Current Journal of Applied Science and Technology

40(28): 38-47, 2021; Article no.CJAST.75080
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Optimization of Protein Extraction from *Arachis hypogaea* L. and *Cucumeropsis mannii* Naud. Seeds for Use as Natural Coagulants in Surface Water Clarification

Hermeline Ntalani[1*], Ravelle Duclérine Ngouanou[1] and Lucien Meril Bassinga Moussounda[1]

[1]Unité de Chimie du Végétal et de la Vie, Faculté des Sciences et Techniques, Université Marien NGOUABI, BP 69, Brazzaville, Congo.

Authors' contributions

This work was carried out in collaboration among all authors. Author HN ensured the writing of this document and coordinated the microbiological analyses. Author RDN carried out the clarification tests of the surface water samples taken by Jar-Test. Author LMBM coordinated the analyses on the determination of the protein contents of the extracts of the 3 vegetable species. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2021/v40i2831532
Editor(s):
(1) Dr. Chen Chin Chang, Hunan Women's University, China.
Reviewers:
(1) Tamador Salem Maayah, Jordan.
(2) Kahsay Tadesse, Aksum University, Ethiopia.
Complete Peer review History: https://www.sdiarticle4.com/review-history/75080

Short Research Article

Received 01 August 2021
Accepted 10 October 2021
Published 16 October 2021

ABSTRACT

The aim of this study was to improve the quality of protein extracts from *Cucumeropsis mannii* Naud. and *Arachis hypogaea* L. The evolution of protein content and microbiological quality of aqueous extracts of *A. hypogaea*, *C. mannii* and *M. oleifera* seed powders were monitored over time during protein extraction. The protein contents of the extracts subjected to decantation and those subjected to centrifugation were determined by the Kjeldahl method. The microbiological quality of the different aqueous extracts was evaluated according to the standards NF EN ISO 7932, NF V 08-059 and NF EN ISO 4833-1. The results obtained show protein contents of 40.6 % for *A. hypogaea*, 25.4 % for *C. mannii* and 36.5 % for *M. oleifera,* after 24 h of decantation. The protein contents of the centrifuged aqueous extracts were higher and 55.9%, 36.7% and 37.6% for

**Corresponding author: E-mail: tabunahermeline@gmail.com;*

A. hypogaea, *C. mannii* and *M. oleifera* extracts respectively. The results of the microbiological analysis showed that the aqueous extracts of *M. oleifera* contain little microbial load. Significant growth of aerobic flora and *Bacillus cereus* was observed in the decanted extracts of *C. mannii* and *A. hypogaea* from 4 h to 24 h of decantation. Extracts obtained by centrifugation have a lower microbial load, because the treatment time was shorter. The aqueous protein extracts of *A. hypogaea* and *C. mannii* seed powders obtained by centrifugation were of better quality than those obtained by decantation, because they have higher protein contents and lower microbial loads. The water solubility of *A. hypogaea* and *C. mannii* seed proteins was also studied on protein suspensions at 2% at pH 3, 4, 5, 6, 7 and 8. The results obtained showed that adjusting the pH to 7 and 8 could improve the degree of protein extraction for *A. hypogaea* and *C. mannii* seeds.

Keywords: Arachis hypogaea; Cucumeropsis mannii; seeds; protein; coagulants; waters.

1. INTRODUCTION

In sub-Saharan Africa, access to drinking water remains a crucial problem, especially in rural areas. 97% of people in rural areas do not have access to piped water supply [1]. These populations use water for household tasks, which can come from rivers, streams, ponds and wells [2]. The quality of this water can be improved by treating it at home with natural substances with coagulant activity and derived from plants in their environment [3,4]. Goal 6, one of the 17 United Nations Sustainable Development Goals, suggests "ensuring the availability and sustainable management of water and sanitation for all [5]". In the framework of the implementation of this goal, we are conducting research on natural substances of plant origin with coagulant activity. Our goal is to expand the range of natural plant coagulants and to help make simple water sanitation processes available to people in rural areas of developing countries through the use of natural coagulants [6,7]. Seed biopolymers, especially proteins are highly cited in the literature for their coagulant activity in water clarification [8]. Our previous works have highlighted high contents of crude proteins and side-chain charged amino acids in *A. hypogaea* and *C. mannii* seeds [9]. This work also showed the coagulant activity of aqueous solutions of the seed powders of these two plants in the clarification of surface water samples of turbidities 89.45 NTU, 94 NTU and 128.60 NTU, with percentages of turbidity reduction higher than 85% for the 3 treated water samples. However, for the preparation of these solutions, the decantation operation that allows to obtain the aqueous solutions of the powders of the seeds of these plants is very slow and lasts several hours. This fact could favor the proliferation of bacteria in the coagulating solutions and thus alter the quality of the water to be treated. In this study, we analyzed the protein extracts resulting from decantation and those obtained by centrifugation to compare their respective qualities. The evolution of the protein content in the aqueous phases and the microbiological quality of the aqueous extracts were monitored over time during the protein extraction. The water solubility profile of the proteins of the seeds of these plant species was also studied. *Moringa oleifera* and aluminum sulfate are used in this study as reference compounds for their scientifically proven coagulant activity [10,11].

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material consists of the seeds of *A. hypogaea*, *C. mannii***,** and *M. oleifera*. The harvesting was done in the month of November of the year 2018. The seeds of *A. hypogaea* and *C. mannii were* collected near the village Mboulankio located 45 kilometers, north of Brazzaville and those of *M. oleifera*, in the arrondissement n°1 of the city of Brazzaville (Congo).

2.2 Preparation of Coagulating Solutions

The coagulant solutions used in this study were prepared according to the protocol below. Seeds of *A. hypogaea*, *C. mannii* and *M. oleifera were* dehulled, dried and ground. For each type of seed, 100 g of the resulting product was dispersed in 1000 mL of distilled water and the resulting mixture was subjected to magnetic stirring. After 10 minutes, the mixture was filtered through an 80-micron sieve and the filtrate was decanted into a separating funnel. The decantation of the filtrate being very slow, after 24 hours of decantation, 3 phases are observed

in the funnel: an organic phase, an aqueous phase which constitutes the coagulating solution and a deposit. The aluminium sulphate solution was prepared at a concentration of 10 g/L.

2.3 Evolution of the Protein Content of Aqueous Extracts during Extraction

The filtrate obtained by mixing the seed powder of each plant with distilled water was subjected to decantation for 24 h. Samples were taken at 0 h; 0.25 h; 0.5 h; 1 h; 2 h; 4 h and 24 h of decantation to monitor the evolution of protein content in the aqueous phase. The total protein content in the aqueous phase during the extraction of proteins from *A. hypogaea*, *C. mannii* and *M. oleifera* seeds was determined from the nitrogen content obtained according to the Kjeldahl method, with a Tecator TM coupled to a Kjeltec 8400 system, Foss® (NF EN ISO 5983-2). In parallel to the decantation, a centrifugation was carried out. The seed powder of each plant was suspended at a concentration of 5 g/100 mL at pH 7 and then solubilized for 30 minutes at 20°C under agitation. The suspension was then centrifuged for 15 minutes at 4000 G.

2.4 Microbiological Analysis of Aqueous Extracts during Protein Extraction

The microbiological quality of an aqueous extract can change over time. The microbiological quality of aqueous extracts during the extraction of proteins from *A. hypogaea*, *C. mannii* and *M. oleifera* seeds was evaluated by determining the contents of *Bacillus cereus*, yeasts, aerobic microorganisms at 30°C and moulds at 25°C, respectively, according to standards NF EN ISO 7932, NF V 08-059 and NF EN ISO 4833-1. The evaluation of the microbiological quality was carried out for the extracts subjected to decantation and for those subjected to centrifugation. Samples were taken at 0.25 h; 0.5 h; 1 h; 2 h; 4 h and 24 h of decantation, for extracts subjected to decantation.

2.5 Study of the Solubility of the Proteins of *A. hypogaea*, *C. mannii*, and *M. oleifera* Seeds

Protein solubility of *A. hypogaea*, *C. mannii* and *M. oleifera* seeds was studied on protein suspensions at 2% protein content at pH 3, 4, 5, 6, 7 and 8. Protein content was determined by the Kjeldahl method on the supernatant after centrifugation at 15000 G for 10 min.

2.6 Study of the Coagulant Activity

2.6.1 Collection of surface water samples

Surface water samples of turbidity 91.06 NTU and 41.45 NTU were collected from the Djoué River in November 2018 and the Congo River in June 2019, respectively. The geographical coordinates indicate 04°18' 34'' South latitude, 015°13' 36'' East longitude and 270 m above sea level, for the Djoué River and 04°18' 55'' South latitude, 015°12' 34'' East longitude and 256 m above sea level for the Congo River.

2.6.2 Clarification tests on surface water samples with turbidity of 91.06 NTU and 41.45 NTU

The clarification tests of raw water samples of turbidity 91.06 NTU and 41.45 NTU were carried out by Jar-Test [11]. For conducting the Jar-Test, 1000 mL of raw water sample was introduced into the beakers of a Lovibond ET 740 flocculator, followed by the addition of different increasing volumes of the powder solutions of the seeds of *A. hypogaea*, *C. mannii*, *M. oleifera* and aluminum sulfate. After rapid agitation of 180 rpm for 3 minutes and slow agitation of 18 rpm for 20 minutes, the treated water samples were subjected to decantation. After 30 minutes of settling, followed by filtration, the residual turbidity was measured for each beaker with a Turbiquant 1100 R turbidimeter. Three Jar-Test treatments were carried out for each raw water sample treated with the different doses of the powder solutions of *A. hypogaea*, *C. mannii*, *M. oleifera* seeds and aluminium sulphate.

2.7 Statistical Analysis

Statistical analysis was performed on the results of clarification tests of surface water samples of turbidity 91.06 NTU and 41.45 NTU, by calculating standard deviation using Microsoft Excel 2013.

3. RESULTS AND DISCUSSION

3.1 Evolution of the Protein Content in the Aqueous Phases during Extraction

The results of the study of the evolution of the protein content of the aqueous phases during extraction are presented in Fig. 1a. This figure shows that during decantation, the total protein

contents in the aqueous extracts decrease over time. At the beginning of decantation, the protein contents in the different aqueous extracts are 54.9%; 52.2% and 52.8%, respectively for the aqueous solutions of *A. hypogaea*, *C. mannii* and *M. oleifera*. At 0.25 h of settling, the protein contents were 47.7%; 35.7%; 40.4%; at 0.5 h of settling, they were 45.5%; 34.2%; 40.1%; and then 44%; 25.4% and 36.5% at 1 h of settling, for *A. hypogaea*, *C. mannii* and *M. oleifera,* respectively. The decrease in protein content continues gradually over time. After 24 hours of decantation, the protein contents in the aqueous extracts are 40.6% for *A. hypogaea*, 25.4% for *C. mannii* and 36.5% for *M. oleifera*. This result can be explained by the insolubility of some proteins of the seeds of these 3 plants [12,13] at the native pH of the solutions of the powders of these seeds (Fig. 2). The insoluble proteins therefore gradually decant over time and the protein contents of the aqueous extracts decrease. This decrease is more limited for *A. hypogaea* because its proteins are more soluble at pH 7 (Fig.1 a and Fig. 2). For *C. mannii,* the decrease in protein content over time is more significant, because its proteins are less soluble at pH 7 (Fig. 1 a and Fig. 2). Throughout decantation, the different values of protein contents of *M. oleifera* are between those of *A. hypogaea* and *C. mannii* (Fig. 1a). In the centrifuged extracts, the protein contents are 55.9%, 36.7% and 37.6% for *A. hypogaea*, *C. mannii* and *M. oleifera* respectively (Fig. 1b). The uncertainty on the different measurements is 1%.

3.2 Microbiological Analysis of Aqueous Phases during Protein Extraction

The results of microbiological analysis of the different extracts subjected to decantation and centrifugation during protein extraction are presented in Table 1. The observation of this table shows that the aqueous extracts of *M. oleifera* contain little aerobic flora, *Bacillus cereus*, yeasts and moulds. Significant growth of aerobic flora and *Bacillus cereus* is observed in the decanted extracts of *C. mannii* and *A. hypogaea,* from 4 h to 24 h of decantation. In the aqueous extracts of *A. hypogaea* and *C. mannii* obtained by centrifugation, the microbial load is lower: less than 100/g for *Bacillus cereus* and 10/g for yeasts and moulds; 60/g and 1200/g for aerobic flora, respectively for *A. hypogaea* and *C. mannii*. This result can be explained by the shorter treatment time for extracts subjected to centrifugation [14].

3.3 Study of the Solubility of the Proteins of *A. hypogaea*, *C. mannii* and *M. oleifera* Seeds

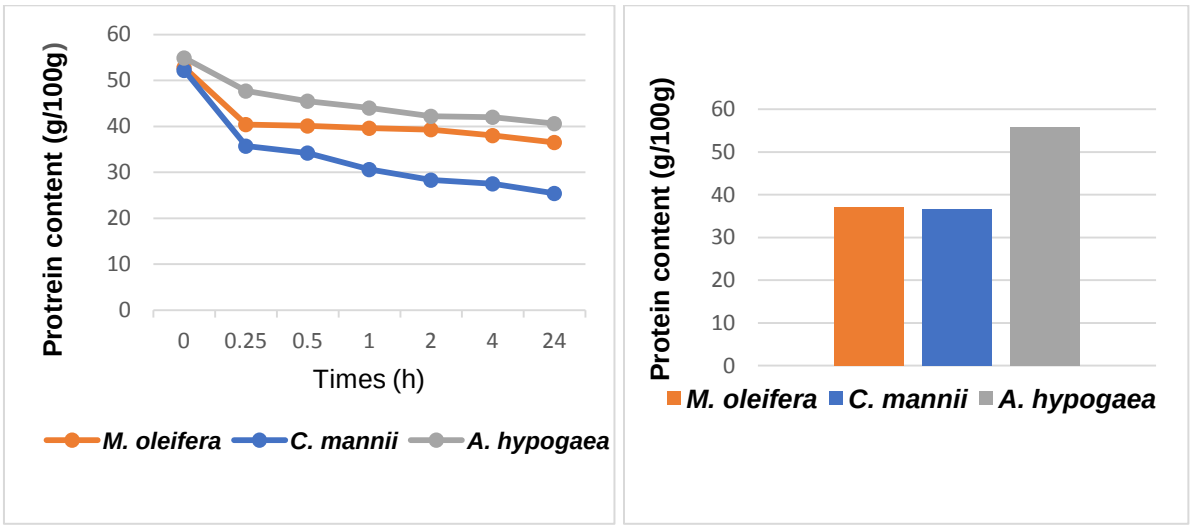
The results of the protein solubility profile study are presented in Fig. 2. The observation of this figure shows that the proteins of *C. mannii* seeds have a solubility rate lower than 10 % from pH 3 to pH 7. Their solubility increases slightly from pH 8 and reaches 28%. The protein solubility of *A. hypogaea* seeds is less than 10% between pH 4 and 6 and increases sharply at pH 7 and 8 to 70%. *M. oleifera* seed proteins have a solubility in the range of 20-25% at all pH. This study showed that pH has little influence on the degree of protein extraction from *M. oleifera* seeds. In contrast, protein extraction from *A. hypogaea* and *C. mannii* seeds is sensitive to pH. Adjusting the pH to 7-8 could improve the degree of protein extraction for *A. hypogaea* and *C. mannii* seeds. The native pH values for the solutions of the powders of these seeds are 5.87, 6.07 and 6.57 for *M. oleifera*, *C. mannii* and *A. hypogaea* respectively. In the literature, we distinguish according to their solubility properties, 4 major families of seed proteins: albumins, globulins, prolamins and glutelins [12].

3.4 Study of the Coagulant Activity

The results of Jar-Test clarification tests of raw water samples of 91.06 NTU and 41.45 NTU turbidity with *A. hypogaea* and *C. mannii* seed powder solutions are presented in Figs 3a and 4a. Observation of these figures shows a decrease in turbidity from 91.06 NTU to 0.09 NTU; and from 41.45 NTU to 0.09 NTU for the treatment with *A. hypogaea* powder solutions. The treatment with *C. mannii* powder solutions shows a decrease in turbidity from 91.06 NTU to 0.6 NTU; and from 41.45 NTU to 0.11 NTU. The percentage of turbidity reduction for *A. hypogaea* seed powder solution is 99.90% and 99.78% for 91.06 NTU and 41.45 NTU raw water samples respectively. For the *C. mannii* seed powder solution, the percentage of turbidity reduction is 99.34% and 99.73% for the 91.06 NTU and 41.45 NTU raw water samples, respectively. The optimal doses for each coagulant correspond to the minimum of each of these curves. They are 7000 mg/L for the *A. hypogaea* seed powder solution and 8000 mg/L for the *C. mannii* seed powder solution (Figs. 3a and 4a).

Table 1. Microbiological parameters of aqueous extracts of *A. hypogaea*, *C. mannii* and *M. oleifera* seeds

Time (h)	*Arachis hypogaea*				*Cucumeropsis mannii*				*Moringa oleifera*			
	Decanted aqueous extracts				Decanted aqueous extracts				Decanted aqueous extracts			
	Bacillus cereus	Yeast	Aerobic flora	Mould	*Bacillus cereus*	Yeast	Aerobic flora	Mould	*Bacillus cereus*	Yeast	Aerobic flora	Mould
0,25	<100/g	<10/g	360/g	50/g	2300/g	<10/g	17000/g	<10/g	<100/g	<10/g	<40/g	<10/g
0,5	<100/g	<10/g	450/g	50/g	2800/g	<10/g	18000/g	< 10/g	<100/g	<10/g	70/g	<10/g
1	<100/g	<10/g	650/g	60/g	3400/g	<10/g	18000/g	<10/g	<100/g	<10/g	70 /g	<10/g
2	<100/g	<10/g	680/g	120/g	3800/g	<10/g	31000/g	< 10/g	<100/g	<10/g	80/g	<10/g
4	<100/g	<10/g	930/g	130/g	5300/g	<10/g	>300000/g	<10/g	<100/g	<10/g	160/g	<10/g
24	33000/g	<10/g	>300000/g	140/g	>150000/g	<10/g	>300000/g	<10/g	<100/g	<10/g	160/g	<10/g
	Centrifuged aqueous extracts				Centrifuged aqueous extracts				Centrifuged aqueous extracts			
	< 100/g	<10/g	60/g	<10/g	<100/g	<10/g	1200/g	<10/g	<100/g	<10/g	<10/g	<10/g



(a). Decanted aqueous extracts **(b). Centrifuged aqueous extracts**

Fig. 1. Protein contents of aqueous extracts of *A. hypogaea*, *C. mannii* and *M. oleifera*

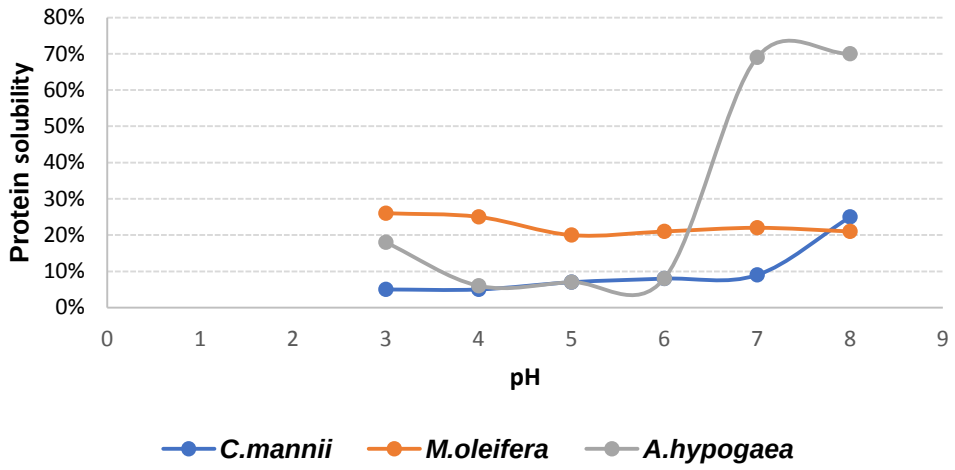


Fig. 2. Protein solubility profiles of *A. hypogaea*, *C. mannii* and *M. oleifera* seeds

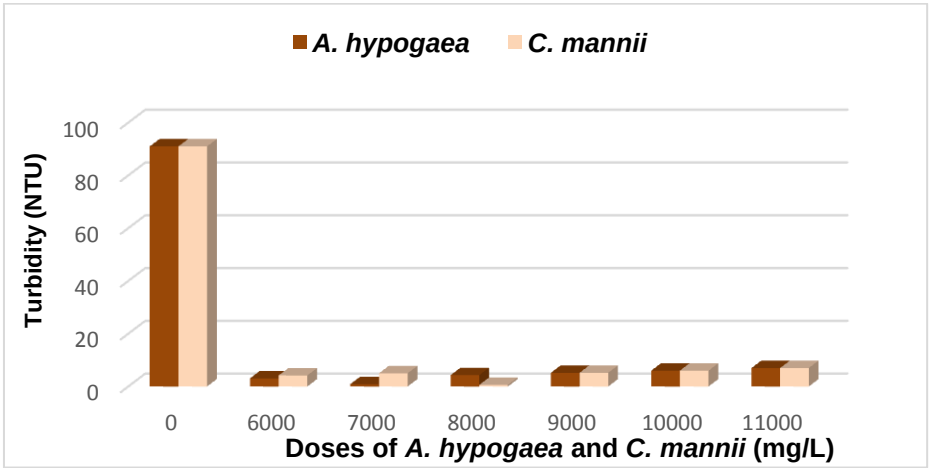
Treatment with *M. oleifera* powder solution resulted in a turbidity reduction of 99.73% and 99.62% for raw water samples of 91.06 NTU and 41.45 NTU, respectively; the respective optimum doses are 1400 mg/L and 1800 mg/L (Figs. 3b and 4b).

Treatment with the aluminum sulphate solution resulted in a turbidity reduction of 99.87% and 99.78% for the 91.06 NTU and 41.45 NTU raw water samples, respectively; the respective optimum doses are 14 mg/L and 35 mg/L (Figs. 3c and 4c).

The residual turbidity values obtained in this study are in accordance with those recommended by the WHO for drinking water, that is residual turbidity values less than or equal to 5 NTU.

These results show the removal of turbidity from the treated water samples by the seed powder solutions of *A. hypogaea* and *C. mannii.* The seed powder solutions of both plants caused coagulation, which is the neutralization of colloidal particles responsible for water turbidity. These results thus highlight the coagulant activity of *A. hypogaea* and *C. mannii* seeds in surface water clarification. These results also show the interest of *A. hypogaea* and C. *mannii* seeds in the clarification of surface water. Indeed, the percentages of turbidity reduction of the solutions of the seed powders of these two plants are close to those of the solution of seed powder of

M. oleifera, which is a natural organic coagulant proven at the scientific level [10], and the solution of aluminum sulfate which is the mineral coagulant most used for the treatment of water intended for human consumption [11]. In the literature, the coagulant activity of *A. hypogaea has* been reported by Mbogo and Prasad [15,16].



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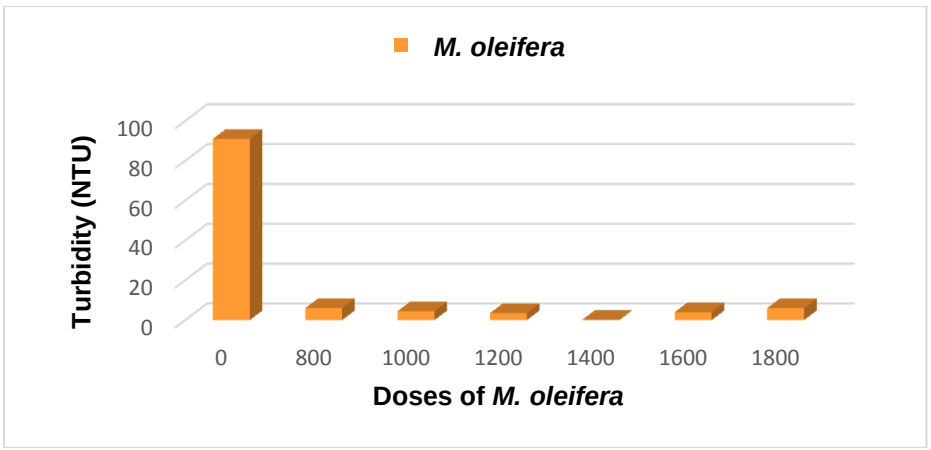

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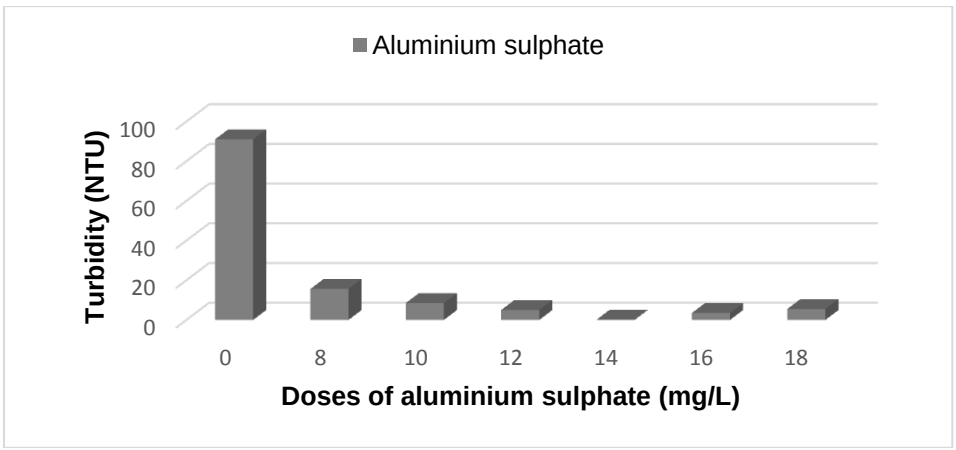
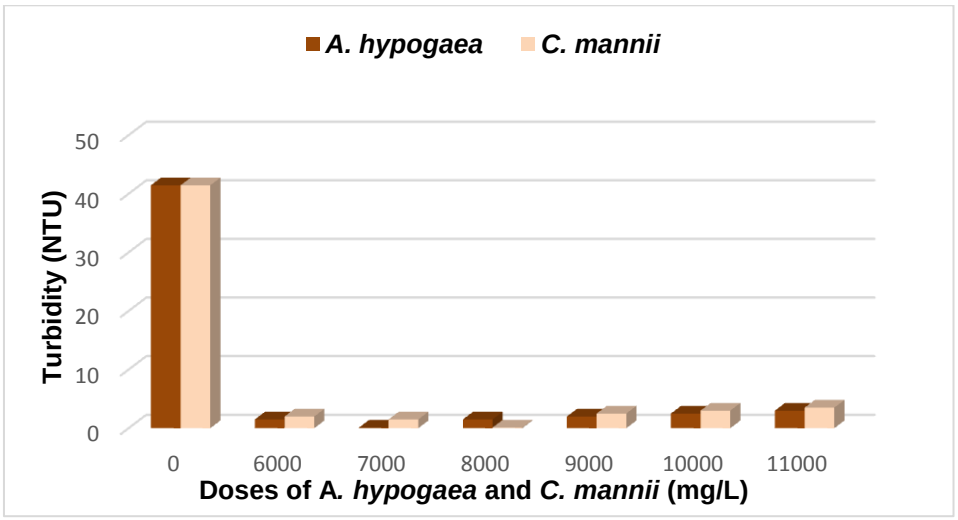


(c)

Fig. 3. Turbidity variation of the raw water sample of 91.06 NTU as a function of coagulant solution doses



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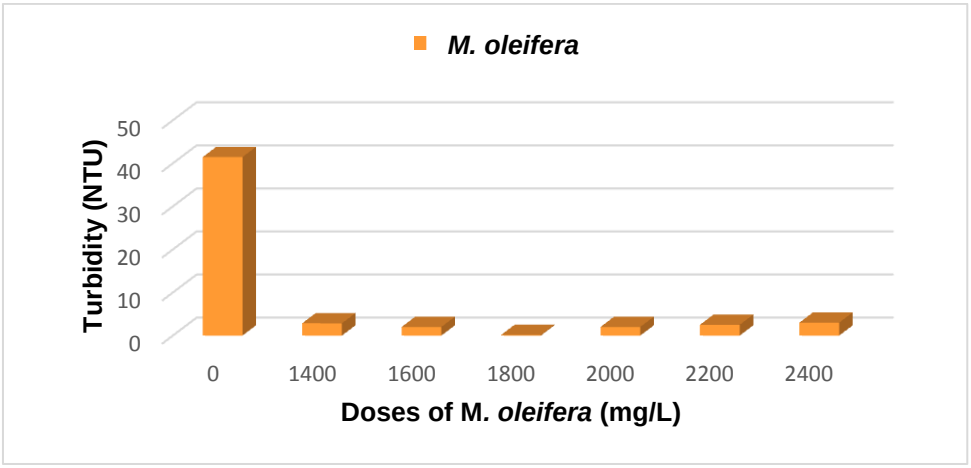

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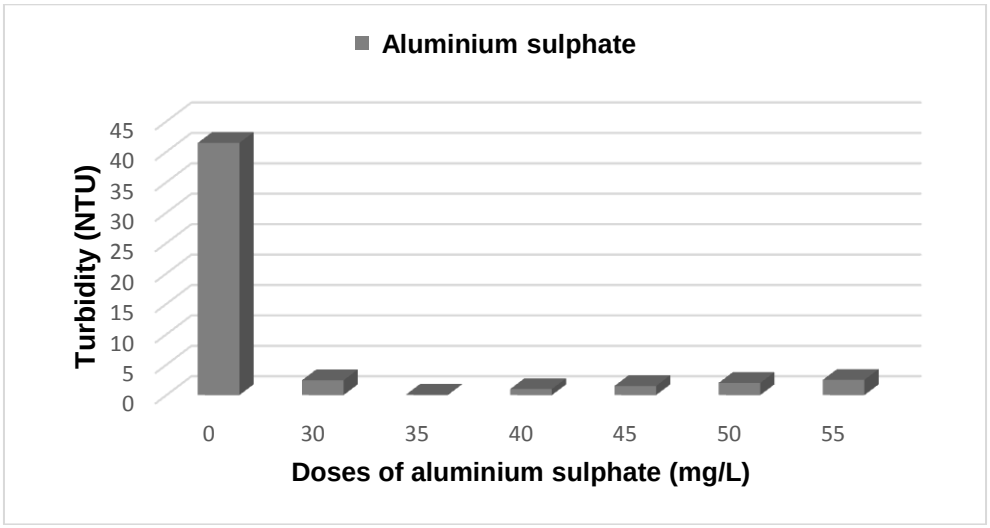

(c)

Fig. 4. Turbidity variation of the raw water sample of 41.45 NTU as a function of coagulant solution doses

3.5 Statistical Analysis

Statistical analysis of the results of the clarification tests on raw water samples of 91.06 NTU and 41.45 NTU showed for *A. hypogaea* a dispersion of 0.01 to 0.02 for turbidity. For *C. mannii*, the dispersion is 0.02 to 0.03 for turbidity.

4. CONCLUSION

This study was conducted to improve the quality of protein extracts of *A. hypogaea* and *C. mannii* seed powders. The results obtained revealed protein contents of 55.9%; 36.7%; 37.6% for centrifuged extracts of *A. hypogaea*, *C. mannii* and *M. oleifera* respectively. These values are higher than those obtained in the decanted extracts; after 24 h of decantation and which are 40.6 % for *A. hypogaea,* 25.4 % for *C. mannii* and 36.5 % for *M. oleifera*. As the microbial load was lower in the centrifuged extracts and the protein contents were higher, these extracts were of better quality than the decanted extracts. The results of this study also showed that adjusting the pH to 7 and 8 could improve the degree of protein extraction *for A. hypogaea* and *C. mannii* seeds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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