

Preservation of Coagulation Efficiency of *Moringa oleifera*, a Natural Coagulant

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Abstract In recent years, there has been an interest to use *Moringa oleifera* as the natural coagulant due to cost, associated health and environmental concerns of synthetic organic polymers and inorganic chemicals. However, it is known that *M. oleifera* as the natural coagulant is highly biodegradable and has a very short shelf life. This research was carried out to investigate the effects of storage temperature, packaging methods, and freeze-drying on the preservation of *M. oleifera* seeds powders. Non freeze-dried *M. oleifera* was prepared into different packaging namely open container, closed container and vacuum packing, whilst, freeze-dried *M. oleifera* was stored in closed container and vacuum packing. Each of the packaging was stored at room temperature (30 to 32°C) and refrigerator (4°C). The turbidity removal efficiencies of stored *M. oleifera* were examined using jar test at monthly interval for 12 months. The results indicated that non freeze-dried *M. oleifera* kept in the refrigerator (4°C) would preserve its coagulation efficiency. In addition, closed container and vacuum packing were found to be more appropriate for the preservation of non freeze-dried *M. oleifera*, compared to open container. Freeze-dried *M. oleifera* retained its high coagulation efficiency regardless the storage temperature and packaging method for up to 11 months. Besides, higher increment in zeta potential values for water coagulated with freeze-dried *M. oleifera* indicated the higher frequency of charge neutralization and better coagulation efficiency of freeze-dried *M. oleifera*, compared to non freeze-dried seeds. As a coagulant, *M. oleifera* did not affect the pH of the water after treatment.

Keywords: *Moringa oleifera*, storage duration, storage temperature, packaging method, turbidity removal, freeze-drying

INTRODUCTION

Coagulants are used in water treatment processes for turbidity removal and are classified into natural, inorganic and synthetic organic polymer [1]. However, serious drawbacks of using inorganic coagulants have been documented by previous researchers [2-6]. Besides, it has also been reported that monomers of some synthetic organic polymers such as acrylamide have neurotoxicity and strong carcinogenic properties [7]. In contrast, naturally occurring coagulants are biodegradable and are presumed safe for human health [1].

Seeds from *Moringa oleifera* have been shown to be one of the most effective primary coagulants for water treatment. *M. oleifera* belongs to small, fast-growing, drought deciduous tree. Fully mature, dried seeds are round or triangular shaped, the kernel being surrounded by a lightly wooded shell with three paper wings [8]. The

active component in aqueous *M. oleifera* extracts [9,10] involves in the coagulation activities is reported to be dimeric cationic protein [11]. The coagulation mechanism of the coagulant protein has been described as adsorption and charge neutralization [11,12] and interparticle bridging [13].

Although there are many studies been carried out on *M. oleifera*'s efficiency, the extensive studies on storage condition of the *M. oleifera*'s seeds and its effluence on performance in coagulation have not been reported. Katayon *et al.* [14] have investigated the influence of storage conditions on the coagulation efficiency of *M. oleifera* stock solution. In their study the aqueous extract of *M. oleifera*'s seeds was kept in different storage durations and temperatures. Their finding indicated that the coagulation efficiency of *M. oleifera* stock solution decreased as storage duration increased [14]. Results of a recent study by Katayon *et al.* [15] revealed that the coagulation efficiency of *M. oleifera*'s seeds declined as storage duration increased from 1 to 3 and 5 months. However, the influence of storage temperature was found to be insignificant for the duration of 5 months.

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Table 1. Storage conditions for the preservation of *M. oleifera* seeds powders

Type of <i>M. oleifera</i> seeds powders	Storage condition	
	Packaging methods	Temperature
Non freeze-dried <i>M. oleifera</i> seeds powders	Open container (100-mL beaker)	Refrigerator (4°C) Room temperature (30~32°C)
	Closed container (50-mL vial)	Refrigerator (4°C) Room temperature (30~32°C)
	Vacuum packing (Vacuum bag with aluminium foil)	Refrigerator (4°C) Room temperature (30~32°C)
Freeze-dried <i>M. oleifera</i> seeds powders	Closed container (50-mL vial)	Refrigerator (4°C) Room temperature (30~32°C)
	Vacuum packing (Vacuum bag with aluminium foil)	Refrigerator (4°C)
		Room temperature (30~32°C)

In the light of the above observation an extension study on the storage of *M. oleifera* was found to be essential in order to ascertain the proper preservation condition to enhance longer shelf life. The proper preservation condition would promise the minimum degradability of the active components and perform the complete mechanism of coagulation to clarify the turbid water. *M. oleifera* with longer shelf life would be more competitive with the conventional chemical coagulants and have better commercialization value. Therefore, this paper is aimed to examine the influence of freeze drying technique on preservation of *M. oleifera* seeds, which are kept in different storage temperatures up to 12 months.

MATERIALS AND METHODS

Preparation of Water Sample

Experiments were carried out using the synthetic turbid water prepared by adding laboratory grade kaolin (R&M, UK) into distilled water [15]. The kaolin suspension was used as stock solution for the preparation of water samples at high turbidity of 200 Nephelometric Turbidity Unit (NTU).

Preparation of *M. oleifera* Seeds Powders

The dry pods of *M. oleifera* were collected from Seri Serdang, Malaysia. The seeds were dried in the oven (Mettler ULE 400, Germany) for 48 h at 40°C. A rice husk removing machine (Satake Rice Machine THU class 35A, Japan) was used to remove the hulls and wings from the kernels. The kernels were ground into a medium fine powder with domestic food blender (Moulinex, Malaysia).

Preparation of Freeze-dried *M. oleifera* Seeds Powders

Different batch of *M. oleifera* dry pods were collected from Serdang Raya, Malaysia. The seeds were processed into powders using the working procedures above. *M. oleifera* seeds powders were prefrozen at -30°C in the

freezer (Sanyo, Chest Freezer MDF435, Japan) for 24 h. The frozen samples were loaded onto a freeze-dryer (Benhay, SB4, UK) and the freeze-drying process was carried out at -40°C for 60 h [16,17].

Storage of Non Freeze-dried and Freeze-dried *M. oleifera* Seeds Powders

The non freeze-dried and freeze-dried *M. oleifera* seeds powders were stored in the different storage temperature and packaging as shown in Table 1. The effects of storage durations were investigated for 12 months. Jar test was carried out to evaluate the coagulation efficiency of each *M. oleifera* groups at monthly intervals.

Preparation of *M. oleifera* Seeds Extract (Stock Solution)

About 1 g of *M. oleifera* powder was mixed with 40 mL distilled water in a beaker. The mixture was blended using domestic blender (Moulinex) for 1 min at high speed in order to extract the active ingredient of *M. oleifera*. The suspension was filtered through muslin cloth. The filtrate was then made up using distilled water to 100 mL to give a stock solution of 10 g/L of *M. oleifera* seeds. The stock solution was used for jar test trials to determine optimum dosages and to run the coagulation tests.

Coagulation Efficiency Test

Coagulation efficiency of each *M. oleifera* group was verified by the jar test. Six beakers were filled with 500 mL of synthetic turbid water and agitated at pre-selected mixing intensity and mixing duration [15]. After settling, 30 mL of the sample was taken from the middle of each beaker using a pipette and placed in small beaker for further analysis.

Analytical Method

Turbidity measurements were conducted using Turbidimeter (Hach, 2100AN, USA). The pH of samples

was measured using Bench pH meter (Cyberscan pH 500, Singapore). The zeta potentials were measured with Zeta-Meter 3.0+ (Zeta-Meter 3.0+, ZM3-001, USA).

Statistical Analysis

The analysis of the interaction between coagulation efficiency of *M. oleifera* kept in different conditions and the storage duration were performed using one-way ANOVA in the SPSS software programme (Pro Series 2005, V13). Tukey's honestly significant difference (Tukey's HSD) test was selected to run the multiple comparison tests and range tests. Differences between the means of samples were analyzed by the least significant different at a probability level of 0.05.

RESULTS AND DISCUSSION

Optimization of *M. oleifera* Dosages

The results from dosage optimization experiments indicated that the optimum dosage for non freeze-dried *M. oleifera* collected from Seri Serdang is 120 mg/L. For the case of freeze-dried *M. oleifera* collected from Serdang Raya, optimization was done before and after freeze-drying and results showed that freeze-drying did not significantly affect the optimum dosage requirement. The optimum dosage of *M. oleifera* before and after freeze-drying was 260 and 240 mg/L, respectively.

The difference in optimum dosage of *M. oleifera* before freeze-drying (120 and 260 mg/L) is attributed to the usage of *M. oleifera* seeds collected from different geographic locations. According to Narasiah *et al.* [18], seeds from different sources may exhibit varying coagulation performance resulted by the differences in the protein content and development of the seed. This is also in agreement with the findings of Muyibi and Evison [10] and Katayon *et al.* [14]. Muyibi and Evison [10] documented the optimum dosages of about 100 and 50 mg/L for moderate to high turbidities (250-550 NTU) while Katayon *et al.* [14] showed the optimum dosage of 300 mg/L in treating high turbid water of 194 NTU.

Effect of Storage Duration and Preservation Condition on Coagulation Efficiency of Non Freeze-dried *M. oleifera*

Fig. 1 shows the results of turbidity removal using non freeze-dried *M. oleifera* seeds powders kept in open container, closed container, and vacuum packing at room temperature and refrigerator for 12 months.

Effect of Storage Duration

The efficiencies in turbidity removal of *M. oleifera* seed decreased with an increase in storage duration. However, the rates of deterioration were different depending on the condition of storage. *M. oleifera* seeds powders stored in open container at room temperature hardly preserved its coagulation efficiency, compared to those stored in other

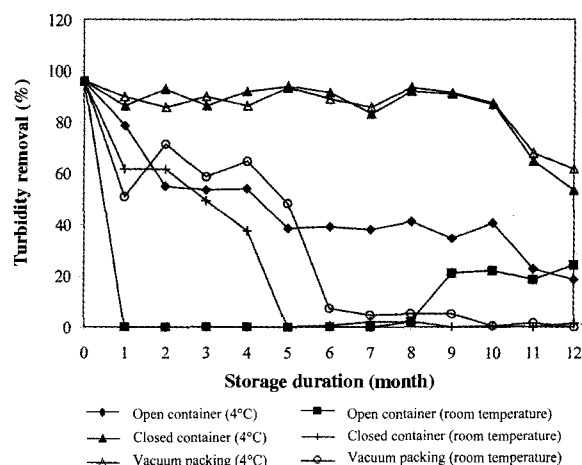


Fig. 1. Turbidity removal efficiency of non freeze-dried *M. oleifera* seeds powders kept in various conditions.

conditions. The percentage of turbidity removal dropped significantly ($p < 0.05$) from the initial value of 95.7% to zero in the first month, marking the end of shelf life for *M. oleifera* as the coagulant. However, there was a significant ($p < 0.05$) increment of about 21% in the turbidity removal from the ninth to twelfth month. The increment of percentage in turbidity removal coincided with the reduction in pH from the average of 6.2 to 5.4. The pH reduction was likely to be the result of organic acids which were produced due to microbial decompositions of organic compounds in the *M. oleifera* seeds [19] during the storage. The presence of oleic acid, palmitic acid, stearic acid, and behenic acid in *M. oleifera* seeds has been documented by Abdulkarim *et al.* [20]. On the other hand, according to Ndabigengesere *et al.* [11], the mechanism of coagulation with *M. oleifera* appears to consist of adsorption and neutralization of the colloidal charges. The produced organic acids may contribute to the charge neutralization and adsorption in the coagulation activity and subsequently increase the percentage in turbidity removal. The interaction between organic acids and colloid has been reported by Nianqiang *et al.* [21].

M. oleifera kept in the open container in refrigerator showed retention of coagulation efficiency at a moderate rate, where the turbidity removal ranged from 95.7 to 18.6% within the 12 months storage duration. The results also indicated that the percentage of turbidity removal of *M. oleifera* kept in closed container and vacuum packing at room temperature decreased significantly ($p < 0.05$) in the fifth and sixth month, respectively. Whilst, *M. oleifera* kept in closed container and vacuum packing in refrigerator retained their efficiency on turbidity removal at a percentage of 95.7 to 83.0% throughout the 10 months storage duration. The efficiency dropped significantly ($p < 0.05$) in the eleventh and twelfth month to the range of 53 to 68%.

In summary, coagulation efficiency of non freeze-dried *M. oleifera* seeds decreased as storage duration increased. This result is in agreement to those reported by Katayon *et al.* [15]. They have documented that an increase in

storage duration of *M. oleifera* seeds from 1 to 3 and 5 months decreased their coagulation efficiency.

Effect of Storage Temperature

Comparison between *M. oleifera* kept at room temperature (30–32°C) and in refrigerator (4°C) revealed that there was a significant difference ($p < 0.05$) between their coagulation efficiency. Refrigerator was found to be more effective in preserving the coagulation efficiency of *M. oleifera* especially in the closed container and vacuum packing. *M. oleifera* kept in closed container and vacuum packing in refrigerator retained the coagulation efficiency at 83% and above within 10 months of storage duration. This result agreed with Kader [22] that storage temperature influences the rate of many deteriorative processes and, in practice, temperature is the most important factor in maintenance of product quality. In addition, as reported by Hawaii Conservation Alliance [23], the key to successful storage of seeds is control of seeds moisture and temperature. This showed that lower storage temperature may preserve the quality of seeds better.

Katayon *et al.* [15] kept the *M. oleifera* seeds for 5 months and monitored the turbidity removal efficiency of *M. oleifera* seeds after 1, 3, and 5 months of storage duration. They found that coagulation efficiency of *M. oleifera* was independent of storage temperature. The difference between their findings with this study could be due to difference in sources of *M. oleifera*. *M. oleifera* used in previous study and those used in current study were collected from different geographical locations and season. According to Narasiah *et al.* [18], seeds from different sources exhibit varying coagulation performance resulted by the differences in the protein content and development of the seed.

Effect of Packaging Methods

M. oleifera kept in open container did not retain its coagulation efficiency neither in the case of storage in room temperature nor refrigerator. The results revealed that storage in closed container and vacuum packing were more appropriate than open container in preventing the degradation of *M. oleifera*. This is critical especially in the refrigerator (4°C) as the statistical analysis indicated that coagulation performance of *M. oleifera* kept in open container at 4°C was significantly ($p < 0.05$) lower than *M. oleifera* kept in closed container at 4°C and vacuum packing at 4°C.

In the case of the *M. oleifera* seeds which were kept in open container the exposure to the environment may cause the degradation of the active agents in the seeds. According to Ndabigengesere *et al.* [11], the active components of *M. oleifera* are proteins. On the other hand protein structures are highly dependent upon the environment. They would assume different conformation and would be denatured as the environmental conditions change. Main external factors which are responsible for denaturation and changes in protein structure are pH, temperature, pressure, and the increase of interfacial surfaces [24]. The changes in protein structure include changes in phase transitions. Proteins have generally four

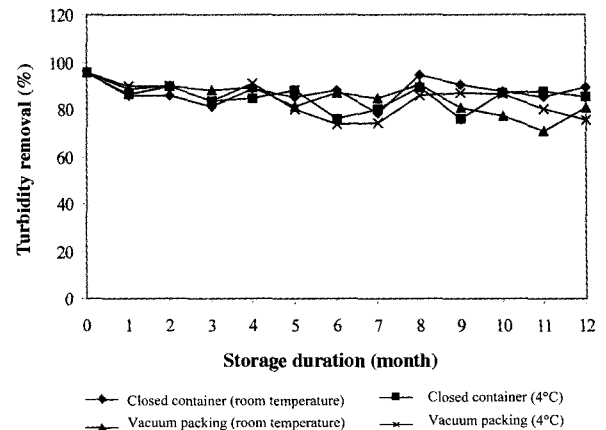


Fig. 2. Turbidity removal efficiency of freeze-dried *M. oleifera* seeds powders kept in various conditions.

phases denoted as immobile, moderately mobile, mobile, and very mobile [24]. Therefore, it could be concluded that exposure to ambient temperature resulted some denaturation of the proteins which became difficult to solubilize [11] and less efficient in coagulation.

Effect of Storage Duration and Preservation Condition on Coagulation Efficiency of Freeze-dried *M. oleifera*

Effect of Storage Duration

Freeze-drying is the common preservation process especially in the aspect of food. The freeze-dried *M. oleifera* seeds powders was found to be more resistant to the changes or alteration of physical, chemical, and biological characteristic during storage. The coagulation efficiencies of freeze-dried *M. oleifera* stored in varying conditions were within 70.0 to 94.3% throughout 12 months storage duration as shown in Fig. 2. The results revealed that there was generally no significant difference ($p > 0.05$) between the coagulation efficiency at different storage duration.

Effect of Storage Temperature and Packaging Methods

As shown in Fig. 2, there was no significant difference ($p > 0.05$) between coagulation efficiency of freeze-dried *M. oleifera* stored at room temperature (30–32°C) and in refrigerator (4°C) neither in the case of closed container nor vacuum packing. Besides, packaging method also did not have significant effect on the preservation of freeze-dried *M. oleifera* as the coagulation efficiency of *M. oleifera* was almost constant in the case of closed container and vacuum packing.

The result is in agreement with the study reported by Ndabigengesere and Narasiah [25] which stated that the lyophilized protein powders of *M. oleifera* remained equally active in coagulation even after a storage period of one year in a plastic bottle without any special precaution. They [25] purified the proteins from the water extract by precipitation, dialysis, ion-exchange, and lyophilization. The end product was a stable white protein powders. However, compared to costly and time consuming protein purification, freeze drying may be the economically feasible

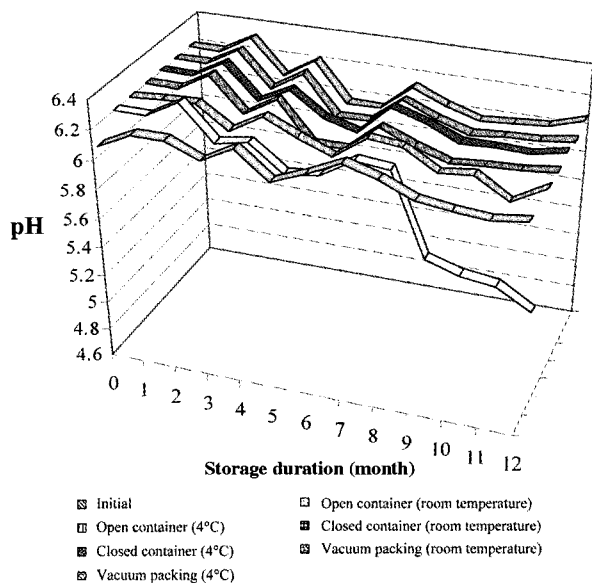


Fig. 3. pH value of water samples treated with non freeze-dried *M. oleifera* stored in different storage durations and conditions.

way to preserve the *M. oleifera* seeds.

Comparison between Coagulation Efficiency of the Non Freeze-dried and Freeze-dried *M. oleifera* Seeds Powders

Comparison between the turbidity removal efficiency of non freeze-dried and freeze-dried *M. oleifera* seeds powders revealed that freeze-drying had positive effect on the preservation of *M. oleifera* in term of coagulation efficiency. The coagulation efficiency of the non freeze-dried *M. oleifera* kept in closed container and vacuum packing degraded significantly to 7% or less in the room temperature after 5 and 6 months, respectively. In contrast, the freeze-dried *M. oleifera* preserved its efficiency as coagulant above 70% for 12 months storage duration in both the closed container and vacuum packing.

On the other hand, the difference between coagulation efficiency of non freeze-dried and freeze-dried *M. oleifera* kept in refrigerator in varying packaging were insignificant within the storage duration except eleventh and twelfth month. The percentages of turbidity removal for non freeze-dried *M. oleifera* kept in closed container (83 to 96%) and vacuum packing (85 to 96%) decreased significantly in eleventh and twelfth month to the range of 53 to 67% (Fig. 1). Whilst for the freeze-dried *M. oleifera*, the turbidity removal did not change throughout the 12 months storage period and remained at the range of 76 to 95% for the case of closed container and 74 to 95% for the case of vacuum packing (Fig. 2). In conclusion, freeze-drying was found useful to preserve the efficiency of *M. oleifera* as natural coagulant.

Effect on pH

Coagulation with the freeze-dried or non freeze-dried

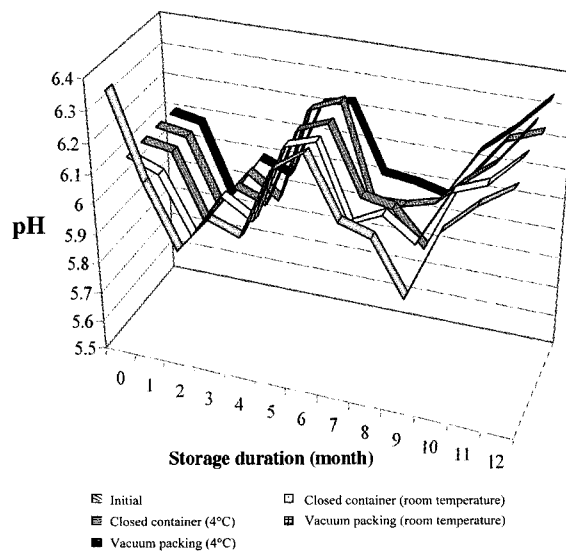


Fig. 4. pH value of water samples treated with freeze-dried *M. oleifera* stored in different storage durations and conditions.

M. oleifera did not significantly affect the pH of treated water samples. The pH values of the raw water and water samples coagulated with *M. oleifera* varied within 5.9 to 6.4 as shown in Figs. 3 and 4. This result is in agreement with Jahn [9] and Olsen [26] that *M. oleifera* coagulants do not change the pH of the water being treated. Therefore, turbidity removal efficiency (Figs. 1 and 2) could not be related to the pH of the water samples. A slight increase and decrease in pH of water sample after coagulation may be due to hydrogen ions of weak acidity of *M. oleifera* stock solution which balanced the hydroxide ions in the raw water.

According to Bina [27], coagulation of kaolin suspension in distilled water using *M. oleifera* was not significantly affected in the pH range of 7 to 9, but that adsorption was high at pH 5 to 6 and the maximum removal of turbidity was however below pH 6 and above pH 9. This is in agreement for the cases of coagulation with *M. oleifera* kept in open container at room temperature from 9 to 12 month. The pH of water samples after coagulation was reduced to the range of 5.3 to 5.5 (Fig. 3). Thus the higher percentages of turbidity removal from water treated with this batch of *M. oleifera* (Fig. 1) is related to lower residual pH as recorded in Fig. 3. The reduction in pH might due to the presence of various organic acids in the *M. oleifera* [20] which are produced through the microbial decomposition of organic compounds [19]. These organic acids are known to take part in charge neutralization [21], thus increasing the coagulation efficiency.

Effect on Zeta Potential

Zeta potential can serve as an excellent tool for coagulation dosage control and also the quick check for coagulation performance. If the turbidity particles have a charge, these particles will move in the field with a speed and direction which is easily related to their zeta potential

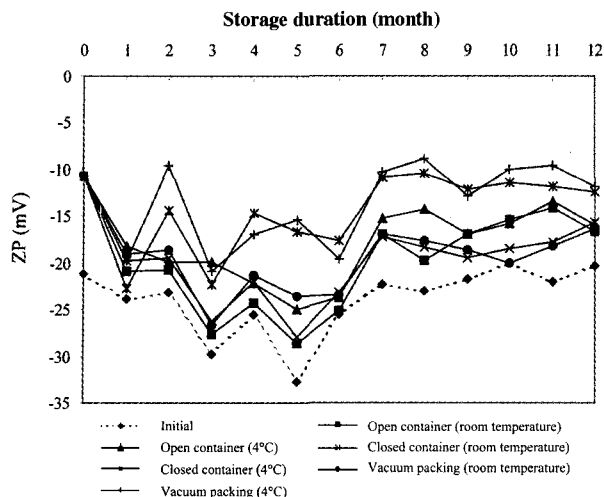


Fig. 5. Zeta potential of water samples coagulated with non freeze-dried *M. oleifera* kept in different storage durations and conditions.

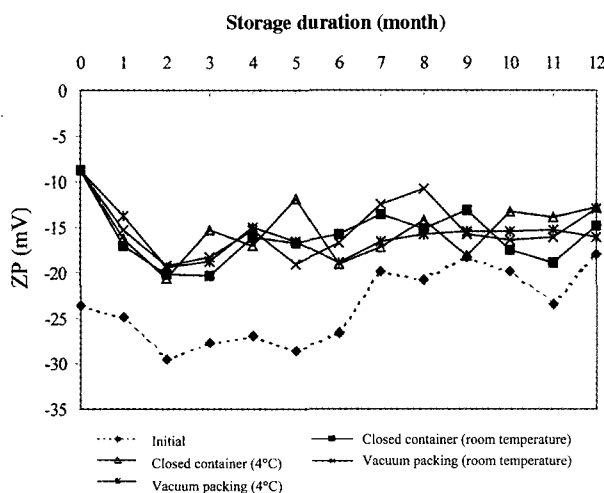


Fig. 6. Zeta potential of water samples coagulated with freeze-dried *M. oleifera* kept in different storage durations and conditions.

[28]. The substantial increase in the absolute zeta potential after coagulation indicates the occurrence of charge neutralization. In other words, the increment in zeta potential shows the presence of coagulation.

Zeta potential of the raw water samples was measured and the initial zeta potential values varied from -17.4 to -32.7 mV (Figs. 5 and 6). The results are in agreement with the previous study which stated that practically all aqueous colloids are electronegative, with the general range of zeta potential between -14 to -30 mV [28].

Figs. 5 and 6 showed the zeta potential of water samples coagulated with non freeze-dried and freeze-dried *M. oleifera*, respectively. The zeta potential values of water samples coagulated with non freeze-dried *M. oleifera* stored at different storage duration and conditions varied between -10.7 to -28.6 mV. Whilst, for water samples

coagulated with freeze-dried *M. oleifera* kept in varying storage duration and conditions, the values of zeta potential were at the range of -10.8 to -20.6 mV. From the results it can be seen that water samples coagulated with freeze-dried *M. oleifera* showed higher increment in zeta potential. The increase of zeta potential indicated the higher frequency of charge neutralization and better coagulation efficiency of freeze-dried *M. oleifera*.

CONCLUSION

Storage temperature and packaging methods are shelf-life limiting factors for *M. oleifera* seeds. Non freeze-dried *M. oleifera* seeds preserved its coagulation efficiency in the refrigerator (4°C), however, in the room temperature ($30\sim 32^{\circ}\text{C}$), it deteriorate rapidly.

There is an interaction effect of packaging methods and temperature on the preservation of non freeze-dried *M. oleifera* seeds powders. Non freeze-dried *M. oleifera* kept in the refrigerator may retain its effectiveness as coagulant with provision that it is kept in a close container or a vacuum packing.

Freeze-drying is advantageous technique to preserve the coagulation efficiency of *M. oleifera* seeds powders regardless the effect of temperature and packaging methods.

Compared to non freeze-dried seeds, freeze dried *M. oleifera* seeds increased the zeta potential values of water thus revealing higher frequency of charge neutralization and better coagulation efficiency.

In coagulation, *M. oleifera* hardly affects the pH of water samples.

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