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Effect of the packaging and storage conditions on the coagulation activity of spray-dried salt-extracted *Moringa oleifera*

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ABSTRACT

Moringa oleifera is one of the natural coagulants considered as an alternative to synthetic coagulants. Several studies were carried out on the usage and extraction of this natural coagulant. In this study, the coagulation activity of spray-dried salt-extracted *M. oleifera* seeds powder was investigated under different storage conditions, packaging forms and storage duration. The spray-dried salt-extracted *M. oleifera* seeds powder was stored at room temperature (29°C) and refrigerator temperature (3°C); under different packaging forms; closed container and vacuum packed stored for 6 weeks. Optimization of spray-dried salt-extracted *M. oleifera* shows that the optimal dosage is half of the nonspray-dried salt-extracted *M. oleifera*. The results of residual turbidity of different packaging and storage conditions of salt-extracted *M. oleifera* show that there was no significant difference between them. The coagualtion activity decreased insiginficantly with the increase of storage duration during the study.

Keywords: Moringa oleifera; Natural coagulant; Salt extraction; Storage condition; Spray drying

1. Introduction

Turbidity removal using different coagulants is one of the important steps in water treatment process [1]. Several coagulants are widely used in conventional water treatment processes and can be classified into inorganic, synthetic organic polymer and natural coagulants [2]. Alum (aluminum sulfate) is most widely used in coagulation [3]. However, recent studies have indicated various serious drawbacks of using aluminum salts, such as Alzheimer's disease and production of large sludge volumes [4]. Several studies have been carried out to introduce the natural coagulants produced or extracted from animals, microorganisms, or plants to replace the use of chemical coagulants [5–7]. Recently, there is an interest in using natural coagulants in water treatment in developing countries [8,9]. Natural coagulants were used before the synthetic chemical coagulants were founded [10]. *Moringa oleifera* seeds are one of the natural coagulants which showed effective coagulation activity [2,3,4,10–13]. Many researches have been done

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towards enhancing the extraction of the active coagulant in *M. oleifera* seeds and to understand the mechanism of coagulation of this coagulant; For example, Ndabigengesere et al. [14] carried out a study on the active agent and mechanism of coagulation using *M. oleifera* seed as a coagulant. The study showed that the active agents are dimeric cationic proteins of molecular weight of 13 kDa and isoelectric points ranging between 10 and 11. The adsorption and neutralization of the colloidal charges were reported to be the mechanisms of coagulation. Okuda et al. [2,3,15] carried out a number of experiments to develop an extraction method to extract the active components from *M. oleifera* seeds by using salt solutions rather than water. The study reported that the salt-extracted *M. oleifera* seeds coagulant showed better coagulation activity with dosages 7.4 times lower compared to the distilled water-extracted one. On the other hand, the purified and isolated active component from an aqueous salt extraction was not a protein, polysaccharide or lipid, but an organic polyelectrolyte with a molecular weight of about 3.0 kDa. This implies that the water and salt extract may be of a different nature. Ghebremichael et al. [16] carried out a study on the purification of water and salt-extracted *M. oleifera* seed coagulant using ion exchange; he found that the active component is a cationic protein with a pI greater than 9.6 and a molecular mass less than 6.5 kDa.

Katayon et al. [17] investigated the effects of storage temperature, packaging methods, and freeze-drying on the preservation of coagulation efficiency of *M. oleifera* seed powders; the results showed that freeze-dried *M. oleifera* retained its high coagulation activity regardless of the storage temperature and packaging method during the study duration. Katayon et al. [17] identified the advantage of the freeze-drying in preserving the coagulation activity of *M. oleifera* seed powder extracted using distilled water. In the present study the spray drying process used as a farther process in the extraction of the effective coagulant in the *M. oleifera* seed salt solution under different operational parameters to study the effectiveness of the spray-dried salt-extracted *M. oleifera* under different storage conditions and packaging forms. Spray drying is the most frequently used technique for dehydration, it is an effective method for preserving biological products because the thermal damage is limited since it does not involve a severe heat treatment, and it allows storage of powders at an ambient temperature [18].

Spray drying due to its simplicity and fast processing has been used in a lot of industrial applications [19]. Spray drying is considered as an efficient drying method because of the large surface area availability for mass and heat transfer during the atomizing of the solution to very small droplets [20].

2. Materials and methods

2.1. Collection of M. oleifera

M. oleifera dry pods were collected from the campus of the University Putra Malaysia. Only good quality pods were collected. Good quality seeds were identified and used raw for extraction. After every collection the wing seeds were dried in the oven for 24 h at 50˚C and stored in desiccators at our laboratory at room temperature.

2.2. Preparation of synthetic turbid water

In all coagulation experiments, samples of turbid water were prepared by adding kaolin produced by R&M Chemicals into tap water [17]. Ten grams of kaolin were added to 1 L of tap water. This suspension was stirred for 1 h at 120 rpm speed for uniform dispersion of kaolin particles by using a Stirrer (R1333, IKA Works, Malaysia) and a Mixer (IKA RW 20 n S2, IKA Works, Malaysia) and then was allowed to stand for 24 h to allow for complete hydration of the particles. This suspension was used as the stock solution to prepare synthetic turbid water of 200 ± 5 NTU initial turbidity for the jar test experiment.

2.3. Preparation of M. oleifera seed extracts for the spray dryer process

The winged seed cover was shelled just before the extraction. The kernel was ground to a fine powder by using a kitchen blender (National De-Luxe Kitchen Family, Japan). 300 g of the fine powder was placed in a beaker containing 1000 mL of 1.0 M NaCl solution and the mixture was blended using a kitchen blender (National De-Luxe Kitchen Family, Japan) for 5 min to allow the extraction of the active component in *M. oleifera* seeds. The suspension was then filtered using a muslin cloth and the filtrate was referred to as the stock solution for spray drying.

2.4. Spray drying process

The spray drying process was done under eight different operational parameters or configurations; in each configuration 100 mL of *M. oleifera* stock solution was used to feed into the spray dryer (BUCHI Mini Spray Dryer B-191) and the resulted powder was col-

Table 1
Spray drying operational parameters

Configuration	Inlet temp. (℃)	Actual temp. (℃)	Outlet temp. (℃)	Pump rate (%)	Aspiration rate (%)
A	120	119–120	90–70	20	50
B	135	134–135	65–72	25	50
C	125	119–125	68–71	20	50
D	130	128–130	75–69	25	50
E	115	114–115	73–75	10	50
F	80	77–79	41–45	20	50
G	100	99–101	50–55	20	50
H	110	108–109	58–60	20	50

lected and packed into closed container, covered with the aluminum foil and stored in a refrigerator at 3℃.

In classical spray dryers the outlet temperature cannot be established at a fixed value; it depends on the inlet temperature, drying gas flow rates, chamber dimensions, and feed flow rate [21].

Since the active component in *M. oleifera* seeds is pertinacious [16,14,22], we suggest the selected parameters to according to literature in spray drying of protein [23–29].

The process parameters were the following: inlet temperature (80–135℃); aspiration rate 50%; pump rate (10–25%); and the outlet temperature was monitored and recorded and it ranged between 41 and 90℃ as shown in Table 1.

2.5. Packaging and storage of spray-dried salt-extracted M. oleifera seeds

After running the first coagulation test (week 0) for spray-dried *M. oleifera* the powder for all configurations were packed into two different packaging forms (closed container, vacuum packed and then stored in two different storage conditions (room temperature 29℃ and refrigerator at 3℃. All the packed forms were covered with the aluminum foil and the closed containers were sealed with laboratory film.

A Vacuum Packaging Apparatus (MASTERPACK, NEW DIAMONED VAC, Model No J-V01M, USA) was used for packaging. The coagulation tests were done for all packaging and storage conditions every week from week 0 to 6.

2.6. Coagulation experiment

A Jar test (BIBBY Stuart Scientific, UK) was used to carry out the coagulation test. The jar test consists of three stages: rapid mixing, slow mixing and settling.

An amount of 300 mL of synthetic turbid water was filled into a 500 mL beaker placed on the slot in a jar tester. During the rapid mixing the coagulant dosages were added into the beaker simultaneously by turning the dosing tubes which were fixed in clips that were made for that purpose. The duration was controlled by stopwatch. After slow mixing the beakers were carefully removed from the jar tester slots and placed in a safe place to allow the settling. Every week test was repeated three times for every configuration and the average was taken as the result of the test. In this study, rapid and slow mixing speeds, time was used according to Katayon et al. [30] as shown in Table 2.

After a settling time of 30 min, the samples were taken 1 cm below the water surface in each beaker, using a pipette. The residual turbidity was measured using a turbidimeter (HACH, Model 2100 AN, USA).

2.7. Coagulation activity of spray-dried salt-extracted M. oleifera

Jar test was used for the coagulation process for the spray-dried salt-extracted *M. oleifera*. The spray-dried salt-extracted *M. oleifera* (0.3 g) was weighed using the analytical balance (model PRECISA 2100 AN, Swiss) and was used to prepare the stock solution for the coagulation test; this amount was used with 200 mL of distilled water to get a 1500 mg/L stock solution concentration. An amount of 300 mL

Table 2
Operating variables used for jar test

Rapid mix velocity (rpm)	100
Rapid mix duration (min)	4
Slow mix velocity (rpm)	40
Slow mix duration (min)	25
Settling time (min)	30

synthetic turbid water of initial turbidity 200 ± 5 NTU was filled into a 500 mL beaker placed on the slot in a jar tester and 8, 12, 16, 24, 32 and 40 mg/L dosages were applied.

2.8. Statistical analysis

One way ANOVA test, with Tukey' HSD test were executed to confirm the significance of variance between the means of residual turbidity of spray-dried *M. oleifera* in different conditions by using SPSS software version 16. All statistical analyses were done with 95% confidence interval ($p < 0.05$).

3. Results and discussion

3.1. Spray drying process

Table 3 shows the different operational parameters (configurations) and the resulting spray-dried *M. oleifera* powder.

The results show that configurations F, G, and H failed to produce a powder. This can be attributed to the operation parameters used that were not suitable to establish the spray drying process (Inlet temperature below 115˚C with fixed pumping and aspiration rates). Ståhl et al. [31] mentioned that aspiration rate, inlet temperature, and pumping rate are the controlling parameters of the spray drying process. For the successful configuration a high inlet temperature from 115 to 135˚C even with the different pumping rates was able to produce the powder.

3.2. Optimization of M. oleifera dosages

The optimization experiments were done for the *M. oleifera* which was distilled water extracted, salt extracted, and spray dried salt extracted. The results from dosage optimization experiments indicated that the optimum dosage for *M. oleifera* that was distilled water extracted is 150 mg/L with 90.7% removal efficiency achieved. For the case of *M. oleifera* that is salt extracted the optimum dosage was 30 mg/L to achieve 95.3% removal efficiency, however for the spray-dried *M. oleifera* salt extracted the optimum dosage was 16 mg/L to achieve 91.05% removal efficiency. The difference in the optimum dosage of *M. oleifera* which is distilled water extracted and salt extracted is attributed to the salt ions that increase the solubility of the active component in *M. oleifera* seed powder solution [15]. On the other hand on comparison of the optimum dosage of spray-dried *M. oleifera* salt extracted (16 mg/L) and the optimum dosage of *M. oleifera* salt extracted (30 mg/L), it is clear that the spray drying process enhances the isolation of the active coagulant so the optimum dosage of spray-dried *M. oleifera* salt extracted was 50% lower than *M. oleifera* salt extracted; however, the comparison of the degree of removal efficiency indicates that the temperature in the spray drying process slightly affects the nature of active coagulant.

3.3. Coagulation performance of spray-dried salt-extracted M. oleifera

The successful spray-dried salt-extracted *M. oleifera* configurations (A-B-C-D-E) were packed into two different packaging forms (closed container, vacuum packed and then stored in two different storage conditions (room temperature 29˚C and refrigerator at 3˚C. the coagulation experiment were carried every week for all configurations under different storage and packaging conditions. Figs. 1–4 show that there was no significant difference between the coagulation performance ($p > 0.05$) of different configurations (A-B-C-D-E) stored in a refrigerator (3˚C) and at room

Table 3
Spray drying configurations and the resulting powder

Configuration	Inlet temp. (˚C)	Actual temp. (˚C)	Outlet temp. (˚C)	Pumping rate (%)	Aspiration rate (%)	Resulted powder (g)
A	120	119–120	90–70	20	50	9.7
B	135	134–135	65–72	25	50	9.92
C	125	119–125	68–71	20	50	9.8
D	130	128–130	75–69	25	50	9.52
E	115	114–115	73–75	10	50	9.6
F	80	77–79	41–45	20	50	Failed[a]
G	100	99–101	50–55	20	50	Failed[a]
H	110	108–109	58–60	20	50	Failed[a]

[a]Failed means no powders formed.

temperature (29˚C) neither in case of closed container nor vacuum packing. The dosage optimization for different configurations under different storage and packaging conditions shows that in the case of a closed container stored in the refrigerator, or at room temperature, and vacuum packed stored in refrigerator (Figs. 1–3); there was no significant difference ($p > 0.05$) between the removal turbidity achieved by the dosages 12, 16, 24, 32 and 40 mg/L for every configuration. The optimum dosage for every configuration under different storage and packaging conditions are shown below in Table 4. However, for the spray-dried salt-extracted *M. oleifera*, vacuum packed, and stored at room temperature (Fig. 4), the results revealed that there was no significant difference ($p>0.05$) between dosage 8, 12, 16, 24, and 32 mg/l for the configurations. The optimum dosage is shown in Table 4.

The performance of all configurations for different storage and packing conditions are shown in Figs. 1–4; Table 4 presents the optimum dosage and the average turbidity removal for every configuration. The results'

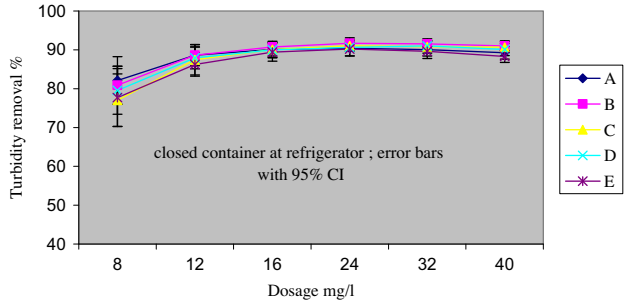

Fig. 1. Average removal turbidity for closed container at refrigerator 3˚C.

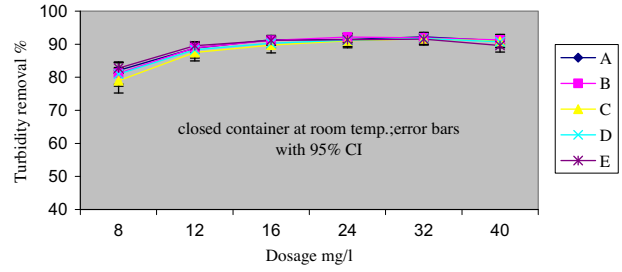

Fig. 2. Average removal turbidity for closed container at room temperature 29˚C.

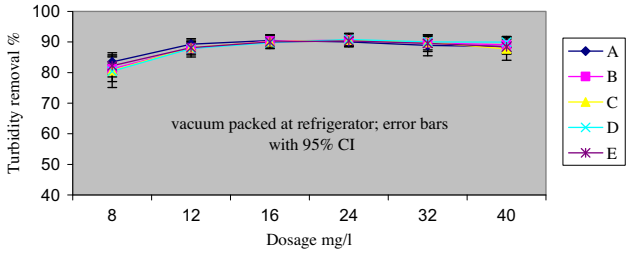

Fig. 3. Average removal turbidity for vacuum packed at refrigerator 3˚C.

Table 4
Summary of the results

Storage condition	Configuration	Optimal dosage (mg/L)	Average turbidity removal (%)
Closed container at refrigerator 3˚C	A	24	90.397
	B	24	91.66
	C	24	91.05
	D	32	90.98
	E	24	90.20
Closed container at room temperature 29˚C	A	32	92.20
	B	24	92.16
	C	32	91.68
	D	32	91.7
	E	32	91.51
Vacuum packed at refrigerator 3˚C	A	16	90.45
	B	24	90.63
	C	24	90.61
	D	24	90.80
	E	24	90.35
Vacuum packed at room temperature 29˚C	A	12	92.35
	B	16	92.06
	C	12	92.31
	D	12	91.34
	E	12	91.21

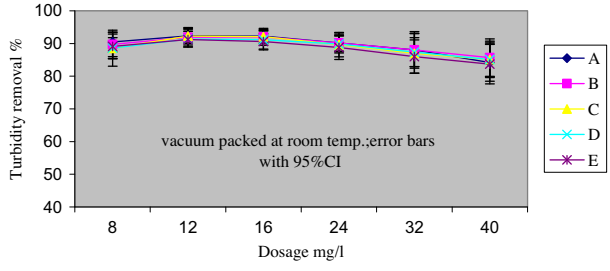

Fig. 4. Average removal turbidity for vacuum packed room temperature 29˚C.

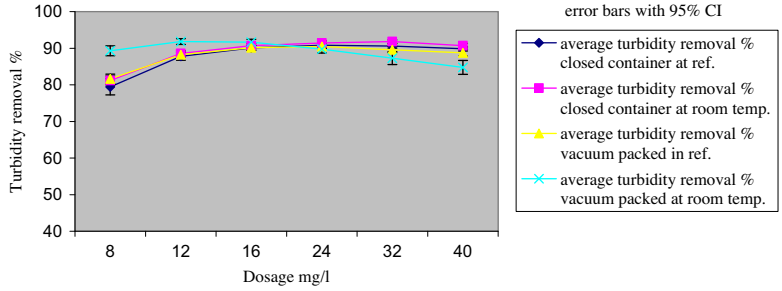

Fig. 5. The average turbidity removal under different storage and packaging conditions.

analysis shows that there was no significant difference ($p > 0.05$) between the performance of all configurations. This reveals that the operational parameters of the spray dying process does not affect the performance of the *M. oleifera* coagulant during the study duration.

3.4. Effect of storage and packaging condition on coagulation performance

For the purpose of comparison of the effect of different storage and packaging conditions the average of all configurations was taken as shown in Fig. 5.

The comparison between the turbidty removal of different packaging forms (closed container and vacuum packing) stored at the same condition (room temperature 29˚C or refrigerator at 3˚C) revealed that there was no significant difference ($p > 0.05$) in the coagualtion performance and the packaging form does not effect the coagulation activity when spray-dried *M. oleifera is* stored at room temperature 29˚C and in a refrigerator at 3˚C and this is in agreement with the study reported by Katayon et al. [17] which stated that packaging method did not have a significant effect on the preservation of freeze-dried *M. oleifera*. However, the comparison between spray-dried *Moringa oleifera* stored at different storage conditions (room temperature 29˚C and refrigerator at 3˚C) and under the same packaging form (closed container or vacuum packing) showed that there was a significant difference ($p<0.05$) between there coagulation activity; closed container and vacuum packing spray-dried *Moringa oleifera* kept at room temperature 29˚C. During the study duration, the last form of packing showed better coagulation activity compared to the closed container and vacuum packing spray-dried *Moringa oleifera* kept in refrigerator at 3˚C. This is likely to be due to the organic acids resulted from the microbial decomposition of organic matter in the spray-dried *Moringa oleifera* during the storage period [32]. Ndabigengesere et al. [14] reported that the mechanism of coagulation using *M. oleifera* consists of adsorption and neutralization of the colloidal charges and the presence of these acids may cause an increment in the charge adsorption and neutralization during the coagulation process which results in an increase in the turbidity removal. The interaction between the colloid and the organic acids has been reported by Wu et al. [33]. But this is not true for long storage duration and this needs to be investigated.

4. Conclusions

The operational parameters of spray drying do not affect the coagulation activity of the spray-dried *M. oleifera*. The coagulation activity is not affected by the packaging condition when it is stored under the same temperature; however, the storage condition (room temperature 29˚C or refrigerator 3˚C) affects the coagulation activity. Room temperature shows better coagulation activity during the study duration. For long duration this may be not true.

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