



Effect of storage and preparation methods of *Moringa oleifera* seeds during the coagulation process

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Received 30 January 2015; Accepted 4 August 2015

ABSTRACT

Many developing countries around the world are now facing a water deficit crisis that worsens with climate change variations. In the particular case of Colombia, water deficit is a major concern in the whole territory, but it gets worse in rural areas. Since 70s, water purification in Colombia is based on conventional physicochemical processes, in which the most common coagulant used is aluminum sulfate (Alum). This study focuses on the behavior of color and turbidity removal of different extraction methodologies of a natural coagulant, *Moringa oleifera*. Results showed that turbidity removal efficiency was not affected by oil extraction. However, oil extraction increases the complexity of the process. Salt addition during coagulant solution preparation increases turbidity and color efficiency removal. No significant difference ($p < 0.05$) on turbidity and color removal was found between coagulant solution storage at 24°C (room temperature) and 4°C. Coagulant solution of *M. oleifera* was found to be very efficient on polluted waters with high concentration of color.

Keywords: Water treatment; Natural coagulants; *Moringa oleifera*; Extraction methodology; Color; Turbidity

1. Introduction

It has been estimated that around 1.1 billion people worldwide currently lives without water supply access and about 2.6 billion lack proper sanitation infrastructures. Different governments, including Colombia, have triggered strategies to secure proper freshwater accessibility and basic sanitation infrastructure for its population [1].

Based on population growth reports, global demand for fresh water continues to increase; it has been calculated at a rate of about 80 million people per year, meaning approximately 64 billion cubic meters of water to fulfill the demand. Additionally, long-life expectancy, globalization trade market, and suggestive advertising strategies that encourage consumption by young people in both developed and developing countries would contribute to this situation [2].

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In South America, specifically in Colombia, lack of proper fresh water for human consumption in urban and rural areas has a big impact on health and social aspects of the country. It has been estimated that water supply coverage in rural areas is 78%; however, the resource has very poor quality standards [3]. In the particular case of Antioquia, an important geopolitical region in Colombia, it is estimated that 54% of the population in rural areas has no access to drinking water and 86% has no sewerage [4].

Appropriate freshwater treatment is a key element to warranty sustainable water quality standards. Conventional water treatment consists of chemical coagulation of non-settleable colloidal particles, flocculation, sedimentation, filtration, and disinfection. Aluminum or iron salts are commonly used in these facilities as effective coagulants to destabilize colloidal particles, however, usage of those chemical coagulants increases, in some cases, the costs of the process (i.e. operation and maintenance) and might result in production of large sludge volumes [5–7]. From the environmental point of view, it has been reported that possible chemical reactions could take place between the coagulants added and other chemicals commonly used during the water treatment process (i.e. ozone and chlorine), generating hazardous substances to human health that cannot be removed from the conventional water treatment system [8,9]. Coagulation process is widely used in Colombia. The most common coagulant employed in Colombia is aluminum sulfate (Alum). Research studies conducted by Castillo et al. [10] and Flaten, P. [11] have shown evidences of neurotoxic effects of alum on human health; especially, it has been reported cases of Alzheimer's disease.

On the other hand, natural coagulants (water-soluble substances from animal or plant constituents) are a suitable alternative for freshwater treatment facilities in rural and urban areas in Colombia. Natural coagulants could reduce pollution caused by chemical coagulants, utilize non-specialized equipment for dosing, and improve accessibility in difficult-access areas because it can be produced and harvested *in situ*.

In Latin America, studies have been conducted on water clarification with species such as potato, cactus, corn, wheat, and cassava [12]. However, many debates on its utilization have raised concerns on food security issues. As an alternative to overcome food security issues for the specific case of Colombia, and to warranty proper water quality standards using natural coagulants, this study has identified *Moringa oleifera* as a suitable option [13].

M. oleifera is a plant variety from India which has a high coagulating power. It is widely known as a

plant with numerous uses in which its plant system can be utilized for beneficial purposes. The plant was reported to contain various amino acids, fatty acids, vitamins, and nutrients and its constituents such as leaf, flower, fruit, and bark have been anecdotically used as herbal medicines in treatments for inflammation, paralysis, and hypertension [14–16]. Toxicological studies reported by Sutherland et al. [17] suggested that *M. oleifera* seeds do not constitute a serious hazard to human health. This effect is very favorable for use in new technologies for water purification [18–21].

Four extraction methodologies were evaluated during this study; two evaluating the increase in ionic strength with salt (NaCl) and two evaluating oil extraction. Techniques were then compared to assess their effectiveness in every case. In addition, five water sources were tested to evaluate removal percentages in terms of turbidity and color for *M. oleifera*. Sludge production and expiration time of the solution were also evaluated.

2. Materials and methods

2.1. Equipment and coagulation procedure

Standard sedimentation jar test equipment was used in this evaluation in order to determine the optimum dosage and color and turbidity removal percentages. The test equipment was manufactured by Aztec Environmental Control Ltd consisting of six 1,000-ml jars that can be used to test each sample simultaneously. Each jar has a paddle with adjustable speed between 20 and 400 rpm. A rapid stirring period of 60 s at 100 rpm was then followed by a slow stirring period of 15 min at 40 rpm for allowing coagulation to occur. Flocs formed were then allowed to settle for 15 min before turbidity and/or absorption measurement of each sample were undertaken. For this measurement, the NTC 3903 methodology was considered [22]. Each test was performed in triplicate. Color was measured using a HANNA 93 monoparametric colorimeter. Turbidity was measured using a hand-held turbidimeter (Hannah Instrument: 93703; range 0–1,000 NTU; accuracy $\pm 2\%$). The experimental trials were performed under controlled conditions in GEMA Laboratory at Medellin University, CO.

2.2. Stock solutions and suspensions

To ensure consistency and replication of the trials, stock solutions were prepared considering different transformation strategies based on the methodologies proposed by Ledo et al. [23], Pritchard et al. [24], and Poumaye et al. [25].

2.2.1. Coagulants

A set of four different techniques for coagulant solution preparation were evaluated during this study. The first method was proposed by Ledo et al. [23]. The authors developed a solution composed of *M. oleifera* powder and distilled water, Solution (S1); the second Solution (S2), was proposed by Ghebremichael et al. [26] in which it was developed a solution composed of *M. oleifera* powder, distilled water, and oil extract. This technique was used to evaluate oil extraction performance. In the third method, Solution (S3) was proposed by Okuda et al. [27]. The authors proposed oil extraction and salt addition to increase efficiency of the coagulant solution. Finally, the fourth solution, Solution (S4), analyzed was prepared using *M. oleifera* powder and adding salt to the mix; oil extraction was not performed during (S4) preparation.

Oil extraction was performed by mixing previously powdered *M. oleifera* seed and ethanol (C₂H₆O) in a ratio of 5% weight/volume during 24 h. Then, the solution was filtered (0.45 µm) separating the liquid and the solid fraction. Solid residues were dried at room temperature and then used for the coagulation solution. The final solution is composed of 1L of distilled water or saline solution (1 M) and 50 g of seeds with extraction or without extraction. The solution was stirred for 1 h, and then was filtered again in a filter of 0.45 µm; the liquid extracted was drawn off for dosing.

2.3. Natural water

Five samples from Picacha brook (water stream located at the southwest of Medellin, nearby the University of Medellin) were evaluated under different environmental conditions. Water quality analysis followed the guidelines established by the Drinking Water and Basic Sanitation Regulations in Colombia, Title B [28].

2.4. Coagulant dosage

Coagulant stock solution was added into the jars by means of a pipet. Jar testing equipment has a maximum capacity of six vessels of 1 L. Geometrical dosage series of *M. oleifera* between 12.5 and 437.5 mg/L (12 incremental steps) were selected to provide a range for determining optimal dosage for turbidity removal.

2.5. Zeta potential

Zeta potential was measured using Zetasizer Z90. Three samples with different turbidity ranges were evaluated.

3. Results and discussion

3.1. Picacha brook characterization

Results obtained from the physicochemical and microbiological characterization from Picacha brook are presented in Table 1. Water samples showed high concentration values of turbidity, color, chlorides, COD, and coliforms, resembling the composition of a typical wastewater.

3.2. Transformation methods for *M. oleifera* seeds

Four seed transformation methods were evaluated for determining the best coagulant solutions. The tests were performed using the jar test method (triplicates). Water samples from Picacha brook were analyzed for turbidity and color removal. Initial turbidity and color concentrations were 5,080 (NTU) and 9,830 (PCU), respectively. Results are presented in Table 2.

3.2.1. Turbidity removal efficiency

Dosage response in terms of turbidity removal for each coagulant solution is presented in Fig. 1. All tested solutions (S1), (S2), (S3), and (S4) presented high turbidity removal performance.

Solution (S1) presented a 97.5% turbidity removal when 25 ml was added. Solution (S2) presented a maximum turbidity removal of 99.1% when 20 ml was added. This result demonstrated that oil extraction could generate slight improvement of turbidity removal efficiency by 2%, while reducing dosage volume from 25 to 20 ml. Although, removal efficiencies during the jar test process are not very significant compared with other solutions, oil extraction from *M. oleifera* seeds has demonstrated to have similar characteristics as edible oil [29]. Tsaknis et al. [30] and

Table 1
Physicochemical and microbiological characterization of Picacha brook

Parameter	Units	Picacha brook
pH		7.37
Turbidity	(NTU)	3,050
Apparent color	(PCU)	7,900
Chlorides	mg Cl ⁻ /L	15,382
Fluorides	mg F ⁻ /L	0.118
BOD ₅	mg BOD ₅ /L	33
COD	mg O ₂ /L	87,783
Total coliforms	NMP/100 mL	3.9 × 10 ⁶
Fecal coliforms	NMP/100 mL	1.2 × 10 ⁶

Table 2
Behavior of coagulant solutions in turbidity removal

Dosage (ml)	Solution 1				Solution 2				Solution 3				Solution 4			
	Final turbidity (NTU)	Turbidity removal (%)	STD	Final turbidity (NTU)	Turbidity removal (%)	STD	Final turbidity (NTU)	Turbidity removal (%)	STD	Final turbidity (NTU)	Turbidity removal (%)	STD	Final turbidity (NTU)	Turbidity removal (%)	STD	
5	588.7	88.4	4.8	354.0	93.0	18.5	4.8	93.0	18.5	99.9	18.5	14.5	99.9	10.1		
10	352.6	93.0	112.1	266.7	94.8	152.9	7.1	94.8	152.9	99.9	152.9	19.3	99.9	7.7		
15	280.1	94.4	72.7	81.3	98.4	20.3	17.2	98.4	20.3	99.7	20.3	21.5	99.6	6.1		
20	193.3	96.2	59.6	46.8	99.1	2.7	16.0	99.1	2.7	99.7	2.7	31.7	99.4	10.2		
25	126.5	97.5	77.5	53.3	99.0	18.4	25.5	99.0	18.4	99.5	18.4	37.4	99.3	8.2		
30	182.2	96.4	172.9	92.9	98.2	99.6	149.7	98.2	99.6	97.1	99.6	45.6	99.1	3.5		

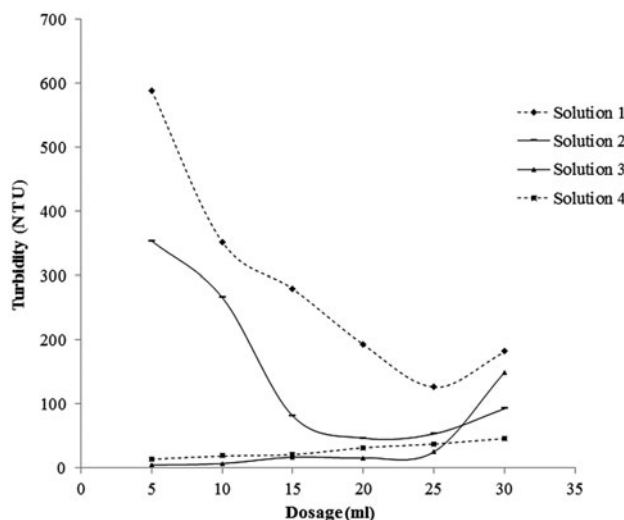


Fig. 1. Turbidity removal for tested *M. oleifera* coagulants solutions.

Ping-Hsien et al. [31] also found that oil extracted from *M. oleifera* has similar characteristics as olive oil. Solution (S3) showed a maximum turbidity removal of 99% when 5 ml of test solution was added. This result suggests that salt addition reduces considerably dosage volume of coagulant solution compared with (S1) and (S2). Solution (S4) presented similar removal efficiency results than the other solutions. It was demonstrated that oil extraction does not affect turbidity removal efficiency. Additionally, dosage incremental concentrations for (S4) did not result in removal efficiency variations. This is important because this method does not need high investments in sophisticated dosing equipment.

Eman et al. [32] have found that organic matter presence in the *M. oleifera* seed could increase the concentration of color, turbidity, and micro-organism activity of the coagulant solution. Oil extraction and salt addition from solution (S3) reduces the possible negative effects as mentioned above. Additionally, solution preparation time for (S3) is lower than the others. For these reasons, this study has selected solution (S3) for evaluating other criteria presented in this article.

3.3. Coagulation efficiency vs. storage conditions (temperature and time)

3.3.1. Turbidity removal vs. temperature

Stock solution (S3) was prepared and stored under two different temperature conditions: in a refrigerator at 4°C and at room temperature (24°C), both for a

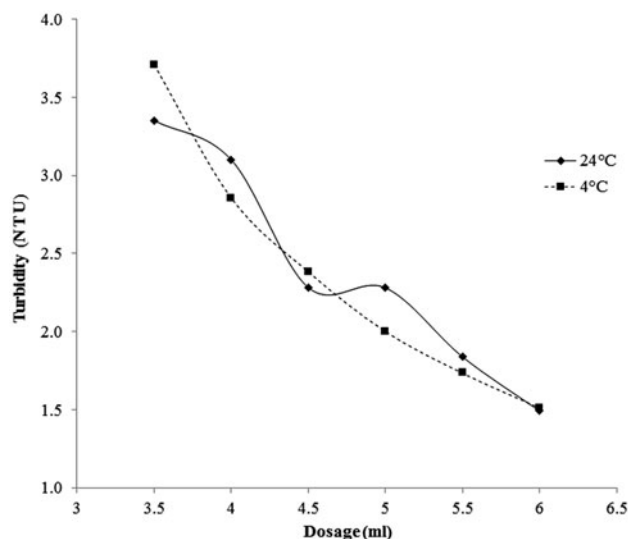


Fig. 2. Effect of temperature storage process on turbidity removal.

period of three months. The test was conducted in triplicate. Results of final turbidity with optimal dosage for each solution (5–6 ml) are shown in Fig. 2.

Results indicate that turbidity removal efficiency presented no significant variation when stock solutions were refrigerated or left at room temperature. This is important considering living conditions in rural zones in Latin America, especially in non-interconnected zones with no refrigeration units.

3.3.2. Turbidity removal vs. time

Stock solution (S3) variations at 4°C during a period of three months were evaluated in terms of turbidity removal. Water samples from Picacha brook were evaluated. Three samples were taken for month

1, 2, and 3 with turbidity values of 3,100, 3,070, and 4,100 NTU., respectively. Results are shown in Table 3.

Results showed that turbidity removal during the evaluation period was above 99% with no significant variation ($p < 0.05$). Dosage response on turbidity removal for a period of three months is presented in Fig. 3.

Maximum turbidity removal (99.6%) was reached at 6 ml of coagulant solution (S3) for months 1, 2, and 3, where no significant variations were found ($p < 0.05$). These results showed that coagulant solution (S3) has very high turbidity removal during the period of evaluation.

3.3.3. Turbidity removal vs. time and temperature

Turbidity removal of stock solution (S3) stored at room temperature and at 4°C was evaluated as a function of time (three months). Three samples from Picacha brook were taken and analyzed for months 1, 2, and 3 with turbidity values of 3,100, 3,070, and 4,100 NTU., respectively. Results are shown in Fig. 4.

Results based on temperature conditions (24 and 4°C) of turbidity removal percentage showed no statistical difference ($p < 0.05$) during the time evaluated (three months). A study conducted by Ndabigengesere et al. [33] found that *M. oleifera* powder could maintain its coagulant properties for more than six months; this confirms the results from this research.

3.3.4. Color removal vs. time

This study has selected solution (S3) for evaluating color removal during a period of three months. Water samples from Picacha brook were evaluated. Three

Table 3
Evaluation of turbidity removal after three months of storage at 4°C

Dosage (ml)	1 month			2 month			3 month		
	Final Turbidity Average 4°C (NTU)	STD	Turbidity removal (%)	Final Turbidity Average 4°C (NTU)	STD	Turbidity removal (%)	Final Turbidity Average 4°C (NTU)	STD	Turbidity removal (%)
3.5	3.7	0.3	99.9	3.8	0.3	99.9	3.6	0.9	99.9
4.0	2.8	0.2	99.9	3.1	0.1	99.9	2.7	0.2	99.9
4.5	2.2	0.2	99.9	2.7	0.2	99.9	2.2	0.3	99.9
5.0	1.9	<0.01	99.9	2.1	0.1	99.9	2.0	0.1	99.9
5.5	1.5	0.15	100.0	1.9	0.1	99.9	1.8	0.1	99.9
6.0	1.1	0.2	100.0	1.7	0.2	99.9	1.7	0.2	99.9

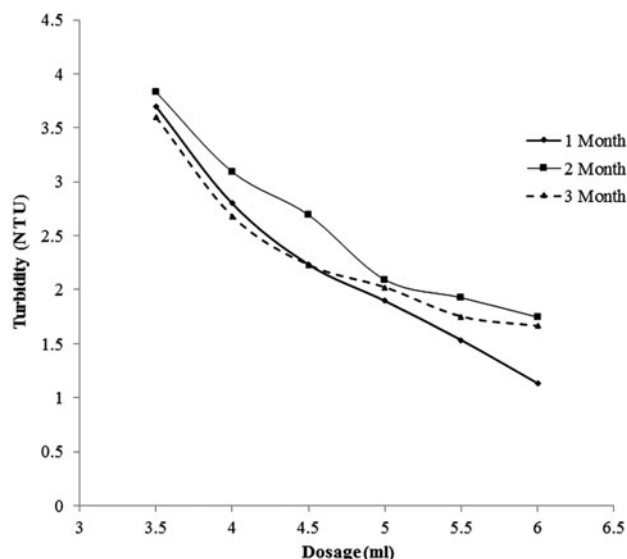


Fig. 3. Performance of coagulant solution by three months.

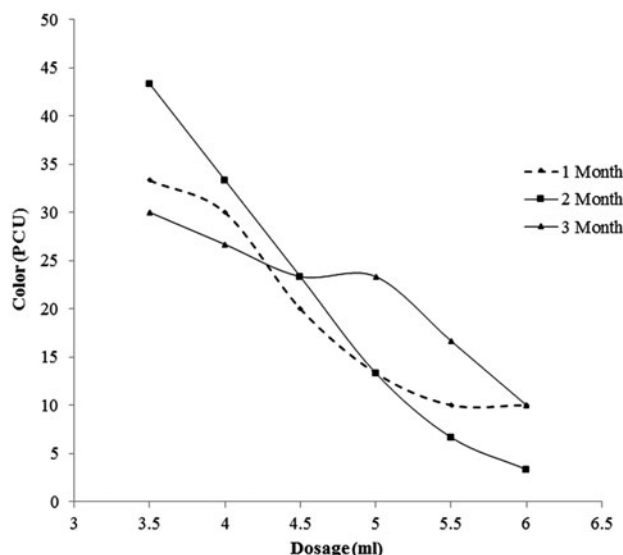


Fig. 5. Performance of coagulant solution by three months.

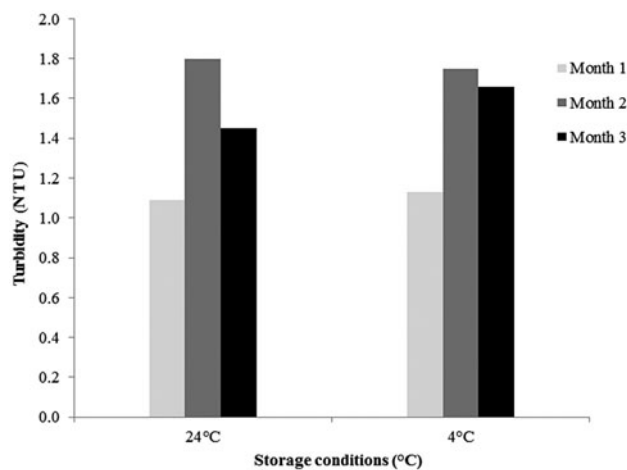


Fig. 4. Effect of temperature in storage process in coagulant solution.

samples were taken for month 1, 2, and 3 with color values of 5,350, 4,050, and 8,900 PCU, respectively. Results are shown in Table 4.

Results showed that color removal for the evaluated period was >99% with no statistical variation ($p < 0.05$). Dosage response on color removal during the evaluated period is presented in Fig. 5.

Maximum color removal percentage was 99.8% when a dosage of 6 ml of coagulant solution (S3) was added. No significant variations were found ($p < 0.05$). These results showed that coagulant solution (S3) was very efficient for color removal during the period of evaluation. Although, color concentration from Month 2 and Month 3 were significantly different (4,500–9,000 PCU), color removal reached >99% with a dosage of 6 ml of (S3) in both cases.

Table 4
Evaluation of color removal after three months of storage at 4°C

Dosage (ml)	Solution 1			Solution 2			Solution 3		
	Final Color Average 4°C (PCU)	STD	Color removal (%)	Final Color Average 4°C (PCU)	STD	Color removal (%)	Final Color Average 4°C (PCU)	STD	Color removal (%)
3.5	33.3	5.8	99.4	43.3	5.8	99.2	30.0	10.0	99.4
4.0	30.0	10.0	99.4	33.3	5.8	99.4	26.7	23.1	99.5
4.5	20.0	10.0	99.6	23.3	5.8	99.6	23.3	20.8	99.6
5.0	13.3	15.3	99.8	13.3	5.8	99.8	23.3	20.8	99.6
5.5	10.0	10.0	99.8	6.7	5.8	99.9	16.7	15.3	99.7
6.0	10.0	0.04	99.8	3.3	5.8	99.9	10.0	10.0	99.8

4. Conclusions

Turbidity removal efficiency was not affected by oil extraction. However, oil extraction increases the complexity of the process. Less dosage volume was required and turbidity and color efficiency removal was improved when salt was added to coagulant solutions. No significant difference ($p < 0.05$) on turbidity and color removal was found between coagulant solution storage (S3) at 24°C (room temperature) and 4°C. Storage time of coagulant solution did not affect turbidity and color removal efficiency negatively. Coagulant solution of *M. oleifera* (S3) was found to be very efficient on polluted waters with high concentration of color. Finally, it was found that oil extraction did not affect coagulation efficiency; instead, it is recommended to extract oil before solution preparation to use as an added value to other applications (edible oil).

Acknowledgment

This research was supported by the University of Medellin. We would greatly like to thank GEMA laboratory for providing us their facilities. Finally, we would like to thank Juan David Cortez for their support during this research study.

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