent to 132 mg/l of raw *M. oleifera* while for 15 min extraction with 65 h freeze-drying, the optimum dosage was 50 mg/l equivalent to 163 mg/l. Shorter extraction and freeze-drying duration achieved a residual turbidity of 14.54 NTU (92.73% removal) at the optimum dosage while longer extraction and freeze-drying duration could only achieve a residual turbidity of 19.82 NTU (90.09% removal). This indicate that for distilled water extraction method, longer extraction duration and freeze-drying duration does not improve the coagulation performance, but it decreases the coagulation efficiency.

On the other hand, the coagulation performances of freeze-dried *M. oleifera* salt extracted is shown in Figs. 3 and 4.

Fig. 3 shows that the optimum dosages for potassium chloride extraction method for 2 min extraction with 24 h, freeze-drying was 60 mg/l equivalent to 97 mg/l of Raw *M. oleifera* while for 15 min extraction with 65 h freeze-drying, it was 60 mg/l equivalent to 70 mg/l of raw *M. oleifera*. The effect of longer extraction duration and freeze-drying duration had improved the coagulation performance in this extraction method. Hence, longer extraction duration and freeze-drying duration need only 70 mg/l of dosage to remove 91.17% of turbidity. On the other hand, shorter extraction and freeze-drying duration would need a higher dosage (97 mg/l) to achieve less turbidity removal (88.27%).

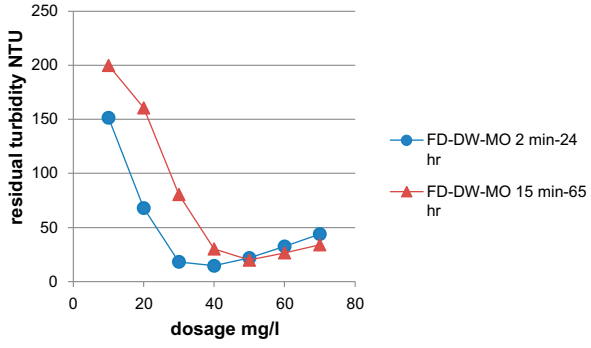

Fig. 2. Coagulation performance of freeze-dried *M. oleifera* distilled water extracted.

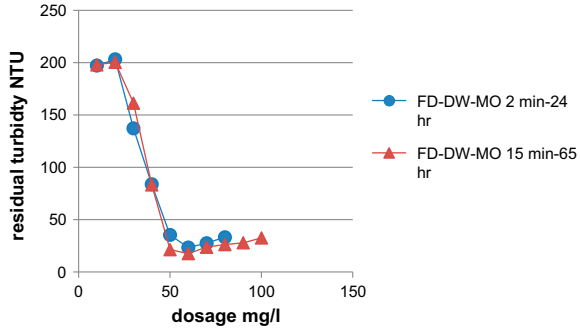

Fig. 3. Coagulation performance of freeze-dried *M. oleifera* potassium chloride extracted.

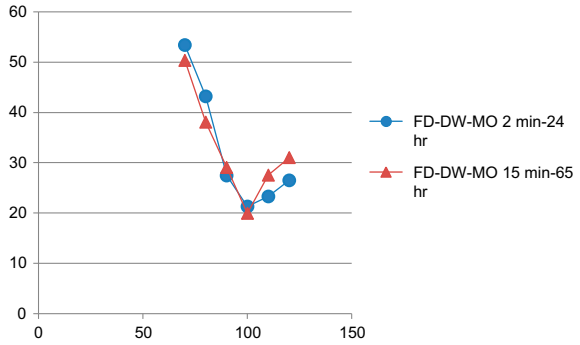

Fig. 4. Coagulation performance of freeze-dried *M. oleifera* potassium nitrate extracted.

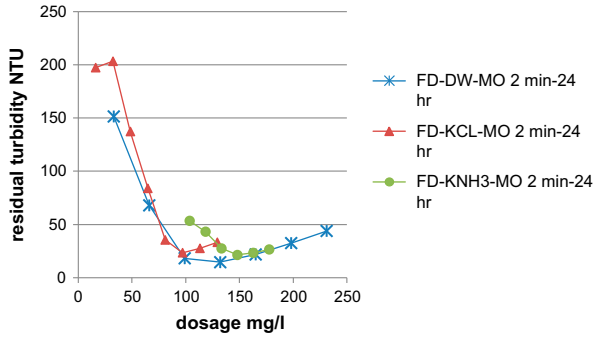

Fig. 5. Coagulation performance of different extraction method with short extraction and freeze-drying duration.

Fig. 4 shows the comparison of coagulation performance of the potassium nitrate extraction method between 2 min extraction with 24 h freeze-drying and 15 min extraction with 65 h freeze-drying. Similar to potassium chloride extraction method, the potassium nitrate extraction method also achieved a better coagulation efficiency with the increase of extraction and freeze-drying duration. The converted optimum dosage decreased to 121 mg/l with the lowest residual turbidity of 19.96 NTU (90.02% removal efficiency) for longer extraction and freeze-drying duration compared to 148 mg/l for shorter extraction duration and freeze-drying duration associated with 21.31 NTU residual turbidity (89.35% removal efficiency).

3.3. *Effects of extraction duration and freeze-drying duration on coagulation performance of* M. oleifera *seeds powder*

To assess the effect of extraction time and freeze-drying time on coagulation performance of freeze-dried *M. oleifera* seeds powder two different extraction time were used 2 and 15 min with two freeze-drying duration 24 and 65 h respectively. For the above durations, three extraction modes were used, distilled water extraction method, potassium chloride extraction method, and potassium nitrate extraction method. To compare the coagulation performance of each mode, the dosage was determined in the form of raw *M. oleifera* used in the extraction.

Fig. 5 shows the coagulation performance of different extraction methods with the short extraction and freeze-drying duration (2 min. extraction duration-24 h freeze-drying duration).

The dosages for each extraction method are shown in Fig. 5 and expressed in mass of raw *M. oleifera* seeds powder (mg) per liter of turbid water sample. Since it is inappropriate to compare the dosage amongst different extraction methods because the freeze-dried *M. oleifera* seeds powder for each extraction method contained different amount of raw *M. oleifera* seeds powder. Based on that and for distilled water extraction method, the converted optimum dosage was found to be 132 mg/l to reach the lowest turbidity of 14.54 NTU. Optimization conducted for potassium chloride and potassium nitrate extraction methods show that the converted optimum dosages were 97 and 148 mg/l with the residual turbidity of 23.46 and 21.31 NTU respectively. This indicates that in terms of coagulation performance, potassium chloride extraction method gave the best result, while potassium nitrate extraction method gives the lowest efficiency among the other extraction methods. Comparison between salt extraction and distilled water extraction methods clearly shows that the salt extraction method is better in coagulation performance [22].

Fig. 6 shows the coagulation performance of the different extraction solutions with the long extraction and freeze-drying duration (15 min extraction duration and 65 h freeze-drying duration).

Results show that for distilled water extraction method, the converted optimum dosage was 163 mg/l with 19.82 NTU of residual turbidity. The converted optimum dosages for potassium chloride and potassium nitrate extraction methods were 70 and 121 mg/l, respectively, with 17.66 and 19.96 NTU of residual turbidity. When comparing the coagulation efficiency in terms of converted optimum dosages for different mode of extraction method, potassium chloride extraction method gave the best result following by potassium nitrate and distilled water extraction method. Comparison between salt extraction and distilled water extraction method clearly show that the salt extraction method give better coagulation activity. This improvement may be due to the salting-in mechanism in protein, where loosening-up of protein associations

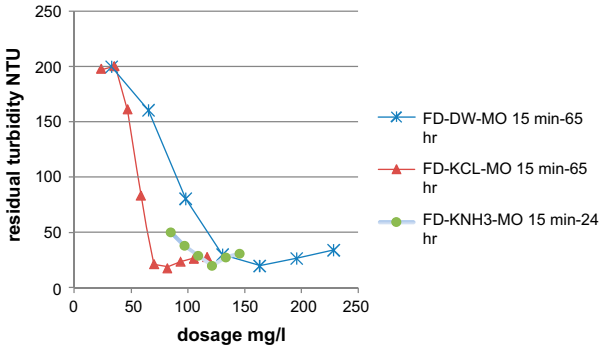

Fig. 6. Coagulation performance of different extraction method with long extraction and freeze-drying duration.

leads to more soluble and coagulation active species in solution [22].

Thus, longer extraction duration and freeze-drying duration has positive effect for salt extraction method (potassium chloride and potassium nitrate extraction) but not for the distilled water extraction method.

4. Conclusions

This study showed that using salt solutions for extraction of active ingredients in *M. oleifera* seeds powder by freeze-drying improves the productivity of the freeze-drying process compared with using distilled water.

For the extraction duration, the amount of resulted freeze-dried powder also increased with the increase in extraction and freeze-drying time. However, the coagulation activity shows that with distilled water extraction a better removal efficiency was achieved compared with salt extraction. But in terms of optimal dosage, the freeze-dried salt extracted *M. oleifera* achieved with less dosage compared to the distilled water case.

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