Evaluation of *Moringa oleifera* seed extract by extraction time: effect on coagulation efficiency and extract characteristic

Youmi Jung, Yoonhee Jung, Minhwan Kwon, Homin Kye, Yirga Weldu Abrha and Joon-Wun Kang

ABSTRACT

The seed of *Moringa oleifera* (MO) is a well-known coagulant used in water and wastewater treatment, especially in developing countries. The main mechanism of MO seed extract in coagulation is the positive protein component for charge neutralization. The method for efficient extraction of MO seed is very important for high coagulation activity. In this study, the effects of extraction mixing speed and extraction time of MO on coagulation activity were evaluated using a distilled water extraction method. Although the rotation per minute for extraction did not affect the coagulation efficiency, the extraction time strongly affected the coagulation efficiency of the extract. To evaluate the characteristic change of MO extract by extraction time, the charge of MO extract and protein characteristic in MO extract were analysed. As the extraction time was short, more positive charge and higher protein content were observed. For detailed protein analysis, the fluorescence spectroscopic study (EEM analysis) was performed. The tryptophan-like peak increased at longer extraction times. For efficient extraction of MO seed, a short extraction time is strongly recommended.

Key words | coagulation, extraction condition, *Moringa oleifera* seed, natural coagulant

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INTRODUCTION

Developing countries are facing water safety problems due to the lack of affordable water treatment technology. To ensure availability and sustainable management of water and sanitation for everyone, the sustainable development goals developed by the United Nations (UN) include clean water and sanitation in developing countries. As one of the key water purification processes, coagulation using natural coagulants is an easy-to-use and cost-effective technology for developing countries. Some studies on natural coagulants have been carried out and various natural coagulants were produced or extracted from microorganisms, animals or plants (Okuda *et al.* 1999). Because the natural coagulant method relies on local materials and local labour, renewable resources and food grade plant materials, this technology can improve the goal of sustainable water treatment technologies (Miller *et al.* 2008).

A water-soluble extract of the dry seeds of *Moringa oleifera* (MO) is well-known natural coagulant used in developing countries (Ndabigengesere *et al.* 1995). MO is a very widespread species and grows quickly at low altitudes in the whole tropical belt including arid zones (Morton 1991). Using this natural coagulant could help developing countries to alleviate their economic situation and allow further extension of water supply to rural areas (Ndabigengesere & Narasiah 1998). Various laboratory studies have so far shown that the MO seeds possess effective coagulation properties (Ndabigengesere *et al.* 1995; Ndabigengesere & Narasiah 1998; Kwaambwa & Maikokera

doi: 10.2166/wh.2018.078

2007; Al-Anizi *et al.* 2014). Coagulation efficiency has been evaluated using various factors such as turbidity, pH, colour and *Escherichia coli*. According to previous research, turbidity removal was influenced by initial turbidity of raw water (Nkurunziza *et al.* 2009). Colour removal showed the same trend as turbidity removal, and *E. coli* removal was also observed as associated with turbidity removal, because the main mechanism of *E. coli* removal is precipitation in the coagulation process.

The active agents of coagulation by MO are dimeric cationic proteins of molecular weight of approximately 13 kDa with isoelectric point between 10 and 11 (Ndabigengesere *et al.* 1995). In addition, another active component was purified from MO seeds by aqueous salt extraction: it was not a protein, polysaccharide or lipid, but an organic polyelectrolyte with molecular weight of about 3.0 kDa (Okuda *et al.* 2001). The seed extract works by adsorption of colloids and subsequent charge neutralization of the resulting compound, allowing for effective precipitation out of solution (Ndabigengesere *et al.* 1995; Miller *et al.* 2008). The crushed seed powder, when mixed with water, yields water-soluble proteins that possess a net positive charge. The solution acts as a natural cationic polyelectrolyte during treatment (Sutherland *et al.* 1990). The water soluble proteins have been proposed to bind to the predominantly negatively charged particles (silt, clay, bacteria, etc. suspended in a colloidal form) that make raw waters turbid (Kwaambwa & Maikokera 2007). The surface activity and fluorescence of the protein component in MO extract have also been studied (Kwaambwa & Maikokera 2007; Maikokera & Kwaambwa 2007). The fluorescence of proteins originates from tryptophan, tyrosine and phenylalanine residues. In aqueous media, the emission peaks of phenylalanine, tyrosine and tryptophan occur at 280, 305 and 348 nm, respectively. The emission of proteins is dominated by tryptophan which absorbs at the longest wavelength.

The toxicity of MO seed has been studied (Ndabigengesere *et al.* 1995; Al-Anizi *et al.* 2014). Although the MO seed is well known as a non-toxic and biodegradable coagulant (Ndabigengesere *et al.* 1995), recently, cytoxicity and genotoxicity of MO was studied by using an *Acinetobacter* bioreporter (Al-Anizi *et al.* 2014). The powdered MO seed showed significant cytoxicity effects at concentrations from 1 to 50 mg/L. The insoluble fatty acidic components of MO seed mainly contributed to the cytoxicity, and limited dissolution of MO seed granule resulted in dominant genotoxicity. Based on these results, more research, such as toxicity to humans and the proper extraction method for reducing toxicity is required.

The storage conditions and performance of MO extract were studied previously (Garcia-Fayos *et al.* 2016). MO extract stored at room temperature generally loses coagulation activity because coagulation protein present decreased. To maintain coagulation protein in extract, the MO extract should be stored at 4 to −18°C.

Sodium chloride solution was used to improve the extraction efficiency of MO (Okuda *et al.* 1999). The MO extract in sodium chloride solution was found to have 7.4 times higher coagulation efficiency than that using distilled water. Because salt increases protein–protein dissociations, protein solubility for coagulation could be increased as the salt ionic strength increases. However, there is lack of studies on extraction condition such as extraction rotation per minute (rpm) and extraction time which could affect the coagulation capacity.

In the present study, we tested the relationship between MO seed extract and extraction conditions. The aims of this study were: 1) to evaluate the effect of extraction rotation speed and extraction time for MO extract as a coagulant, 2) to characterize the MO extract depending on the extraction conditions and 3) to suggest the optimum MO extraction conditions for use of coagulant.

MATERIALS AND METHODS

MO seed extraction

The MO seeds used in this study were brought from India. The MO seeds were stored with winged seed covers in our laboratory at room temperature. Prior to use, the winged seed cover was removed and the kernel was ground to fine powder by mortar and pestle and then sieved using a 1.18 mm sieve. The active agents of coagulation were then extracted from the powder using distilled water. A concentration of 5% (5 g/100 mL) was used based on previous research (Ndabigengesere & Narasiah 1998). In this study, we focused on revolutions per minute and extraction time

for optimal extraction condition. The MO suspension was stirred using a magnetic stirrer by revolutions per minute from 100 to 800 rpm. The extraction time was varied from 1 to 120 min. The suspension was then filtered firstly through 10 μm nylon filter and then through a 0.45 μm membrane. The prepared MO seed extract was stored for 2 weeks at room temperature before the coagulation test.

Coagulation test

Preparation of turbid water

Turbid water for coagulation tests was prepared by adding kaolin into wastewater from Yonsei University in Wonju, Korea. After adding kaolin (Ducksan, Korea) into distilled water, the suspension was stirred for 30 min to achieve uniform dispersion of kaolin particles, and then was allowed to settle for 24 h for complete hydration of the particles. The supernatant was carefully collected and mixed with wastewater. Table 1 shows the characteristics of the synthetic turbid water.

Jar test

The jar test has been widely used to evaluate coagulation efficiency (Hudson 1981; Ndabigengesere *et al.* 1995). Glass beakers each containing 1 L of turbid water were placed in the slots of a jar tester. This study consisted of batch experiments including rapid mixing, slow mixing and sedimentation. The MO seed extract was added to test beakers at various doses and agitated at 100 rpm for 2 min for rapid mixing. The mixing speed was then reduced to 40 rpm for 30 min. After sedimentation for 30 min, an aliquot of 10 mL was sampled from the mid depth of the beaker and residual turbidity was determined. The same coagulation test was conducted with no coagulant as a control.

Table 1 | Characteristics of tested turbid water

Parameter	Value
pH	7.7
Turbidity	325 ± 5 NTU
DOC	21 mg/L
A_{254}	0.141 cm^{-1}
Alkalinity	192 mg/L as $CaCO_3$

Analytical methods

Turbidity was measured using a turbidimeter (2100QIS01 HACH, USA) and pH was determined using a pH meter (Thermo Fisher, USA). UV absorbance was measured in a Cary 50 spectrophotometer (Varian, USA). *E. coli* was enumerated on desoxycholate agar (BD Co., USA) which is a selective medium for *E. coli*. To measure total dissolved organic carbon (DOC), the collected sample was passed through a 0.45 μm filter and diluted 5-fold. The prepared sample was determined using a total organic carbon analyser (TOC-V CPH/CPN, Shimadzu). Zeta potential was measured with a particle electrophoresis apparatus (Photal Otsuka Electronics, ELSZ- 1000, Japan) after 5-fold dilution. To measure the concentration of organic compounds, protein was measured by the Bradford method (Thermo Fisher, USA) (Bradford 1976), which is a colorimetric protein assay based on an absorbance shift from red to blue using a dye Coomassie Brilliant Blue G-250 (Bradford reagent). To measure the spectrum of MO extract, the sample was passed through a 0.45 μm filter and diluted 20-fold. The range of the spectrum was measured from 200 nm to 800 nm. To analyse the characteristic of organic matter in the MO extract, the fluorescence excitation–emission (EEM) was measured by scanning over an excitation range of 240–440 nm in 10-nm increments and an emission range of 290–530 nm in 10-nm increments using a LS-55 (Luminescence Spectrometer, PerkinElmer, USA). The excitation and emission bandwidths were 5 nm each and the scanning speed was set at 1,000 nm min^{-1}.

RESULTS AND DISCUSSION

Effect of extraction condition for MO extract as a coagulant

The MO seed was ground and prepared as a powder for further use. To focus on the effect of extraction conditions, distilled water was used for extraction of MO seed. Solvent or other additives were not used as they would not be easy to

apply in rural communities in developing countries. There are two important parameters for MO seed extraction: the rpm and extraction time.

Mixing speed for MO seed extraction

Mixing speed is related to energy consumption in the extraction procedure. It is difficult to find the optimum mixing speed for MO extraction. The effect of mixing speed was evaluated prior to the effect of extraction time experiment. In previous research, the extraction time was generally 30 min (Ndabigengesere *et al.* 1995; Ndabigengesere & Narasiah 1998; Baptista *et al.* 2015; Petersen *et al.* 2016), so the extraction time was fixed at 30 min in the test for mixing speed. The amount of MO seed powder was also fixed at 5 g in 100 mL of distilled water. The MO seed was extracted with extraction rotation speed of 100, 200, 400, 600 and 800 rpm.

To evaluate the coagulation efficiency of MO seed extract with various extraction mixing speeds, synthetic wastewater of 327 NTU was prepared using public wastewater and kaolin (Ducksan, Korea). A general jar test was performed with injection of 10 mL extracted MO into 1 L of synthetic wastewater. Figure 1 shows turbidity removal efficiency by extraction mixing speed. In the case of no injection of MO seed extract, turbidity removal efficiency was only 10%. However, regardless of extraction speed, 95% turbidity removal average value was achieved in all case of MO seed extract injection, i.e. rotation speed did not affect turbidity removal. To optimize the energy consumption, 100 rpm was used to evaluate the effect of extraction time on MO coagulation efficiency.

Extraction time

The MO seed extract was prepared by using distilled water and varying the extraction times from 1 min to 120 min. The stirrer speed was fixed at 100 rpm. For the coagulation test, the prepared MO extract was injected in to 1 L turbid water of 325 ± 5 NTU turbidity. The dose of MO extract solution (volume/volume) was 1, 5 and 10 mL/L. To evaluate the coagulation activity, the turbidity was measured after the coagulation test.

Figure 2 shows turbidity removal efficiency using the MO seed extract of various extraction times as a function of MO seed extract dose. In the control test (no injection of MO seed extract), about 19% of turbidity removal by natural flocculation was found in raw turbid water. The results showed that the turbidity removal efficiency increased with increasing MO seed extract dose (1 mL/L < 5 mL/L < 10 mL/L). The optimum dose of MO extract was not observed in the seed extract dose range 1 mL/L to 10 mL/L.

For the effect of extraction time of MO seed, it is very important to note that turbidity removal efficiency surprisingly decreased with increasing the seed extraction time at all doses of seed extract. When the MO seed extract dose was 1 mL/L, the extract with over 30 min extraction showed the same turbidity removal efficiency as the control (=no injection of MO extract). With <10 min extraction time, 50% turbid removal efficiency was observed. With

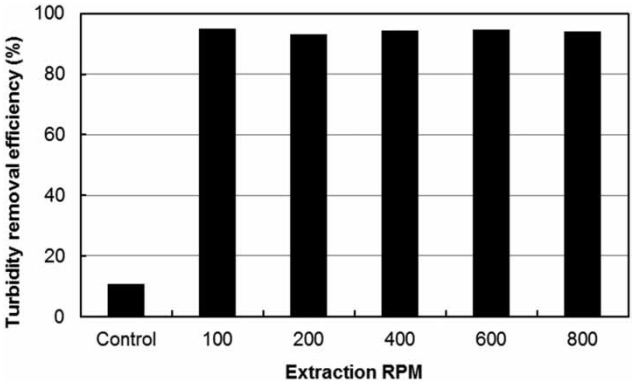

Figure 1 | Turbidity removal efficiency by rotation speed of MO seed extraction (Extraction condition: 5 g MO/100 mL, 100–800 rpm, 30 min, Dose of MO seed extract: 10 mL/L).

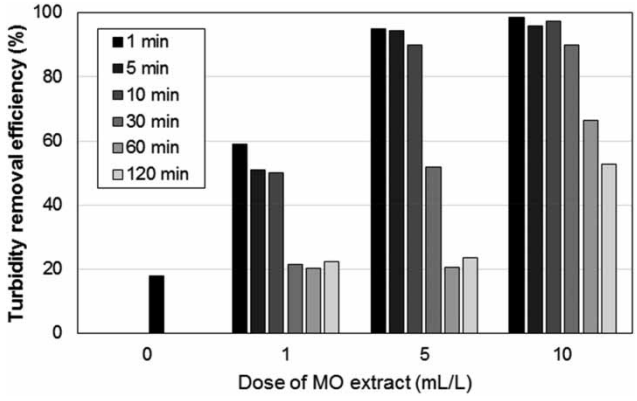

Figure 2 | Turbidity removal efficiency in coagulation test using MO seed extract made by various extraction time (Extraction condition: 5 g MO/100 mL, 100 rpm, 1–120 min, Dose of MO extract: 1–10 mL/L).

5 mL/L and 10 mL/L MO extract and 1 min extraction time, the turbidity removal efficiency was over 90%, which was similar to the removal efficiency at 5 and 10 min extraction time. However, the turbidity removal efficiency decreased with increasing extraction time from 30 min to 120 min. Thus, in these experimental conditions, the 5 mL/L of the MO seed extract with 1 min extraction time could be suggested as the optimum point.

Coagulation processes can remove microorganisms through coagulation and precipitation (Anwar *et al.* 2007; Nkurunziza *et al.* 2009). Since the turbid water for coagulation test was prepared with wastewater, the turbid water contains various types of organisms. Among them, the concentration of *E. coli* was measured by plate count method using a selective culture medium (desoxycholate agar). The average initial concentration of *E. coli* was 1.9×10^5 CFU/mL (range 8.5×10^4 to 3.5×10^5 CFU/mL). After injecting the MO seed extract with various extraction times, the concentration of *E. coli* was measured (Figure 3). The trend of *E. coli* removal was associated with the turbidity removal depending on the extraction time. Correlation between *E. coli* reduction and turbidity removal shows $R^2 = 0.8911$ according to linear Equation (1)

$$\left(-\log\left(\frac{N}{N_0}\right) = 0.0114 \times \text{turbidity removal rate} - 0.0942\right) \quad (1)$$

The *E. coli* in turbid water was mainly removed by coagulation and precipitation as shown in previous studies (Anwar *et al.* 2007; Nkurunziza *et al.* 2009).

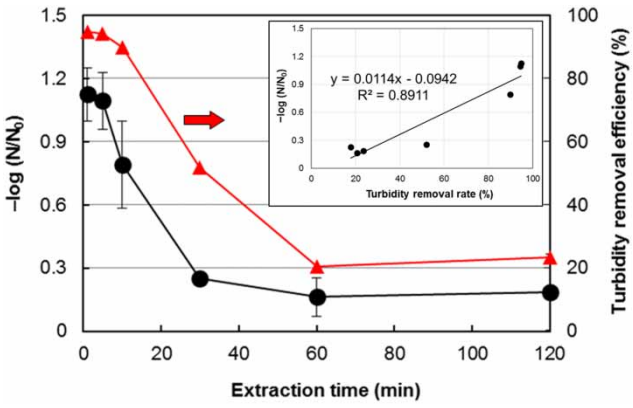

Figure 3 | Removal of *E. coli* in coagulation test using MO seed extract (Extraction condition: 5 g MO/100 mL, 100 rpm, 1–120 min, Dose of MO extract: 5 mL/L).

After coagulation, the effect of MO seed extract on pH and alkalinity was also evaluated with turbidity removal efficiency. The pH and alkalinity of initial turbid water was pH 7.7 and 192 mg/L as $CaCO_3$, respectively. After coagulation, pH and alkalinity were unchanged, the same as shown in previous research (Ndabigengesere & Narasiah 1998).

Characteristics of MO extract by extraction time

The previous tests showed that the extraction time of the MO seed strongly affects coagulation efficiency. The characteristic change was evaluated depending on the extraction time of MO seed, zeta potential, protein type and concentration, and UV absorption. The dose of MO seed extract of 5 mL/L was selected based on turbidity removal tests.

Zeta potential

Charge neutralization is the main coagulation mechanism for MO seed extract (Ndabigengesere *et al.* 1995; Miller *et al.* 2008). Most particulates in natural waters are negatively charged in the natural pH range (pH 6 to 8). The turbid water in this study was negatively charged with -9.38 ± 0.87 mV. When the positively charged coagulant is added, the repulsive charges are neutralized (close to zero). The van der Waals force causes the particles to agglomerate and settle (Snodgrass *et al.* 1984; Jarvis *et al.* 2006; Li *et al.* 2006).

The charge of MO seed extract was measured by zeta potential (Figure 4). All the MO seed extract was charged positively. As the extraction time increased, the charge of MO seed extract decreased. It is very interesting to note that the decreased positive charge had the same trend as turbidity removal efficiency. Since the positive charge of MO extract mainly affected the coagulation, the short extraction time is recommended for extraction of MO extract.

Protein concentration and component

Regarding the active component of MO extract for coagulation, researchers have mainly suggested cationic proteins acting through the mechanism of charge neutralization via the isoelectric point, and adsorption (Ndabigengesere *et al.* 1995; Ghebremichael *et al.* 2005; Miller *et al.* 2008; Joseane

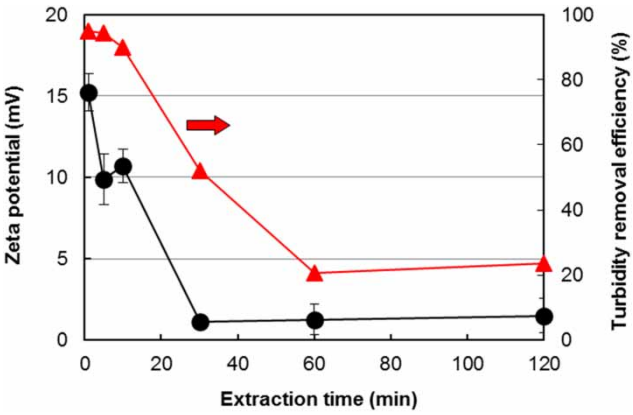

Figure 4 | Zeta potential of MO seed extract made by various extraction time (Extraction condition: 5 g MO/100 mL, 100 rpm, 1–120 min, Dose of MO extract: 5 mL/L).

et al. 2013). To evaluate the protein change by extraction time and its effect on coagulation efficiency, the concentration of protein and the main component of MO seed extract were measured and compared with its coagulation efficiency by extraction time.

The Bradford method was used to measure protein concentration. Figure 5(a) shows the protein concentration of MO seed extract at various extraction times from 1 min to 120 min. With increasing extraction time, the protein concentration decreased from 5 to 0.5 mg/mL, which shows same trend as turbidity removal efficiency shown in red triangles. This is consistent with the protein being the main mechanism for coagulation using MO seed extract.

To better understand the characteristics of the MO seed extract, the excitation emission matrix (EEM) of the MO seed extract was measured (Figure 5(b)). The EEM depends on the excitation (*y*-axis) and emission (*x*-axis) wavelengths. There are three main fluorescent components depending on the excitation and emission wavelengths: protein-like, humic acid-like and fulvic acid-like. According to previous studies, there are two main peaks in the EEM results: tryptophan-like fluorescence (280 nm for excitation and 348 nm for emission) and tyrosine-like fluorescence (275 nm for excitation and 305 nm for emission) (Edelhoch 1967; Coble 1996; Lakowicz 2006). The two peaks showed completely different trends depending on the extraction time. In the case of MO seed extract with 1 min extraction time, a strong tryptophan-like peak is seen, indicated in red. The tryptophan-like peak decreased as extraction time increased as show in Figure 5(c), i.e. the same trend as turbidity removal efficiency (see Figure 2). Maikokera & Kwaambwa (2007) have previously been shown the tryptophan in coagulant protein from *M. oleifera* to be an active component in coagulation. As the stirring time for extraction increased, the important coagulation protein (tryptophan-like protein) decreased. Mechanical force, such as vigorous stirring, whipping, heating, or radiation can weaken or break down the protein structure causing it to unfold and lose its properties (Patel *et al.* 1988; Isralewitz *et al.* 2001). Since the stirring method for extraction could affect protein denaturation, protein concentration and zeta potential from extracted protein could begin to decrease. On the other hand, with increase of extraction time, the tyrosine-like peak appeared in EEM spectrum. It shows the opposite trend to turbidity removal efficiency depending on the extraction time, which means no effect on coagulation.

UV absorption

The UV spectrum (250 to 300 nm) of the MO extract which contained many water-soluble components was measured (Figure 6). To compare UV absorbance depending on the extraction time, the stock of MO seed extract was diluted 20-fold. The UV spectrum showed the highest absorbance at about 270 nm and increased with longer extraction times. This is significantly different from protein concentration in MO extract measured by Bradford method in Figure 5(a), which is based on the formation of a complex between Brilliant blue G dye and proteins in solution (Bradford 1976; Roberts & Jones 2008). Because the UV absorbance reveals the total content of the MO seed extract, it is unsurprising that the results of the Bradford method were different. The MO seed extracted into water may include components that are efficient and inefficient for coagulation.

Correlation of turbidity removal with MO extract characteristics

To evaluate the main MO extract characteristic for coagulation, correlation of turbidity removal rate with MO extract characteristics is shown in Figure 7. Three MO

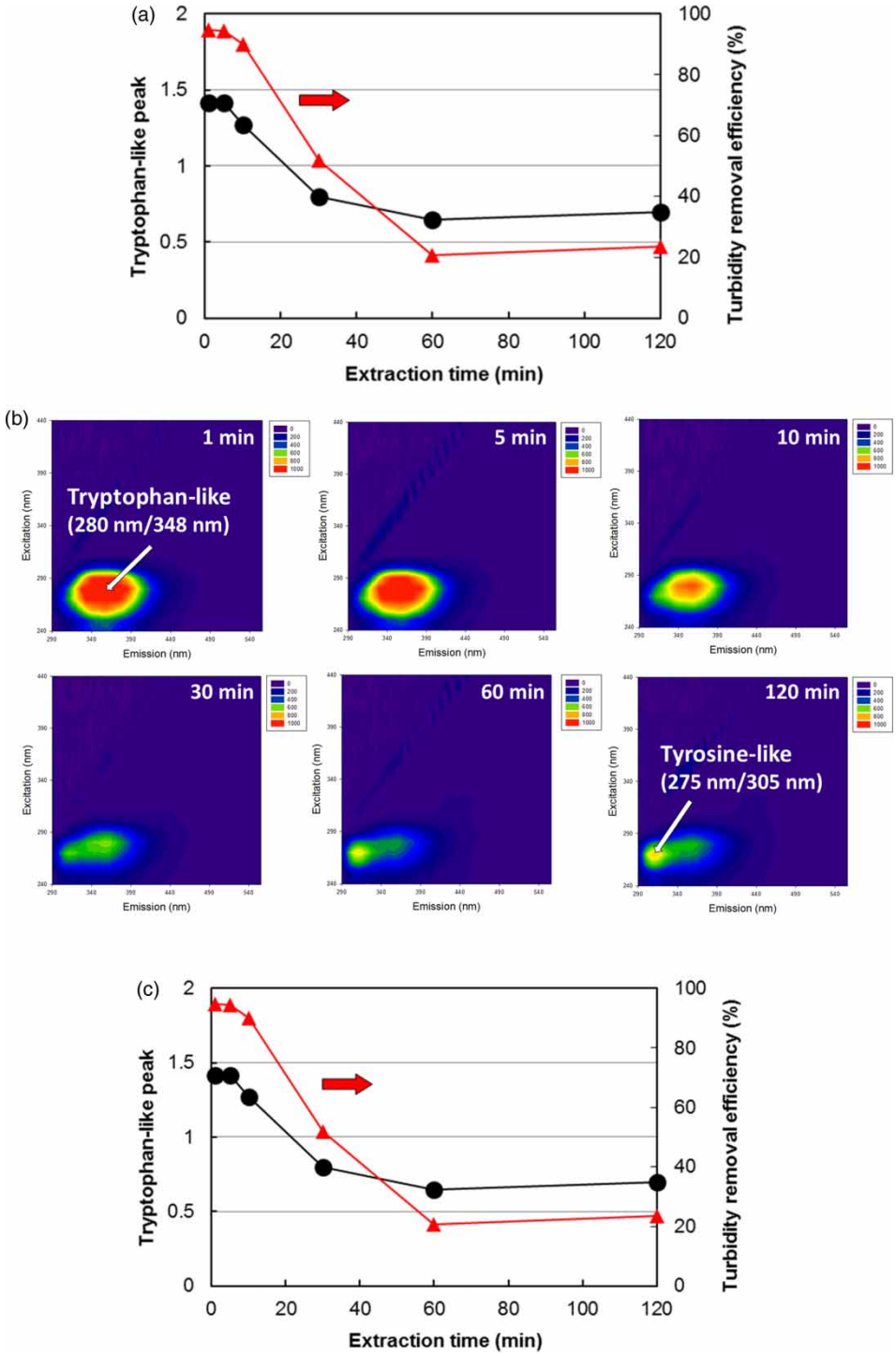

Figure 5 | Excitation emission matrix (a) and Tryptophan-like peak (b) of MO seed extract by extraction time (Extraction condition: 5 g MO/100 mg, 100 rpm, 1–120 min time).

extract characteristics were used: zeta potential, protein by Bradford method, and tryptophan-like protein from EEM analysis. R^2 values of each factor were 0.8242, 0.923 and 0.9569, respectively. Although the positive zeta potential might contribute to coagulating turbid water (Figure 4), protein components contribute more to turbidity removal

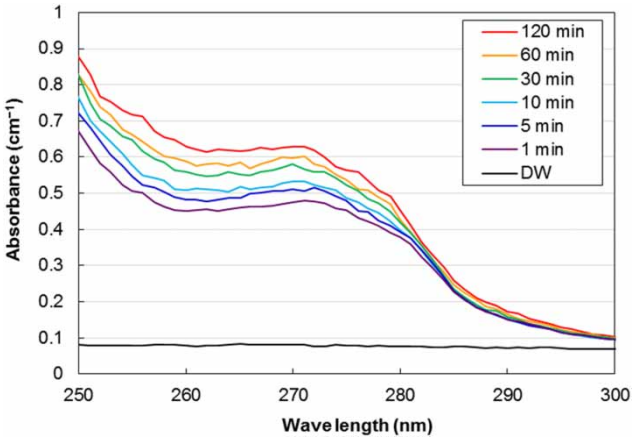

Figure 6 | Absorbance of MO seed extract by extraction time (Extraction condition: 5 g MO/100 mg, 100 rpm, 1–120 min time).

with over 0.9 of correlation value. In particular, the tryptophan-like protein in the MO extract strongly affects coagulation mechanism. Since protein could be denatured by physical stress (Patel *et al.* 1988; Isralewitz *et al.* 2001), it is assumed that the tryptophan-like protein of MO extract decreased with longer extraction times. In fact, the extraction time strongly affects the tryptophan-like protein concentration to decrease the coagulation efficiency. To reduce the energy consumption and increase the coagulation efficiency for MO extraction, the short extraction time should be strongly recommended.

Turbidity and DOC caused by MO extract

The water soluble extract of MO seed contains organic compounds, which can affect turbidity and DOC in the coagulation test. For the evaluation of increasing turbidity and DOC by MO extract, the MO extract was added to distilled water in the same conditions as the coagulation test. Figure 8(a) and (b) shows turbidity and DOC in distilled water, respectively, which is only from the MO extract. The MO extract produced by 120 min extraction shows significantly high turbidity (9.0 NTU in 10 mL/L dose of MO extract). DOC concentrations of 3.1, 3.8, and 4.2 mg/L when only 1 mL/L dose of MO extract was used.

At the optimum dose of MO (1 min of extraction time and 5 mL/L of MO extract dose), the extract caused low turbidity, which does not affect the water turbidity in the coagulation test. The optimum dose of MO extract increased DOC to 15 mg/L of, which could affect by-product formation during chlorination, e.g. trihalomethanes, haloacetic acids. When extraction time varied, there was no difference of DOC concentration. The DOC was only affected by extract dose.

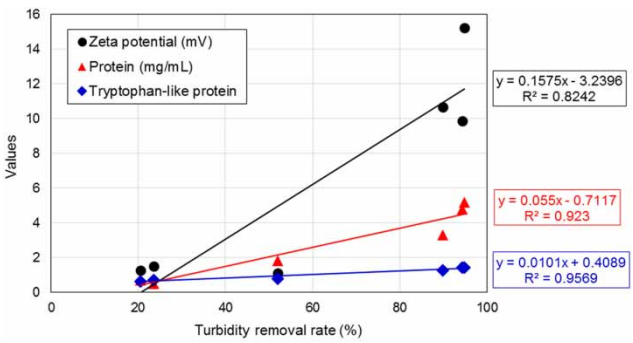

Figure 7 | Correlation of turbidity removal rate with zeta potential, protein, and tryptophan.

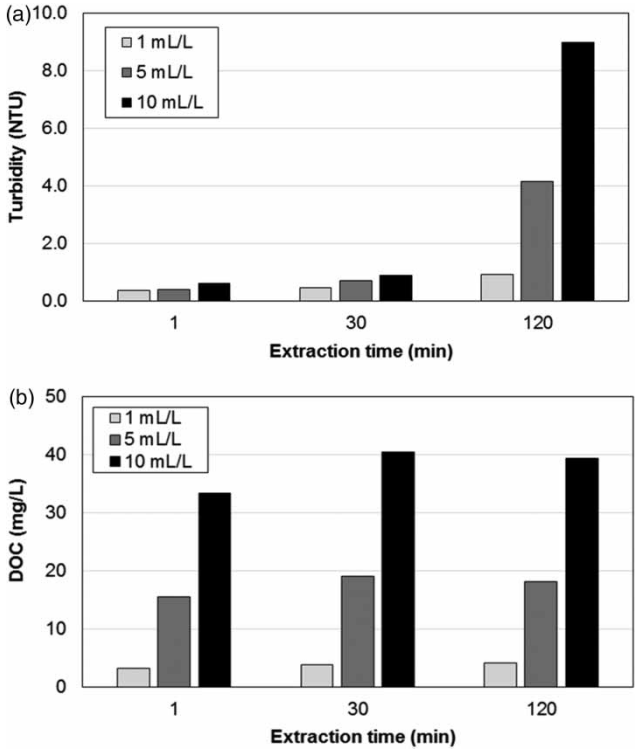

Figure 8 | Turbidity (a) and DOC (b) with adding the MO seed extract by extraction time to distilled water (Extraction condition: 5 g MO/100 mL, Extract dose: 1, 5, and 10 mL/L, Extraction time: 1, 30, and 120 min).

CONCLUSION

The seeds of MO have been widely studied for coagulation in water treatment. This study was performed to find efficient extract conditions of MO seed for coagulation. The results obtained in this study lead to the following main conclusions:

1) The rotation speed for MO extraction did not affect the coagulation activity from 100 rpm to 800 rpm so 100 rpm was used for energy efficiency. The MO extract produced by a short extraction time showed higher coagulation activity in the range from 1 min to 120 min.
2) The characteristics of the MO extract by extraction time were measured by zeta potential and protein content. As the extraction time increased, the positive charge of MO extract decreased, and it reduced the charge neutralization. Decreased protein concentration by the Bradford assay was observed with increasing the extraction time. The tryptophan-like peak in the MO extract, which is well known main mechanism for coagulation, was also observed to decrease. The extraction time for MO seed strongly affect the coagulation activity of MO extract. The short extraction time (1 min) could be suggested for efficient extraction condition of MO extract.
3) The MO extract contains complex components including positive protein for coagulation. When added to raw water, the MO extract can produce turbidity and DOC. The organic compounds in MO extract could affect some steps in the water treatment process, which means that organic matter from MO extract can be a precursor of disinfection by-product. For safe use of MO extract, an appropriate amount of MO extract should be used to reduce the residual organic compound.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education of the Republic of Korea and the National Research Foundation of Korea (NRF-2016S1A5B8925203).

REFERENCES

Al-Anizi, A. A., Hellyer, M. T. & Zhang, D. Y. 2014 Toxicity assessment and modelling of *Moringa oleifera* seeds in water purification by whole cell bioreporter. *Water Res.* **56**, 77–87.

Anwar, F., Latif, S., Ashraf, M. & Gilani, A. H. 2007 *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytother. Res.* **21**, 17–25.

Baptista, A. T. A., Coldebella, P. F., Cardines, P. H. F., Gomes, R. G., Vieira, M. F., Bergamasco, R. & Vieira, A. M. S. 2015 Coagulation-flocculation process with ultrafiltered saline extract of *Moringa oleifera* for the treatment of surface water. *Chem. Eng. J.* **276**, 166–173.

Bradford, M. M. 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dy binding. *Anal. Biochem.* **72**, 248–254.

Coble, P. G. 1996 Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chem.* **51**, 325–346.

Edelhoch, H. 1967 Spectroscopic determination of tryptophan and tryrosine in proteins. *Biochemistry* **6**, 1948–1954.

Garcia-Fayos, B., Arnal, J. M., Ruiz, V. & Sancho, M. 2016 Use of *Moringa oleifera* in drinking water treatment: study of storage conditions and performance of the coagulant extract. *Desalin. Water Treat.* **57**, 23365–23371.

Ghebremichael, K. A., Gunaratna, K. R., Henriksson, H., Brumer, H. & Dalhammar, G. 2005 A simple purification and activity assay of the coagulant protein from *Moringa oleifera* seed. *Water Res.* **39**, 2338–2344.

Hudson, H. E. 1981 *Water Clarification Processes: Practical Design and Evaluation.* Van Norstrand Company, New York.

Israelewitz, B., Gao, M. & Schulten, K. 2001 Steered molecular dynamics and mechanical functions of proteins. *Curr. Opin. Struct. Biol.* **11**, 224–230.

Jarvis, P., Jefferson, B. & Parsons, S. A. 2006 Floc structural characteristics using conventional coagulation for a high doc, low alkalinity surface water source. *Water Res* **40**, 2727–2737.

Joseane, D. P. T., Guilherme, F. L., Ricardo, F. Z. & Rosangela, B. 2013 Coagulants and natural polymers: perspectives for the treatment of water. *Plastic Polymer Technol.* **2**, 55–62.

Kwaambwa, H. M. & Maikokera, R. 2007 A fluorescence spectroscopic study of a coagulating protein extracted from *Moringa oleifera* seeds. *Colloid Surface B* **60**, 213–220.

Lakowicz, J. R. 2006 *Principles of Fluorescence Spectroscopy.* Springer, New York.

Li, T., Zhu, Z., Wang, D. S., Yao, C. H. & Tang, H. X. 2006 Characterization of floc size, strength and structure under various coagulation mechanisms. *Powder Technol.* **168**, 104–110.

Maikokera, R. & Kwaambwa, H. M. 2007 Interfacial properties and fluorescence of a coagulating protein extracted from *Moringa oleifera* seeds and its interaction with sodium dodecyl sulphate. *Colloid Surface B* **55**, 173–178.

Miller, S. M., Fugate, E. J., Craver, V. O., Smith, J. A. & Zimmerman, J. B. 2008 Toward understanding the efficacy and mechanism of Opuntia spp. *as a natural coagulant for potential application in water treatment*. *Environ. Sci. Technol.* **42**, 4274–4279.

Morton, J. F. 1991 The horseradish tree, Moringa pterygosperma (Moringaceae) – a boon to arid lands. *Econ. Bot.* **45**, 318–333.

Ndabigengesere, A. & Narasiah, K. S. 1998 Quality of water treated by coagulation using *Moringa oleifera* seeds. *Water Res.* **32**, 781–791.

Ndabigengesere, A., Narasiah, K. S. & Talbot, B. G. 1995 Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera*. *Water Res.* **29**, 703–710.

Nkurunziza, T., Nduwayezu, J. B., Banadda, E. N. & Nhapi, I. 2009 The effect of turbidity levels and *Moringa oleifera* concentration on the effectiveness of coagulation in water treatment. *Water Sci. Technol.*, **59**, 1551–1558.

Okuda, T., Baes, A. U., Nishijima, W. & Okada, M. 1999 Improvement of extraction method of coagulation active components from *Moringa oleifera* seed. *Water Res.* **33**, 3373–3378.

Okuda, T., Baes, A. U., Nishijima, W. & Okada, M. 2001 Isolation and characterization of coagulant extracted from *Moringa oleifera* seed by salt solution. *Water Res.* **35**, 405–410.

Patel, P. D., Stripp, A. M. & Fry, J. C. 1988 Whipping test for the determination of foaming capacity of protein: a collaborative study. *Int. J. Food Sci. Technol.* **23**, 57–63.

Petersen, H. H., Petersen, T. B., Enemark, H. L., Olsen, A. & Dalsgaard, A. 2016 Removal of *Cryptosporidium parvum* oocysts in low quality water using *Moringa oleifera* seed extract as coagulant. *Food Waterborne Parasitol.* **3**, 1–8.

Roberts, P. & Jones, D. L. 2008 Critical evaluation of methods for determining total protein in soil solution. *Soil Biol. Biochem.* **40**, 1485–1495.

Snodgrass, W. J., Clark, M. M. & Omelia, C. R. 1984 Particle formation and growth in dilute aluminum(III) solutions – characterization of particle-size distributions at pH 5.5. *Water Res.* **18**, 479–488.

Sutherland, J. P., Folkard, G. K. & Mtawali, M. A. 1990 Natural coagulants for appropriate water treatment: a novel approach. *Waterlines* **8**, 30–32.

First received 8 April 2017; accepted in revised form 3 August 2017. Available online 14 February 2018