



## Enhanced coagulant extraction from *Jatropha curcas* in aqueous solutions and its application in turbidity removal

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### ABSTRACT

In this study, the effect of the extraction medium on the properties and efficiency of bio-coagulant, extracted of *Jatropha curcas* (Jc), in turbidity removal from aqueous solutions has been investigated. The optimized values of NaCl concentration (i.e., NaCl), solution pH and solution temperature were identified to improve the extraction of the coagulant. The optimized conditions were associated with an optimum coagulant dosage and a maximum turbidity removal from the synthetic aqueous solutions. The highest turbidity reduction was achieved with the coagulant extracted at a solution pH of 10 and an extraction temperature of 60°C (pH10/60°C-Jc press cake). Under these conditions, the coagulant dosage required was reduced by 80%–90%, depending on the coagulation pH. At the coagulation pH = 6, the pH10/60°C-Jc press cake well reduced the turbidity by 85%. However, the distilled water-based extract failed to lower the turbidity. Several analytical techniques were employed to characterize the nature of the active components derived from Jc. SDS-PAGE electrophoresis showed that Jc extract was mainly made up of proteins with molecular weights between 20 and 35 kDa. The optimized extraction conditions significantly improved the efficiency of this promising bio-derived coagulant in turbidity reduction. This study demonstrates the potential employability of these enhanced bio-coagulants. This can be a step ahead in helping with the development of sustainable processes in (waste)water treatment, particularly in tropical regions, for example, Malaysia with an abundant access to Jc.

*Keywords:* *Jatropha curcas*; Natural coagulant; Water treatment; Biomass; Protein; Turbidity removal

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## 1. Introduction

Today, one of the key changes in the process industries is waste minimization in the conversion of waste to value-added end products. Furthermore, the continual depletion of available resources has led to a rising growth in research opportunities, for example, the use of waste biomass in energy production and water/wastewater treatments. Natural materials are known to be effective coagulants. They are of great importance especially in countries where access to conventional chemicals has proven to be difficult or not economical [1,2]. These easily-biodegradable biomaterials are readily found in abundance as waste by-products with practically little or no secondary large-scale application [3,4].

Active components derived from natural coagulant can be produced or extracted from microorganisms, animals [5] and/or plants [6]. Plant-based coagulants are categorized into three main groups depending on the nature of their active components. The first group comprises oilseeds of which press cake has shown a good coagulation activity with protein as their active component. The next group is mucilage of the cactus family plants with a mixture of polysaccharides, capable of treating water via floc forming. And finally, tannin, a general name given to large polyphenolic compounds derived from natural materials [6].

Among plant-based coagulants, the active component in *Moringa oleifera* seeds is a promising alternative for the conventional coagulants. The corresponding coagulation mechanism has been thoroughly investigated in the literature [7,8]. It was shown that the active components in the water extract are the dimeric cationic polypeptides (6–17 kDa) with 10–11 isoelectric points [9]. Another potential source of bio-coagulant is *Jatropha curcas* (Jc), sometimes referred to as the “physic nut”. The seeds are made up of about 30%–35% oil. Jc has been widely grown for oil extraction from its seeds [9–11]. A solid waste residue, called press cake (Pc), with a good protein content is generated after the oil extraction process [10–12].

In comparison to *M. oleifera*, it has been reported [13] that the aqueous extracts of Jc kernel demonstrate an acceptable coagulation activity when tested on river water samples. The active coagulant component in the Jc is believed to be a soluble protein (Jc is an oilseed with a high protein content). It has also been reported that the key factors in increasing the solubility of protein in aqueous solutions are the ionic strength, solution pH and temperature [14–16].

The first objective of the present work is to investigate and optimize the extraction conditions in order to reduce turbidity in aqueous solutions with a minimum dosage of bio-coagulant. This study also seeks to specify and characterize the produced extract. To the best of our knowledge, the composition, nature and properties of Jc extract have not been reported elsewhere. Elemental analysis, FTIR analysis, enzymatic hydrolysis and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) measurements were employed in order to characterize the active components.

## 2. Materials and methods

### 2.1. Materials

The Jc seeds were supplied by the Malaysian Agricultural Research and Development Institute (MARDI). The raw

plant was freshly collected from Universiti Putra Malaysia's farm. Kaolin, sodium hydroxide pellets and sodium chloride were purchased from R&M Chemicals, Essex, UK. Hexane from System Co., Shah Alam, Malaysia; Alcalase from Novozymes A/S, Bagsværd, Denmark and hydrochloric acid (HCl, 36%) from Fisher Scientific, UK, were the other chemicals used in the present work.

### 2.2. Characterization of Jc

A hexane-based solid–liquid extraction process was employed in order to prepare the Jc press cake [17]. The results from the proximate and elemental analyses (Truspec CHNS/CHNSO elemental analyzer [LECO, USA]) of the shelled Jc seeds as well as the press cake are shown in Table 1. All chemicals used in the experiments were of analytical grade and were used as received without any further purification. In addition, the functional groups in Jc and JcPc were detected on a Spectrum 100 FTIR spectrometer (Thermo Nicolet) within a spectral range of 4,000–400  $\text{cm}^{-1}$ . The spectra of the kernel and the press cake were similarly recorded.

### 2.3. Extraction of the coagulant's active component

In order to extract the active component, two grams of the sieved JcPc (100–550  $\mu\text{m}$ ) was mixed with 100 mL of the extraction solution. In order to determine the optimum extraction conditions, the effects of three experimental parameters, that is, solution temperature, NaCl concentration and solution pH (i.e., the extraction pH), were studied. To achieve this, different aqueous media were used: distilled water (Favorit Water Still W4L) at neutral pH (i.e., pH 7) as well as at pH values of 9 and 10 (adjusted by using 0.1 and 1 M NaOH) and NaCl solutions (0.1, 0.2, 0.3, 0.5, 1.0, 2.0 M) [11,18]. The extraction temperature was studied at three levels: the room temperature (RT), 40°C and 60°C [10]. All mixtures were continuously stirred for 5 min on a heated hotplate, using a magnetic stirrer (Fisher Scientific Isotemp). The suspension was next filtered through a muslin cloth and cooled down to room temperature. The supernatant was then centrifuged (high speed refrigerated centrifuge CUBOTA 6500) at 3,000 rpm for 15 min. These coagulant solutions, extracted in distilled water (DW) and NaCl solutions, were referred to as DWE-JcPc and SCE-JcPc (i.e., distilled water extract/sodium chloride extract of JcPc), respectively. A fresh solution was prepared for each sequence of experiments to prevent any change in coagulation activity due to microbial decomposition of the organic compounds during storage. The coagulant extract from JcPc at optimum extraction condition was labeled as pH10/60°C-JcPc indicating that the extraction solution pH and solution temperature were adjusted at 10°C and 60°C, respectively.

### 2.4. Isolation, partial purification and characterization of the active component

In order to identify the nature of the active components in Jc, enzymatic hydrolysis was performed by adding Alcalase to pH10/60°C-JcPc. The solution pH of the pH10/60°C-JcPc was adjusted at 8 using HCl (0.1 M and 1 M) prior to the

Table 1  
Elemental and proximate analyses of shelled Jc seed

Elemental analysis (dry wt%)						
	Nitrogen	Hydrogen	Carbon	Sulfur	Oxygen	
Kernel	4.98 ± 0.03	8.87 ± 0.17	57.05 ± 0.24	2.20 ± 0.06	22.56 ± 0.16	
Press cake	8.38 ± 0.30	6.80 ± 0.12	47.69 ± 0.15	0.18 ± 0.02	33.11 ± 0.23	
Proximate analysis (raw wt%)						
	Crude protein	Crude lipids	Ash content	Moisture content	Crude fiber	Total carbohydrates <sup>a</sup>
Kernel	31.52 ± 0.35	51.79 ± 0.09	3.30 ± 0.14	3.95 ± 0.04	6.56 ± 0.27	2.88 ± 0.06
Press cake	54.04 ± 0.43	16.19 ± 0.02	6.03 ± 0.03	5.20 ± 0.02	7.64 ± 0.13	10.90 ± 0.12

<sup>a</sup>Calculated from difference.

addition of the enzyme (pre-adjusted medium pH). The mixture was then continually stirred inside a water bath regulated at 50°C for 6 h [19,20]. During this process, the entire peptide bonds were broken down [21]. This would then allow a coagulation test to potentially prove the quality of the extracted active component in the solution [19].

In order to study the nature of the active component, three samples from the original pH10/60°C-JcPc solution (30 mL, pH = 8) were prepared in three volumetric flasks (100 mL). Alcalase (500 µL) was next added to one of the three samples. The second sample was used as the control experiment with no added Alcalase. Finally, the third sample (Alcalase free) was placed and kept inside the refrigerator (4°C–7°C) in order to prevent any microbial decomposition of the organic compounds. An enzyme control test was also employed by adding of Alcalase (500 µL) to distilled water (30 mL, pH = 8). After the completion of the hydrolysis reactions, the coagulation activities of all four samples were tested via the jar test.

Active proteins were isolated and characterized using a range of techniques including isoelectric precipitation, dialysis and electrophoresis. A modified version of an existing method [10] was employed in order to precipitate the active proteins from the JcPc solutions. In order to isolate the protein from the pH10/60°C-JcPc, the pH of the coagulant solution was adjusted to 5 using HCl (Fisher Scientific 36%) before being centrifuged at 3,000 rpm for 20 min at room temperature (high speed refrigerated centrifuge CUBOTA 6500). The generated white suspension was then dissolved in distilled water and was continually stirred for 4 h on a magnetic hot-plate (Fisher Scientific Isotemp). Finally, the non-soluble residue was filtered first through a Whatman No. 1 filter paper and then through a 0.45 and 0.22 µm nylon membrane. The supernatant was dialyzed overnight at 4°C against deionized water in a dialysis bag (UC36-32-100, Viskase Sales Corp) with a molecular weight cut-off of 12–14 kDa.

The protein's molecular weight distribution of the distilled water extract and the purified pH10/60°C-JcPc samples were monitored by SDS-PAGE on 12.5% (w/v) polyacrylamide gel. Protein bands were visualized via Coomassie brilliant blue R-250 staining [22].

The total sugar and protein contents of the natural coagulant solutions were measured via the phenol–sulfuric acid method with glucose as the standard [23] and the Bradford method with bovine serum albumin as the standard, respectively [12].

### 2.5. Turbid water samples

In order to identify the optimized experimental condition for the extraction of the active component of Jc, synthetic kaolin turbid water was prepared and used in the subsequent turbidity removal experiments. Kaolin was used to establish a desired level of turbidity. By adding 7.5 mL of kaolin stock solution [24] to 500 mL of tap water and storing the solution overnight to maintain a constant temperature and remove any residual chlorine, a 200-NTU turbid water was successfully synthesized. The turbidity was determined using a turbidimeter (Model 2100 N, HACH, Colorado, USA). The original pH of the turbid water samples was  $7.41 \pm 0.07$  and the desired pH (i.e., coagulation pH) was adjusted by adding hydrochloric acid (0.1 M and 1 M) and sodium hydroxide (0.1 M and 1 M).

### 2.6. Coagulation experiments

Jar flocc test, comprising a series of batch experiments, was employed in order to study the coagulation process. All experimental parameters were kept constant during the tests except for the target variable. The coagulation activities of the different extracts were evaluated in a jar tester (VELP, model JLT6). Samples were added to beakers filled with turbid water in different dosages (10–400 mg L<sup>-1</sup>). The solutions were then constantly stirred (100 rpm) for 4 min, followed by a slow mixing phase (40 rpm) for 25 min. After about 30 min of sedimentation, an aliquot of the clarified sample (20 mL) was collected from the top of the beaker and the residual turbidity was measured. The final (residual) turbidity of the treated water samples was measured and the turbidity removal percentage was calculated by the difference between the initial and the final turbidity values divided by the initial turbidity, multiplied by 100. All experiments were run at room temperature (25°C ± 1°C).

In order to investigate the reproducibility of the results, the extraction experiments in pH 10 and temperature 60°C were repeated in quintuples and in irregular intervals for 2 months followed by the subsequent analyses of the efficiency of the pH10/60°C-JcPc in turbidity removal at coagulation pH of 4. Each experiment was run in triplicates. The residual turbidity of these runs, that is,  $4.22 \pm 0.43$ ,  $4.02 \pm 0.20$ ,  $4.63 \pm 0.34$ ,  $5.48 \pm 0.45$ ,  $4.65 \pm 0.31$  NTU, indicates that the extracted natural coagulant could successfully reduce the turbidity of the synthetic turbid water with a high degree of reproducibility.

### 3. Results and discussions

#### 3.1. Optimization of the extraction conditions

The coagulation activity of the DWE-JcPc was investigated in a synthetic aqueous kaolin solution. Through a series of initial tests, it was concluded that acidic pH facilitates turbidity reduction from synthetic water. Fig. 1 presents the effect of DWE-JcPc dosage on residual turbidity of synthetic aqueous kaolin solution at coagulation pH values of 4, 5 and 6.

The residual turbidities of water treated with the optimum dose of the coagulating solution – corresponding to the highest turbidity reduction – were found to be 5 and 33 NTU at pH 4 and 5, respectively. The optimum doses of coagulant at pH 4 and 5 were 100 and 200 mg L<sup>-1</sup>, respectively. However, at a coagulation pH of 6, DWE-JcPc could not reduce the turbidity to an acceptable level (i.e., >30% [25]) and therefore, distilled water extraction proved not to be suitable for successful coagulation at coagulation pH of 6.

The elemental analysis on the JcPc (Table 1) showed an enrichment of protein in the sample: 8.38 wt% nitrogen (7.69 wt% carbon) as compared with the kernel, which contained only 4.98 wt% nitrogen (57.05 wt% carbon). The crude protein contents of Jc's kernel and JcPc are 31.52% and 54.04%, respectively (Table 1). Both kernel and press cake were further analyzed via FTIR to confirm the presence of the protein. As illustrated in Fig. 2, FTIR analysis for Jc kernel and press cake showed two strong absorption bands at 1,635 cm<sup>-1</sup> and another at 1,535 cm<sup>-1</sup>. Another band was also recorded in the range of 1,240–1,246 cm<sup>-1</sup>. These peaks are characteristic of carbonyl C=O stretching vibrations in primary, secondary and tertiary amides, respectively [26,27]. This suggests that the Jc seeds hold up a high percentage of protein within them. The comparison between spectra of the press cake and the kernel demonstrated that the amide infrared absorbance has not been remarkably altered in the press cake due to the structural changes in protein after solvent extraction of lipids.

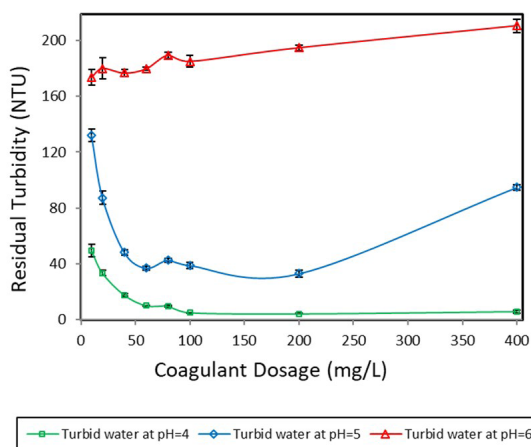


Fig. 1. Effect of water-extracted coagulant (DWE-JcPc) dosage on turbidity reduction of synthetic kaolin/water at coagulation pH values of 4, 5 and 6.

Therefore, the active component of Jc press cake as a coagulant might be protein molecules. In both laboratory and commercial-scale extraction processes, water with sodium or potassium hydroxide are used to modify the pH and to ultimately extract protein from press cake [10,28]. On this ground, the same approach was adapted throughout the JcPc active component extraction process.

With NaCl solution, it was observed that the optimum dosage of JcPc extract was halved compared with when water was used as the extraction medium at coagulation pH 4. The corresponding amount of SCE-JcPc was 40 mg L<sup>-1</sup>. On the other hand, the lowest concentration of NaCl showing the same degree of turbidity reduction was 0.3 M. Additionally, higher concentrations of up to 0.5 M did not show any significant difference while elevated salt concentrations (1.0 and 2.0 M NaCl) had a negative impact on turbidity reduction. The decrease in coagulation performance at 1.0 and 2.0 M could be due to the “salting-out” phenomena [10].

In the next phase of the experiments, the effects of temperature and pH on extraction were investigated. Although the temperature of the extraction solution was expected to improve the extraction yield (increasing temperatures

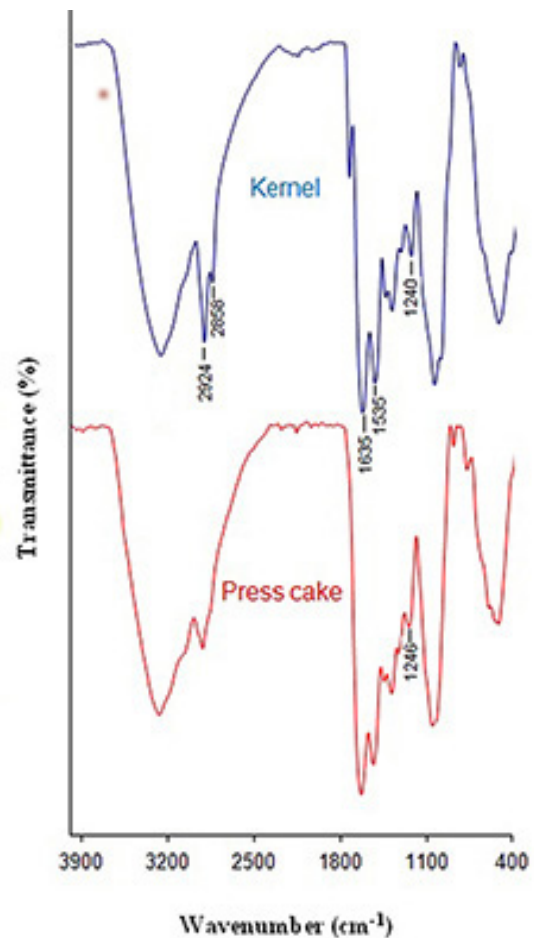


Fig. 2. FTIR spectra of Jc kernel and press cake. The classical bands at 2,924 and 2,858 cm<sup>-1</sup> are typical features of compounds containing long alkyl chains [10]. The absence of 2,858 cm<sup>-1</sup> band in the press cake spectrum validates the fat extraction from the Jc.



facilitated the molecular dissolution of solutes in the solvents), the results indicated that solely preheating the coagulant solution would not have a significant effect on the coagulating efficiency. It has been observed that in some cases, an increase in temperature could decrease the protein solubility at isoelectric point (pI); however, at other pH values (below and above pI), the effect of temperature on solubility of protein was strongly affected by solution pH [28]. On the other hand, Saetae et al. [16] reported that Jc protein solubility increases with temperatures within the range of 30°C–50°C at an extraction pH of 12. In general, it is understood that the effect of temperature on solubility is strongly dependent on the pH of the extracting solution and therefore, there exists an interaction between these two experimental factors. Hence, in the subsequent experiments, the effects of temperature and pH of the media were studied together.

Fig. 3 demonstrates the effects of ionic strength, pH and temperature of the extraction solution on the optimum coagulant dosage to achieve a residual turbidity of  $\leq 5$  NTU in synthetic water at a coagulation pH of 4.

The optimum dosage was observed to be 100, 40 and 20 mg L<sup>-1</sup> for DWE-JcPc, SCE-JcPc and pH10/60°C-JcPc at a coagulation pH of 4, respectively. A notable achievement was the improvement in coagulation activity of JcPc extract at coagulation pH values of 5 and 6 by changing the extraction pH and the temperature of the extraction media. Fig. 4 draws a comparison among various extracting solutions with respect to turbidity removal at coagulation pH values of 5 and 6. The results show that the extraction of active components at an extraction pH of 10 and higher temperature substantially improved the performance of coagulant at coagulation pH values greater than 4.

The efficiency of different extracting solutions at coagulation pH = 4 was understood to be the same in terms of the observed residual turbidity. They all showed residual turbidity of  $\leq 5$  NTU. However, the optimum coagulant dosages dropped by 80% and 90% for coagulation pH 4 and 5, respectively. Additionally, a higher coagulation activity (by 91.6%) with the pH10/60°C-JcPc was observed at coagulation pH = 5 comparing with 83% when using DWE-JcPc. Therefore, regarding the optimum dosage improvement, the highest achieved turbidity removal with pH10/60°C-JcPc at coagulation pH = 5 was almost 11 times higher than that of distilled water extract. Interestingly, at the coagulation pH = 6, and with the use of an alkaline medium extract, the coagulation activity increased by 85% despite the absence of coagulation activity with distilled water extract. Therefore, it is concluded that extraction at pH 10 and at 60°C presents two major advantages: a reduction in the optimum dosage to achieve the maximum turbidity removal and the improvement of coagulation activity at coagulation pH 5 and 6. Although salt extraction solution showed its capability in reducing the optimum dosage at coagulation pH = 4 and it also indicated an improvement in coagulation activity at coagulation pH = 5 compared with DWE-JcPc, it was still not as efficient as the solution extracted at pH 10 and at 60°C.

Since the active coagulant component was now believed to be protein, the protein and carbohydrate content of the extractant was subsequently analyzed. The results indicate a significant increase in the yield of protein extraction from JcPc through alkaline extraction. Extraction at 60°C and

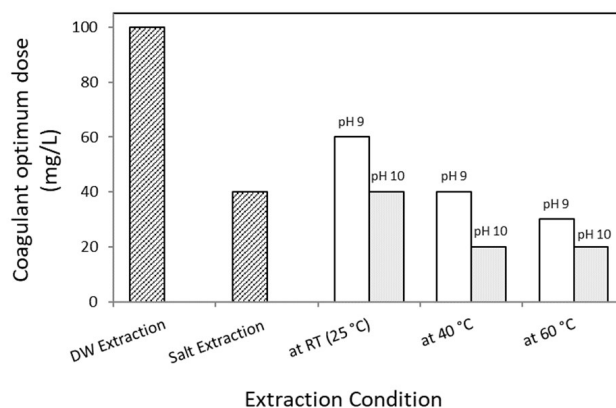


Fig. 3. Optimum dose corresponding to a residual turbidity of  $\leq 5$  NTU of water at coagulation pH 4 using various extracting solutions.

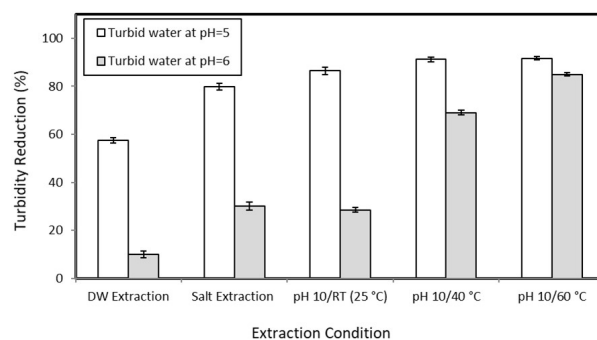


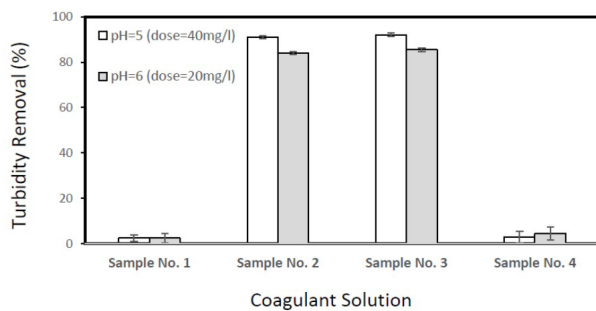
Fig. 4. Turbidity reduction of synthetic water at two coagulation pH values of 5 and 6 with various extracting solutions with a constant coagulant solution dosage of 20 mg L<sup>-1</sup>.

a pH of 10 corresponded to a higher protein content (i.e.,  $6.65 \pm 0.20$  g L<sup>-1</sup>) than extraction in a water-only medium (i.e.,  $0.81 \pm 0.06$  g L<sup>-1</sup>). Furthermore, the total amount of carbohydrate in DWE-JcPc and pH10/60°C-JcPc was  $1.35 \pm 0.17$  g L<sup>-1</sup> and  $1.63 \pm 0.02$  g L<sup>-1</sup>, respectively. Similar findings by other researchers [10,29] have also been reported in the literature for optimizing the extraction of protein from oil seed biomass.

### 3.2. Isolation and identification of pH10/60°C-JcPc active component

In order to characterize the extracts, several characterization techniques were employed. Protein hydrolysis and SDS-PAGE electrophoresis were used to characterize the coagulation active components in Jc.

The hydrolysis process is a reaction in which a substance is degraded. Enzymatic hydrolysis involves minimum side reactions and leads to a limited hydrolysis of selected bonds. Proteases hydrolysis breaks down proteins into various peptides which are further reduced into amino acids. This leads to a complete degradation of protein



Sample:  
 No. 1: pH10/60°C-JcPc+Enzyme      No. 2: pH10/60°C-JcPc inside water bath  
 No. 3: pH10/60°C-JcPc inside refrigerator      No. 4: Distilled water +Enzyme

Fig. 5. Residual turbidity of kaolin water at two coagulation pH values of 5 and 6 by using pH10/60°C-JcPc solution under protein hydrolysis reaction.

structure [19]. The results could support our hypothesis that the active component of Jc-derived coagulant is a protein if the hydrolyzed sample does not show a significant coagulation activity. Fig. 5 compares the performance of the pH10/60°C-JcPc and the hydrolyzed pH10/60°C-JcPc solutions for their turbidity removal.

Samples no. 1, 2 and 4 were maintained inside a water bath under identical Alcalase hydrolysis conditions. The hydrolyzed pH10/60°C-JcPc solution did not show any coagulation activity. The turbidity removal of the treated water using the hydrolyzed pH10/60°C-JcPc was measured to be 2%–5% compared with the turbid water treated with sample no. 2 and 3 (i.e., 85%–92%). This indicated that sample no. 1 completely failed to reduce the turbidity and therefore, no coagulation activity was observed. Alcalase just reacts with protein components and breaks down peptide bonds, resulting in new components such as amino acids with completely different properties from proteins. In addition, the results of sample no. 4 confirmed that Alcalase did not demonstrate any coagulation activity.

The SDS-PAGE was used to determine the molecular weight profile for the isolated proteins. The SDS-PAGE patterns of pH10/60°C-JcPc (Fig. 6(a)) contain three major bands indicating the presence of various types of proteins with different molecular weights. The highest protein intensities of pH10/60°C-JcPc are grouped into two classes of molecular weights: <25 kDa and >25–35 kDa. In general, a minimum number of 25 protein bands are present in the Jc seed protein [18]. The number of proteins extracted in distilled water was much larger than the optimum extraction conditions while the concentration of the target protein was significantly higher in the pH10/60°C-JcPc solution. These results were consistent with the findings by Hamarneh et al. [22]. They stated that the molecular weight of the protein in the Jc extract (under isoelectric conditions) contains three major bands between 20 and 45 kDa (Fig. 6(b)). In our study, this was between 20 and 35 kDa.

The total protein content in pH10/60°C-JcPc after isoelectric precipitation was measured to be  $1.5 \pm 0.06 \text{ g L}^{-1}$ . However, the total amount of carbohydrate was observed to be negligible. This also confirmed that the protein isolation procedure

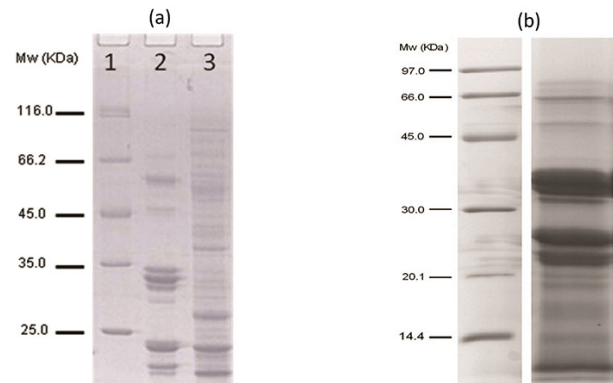


Fig. 6. Protein molecular weight distributions of JcPc extracts: (a) isolated protein from pH10/60°C-JcPc by isoelectric precipitation and dialysis (2), DWE-JcPc (3), and protein marker (1); and (b) Jc proteins extracted by isoelectric precipitation [22].

was successful. The protein content of the pH10/60°C-JcPc after isoelectric precipitation was 77% less than that of the pH10/60°C-JcPc, owing to the loss of proteins during the precipitation and dissolution stages.

#### 4. Conclusion

The purpose of this study was to optimize the extraction method of the active coagulation component from Jc seeds. In order to identify the extracted species, a range of characterization techniques were employed. The key findings in this study are summarized as follows:

- (1) Among various extraction media employed to extract the active components, an extraction medium with a pH of 10 and a temperature of 60°C was found to be the optimized condition for both maximum turbidity removal and optimum coagulant dosage required. In the removal of kaolin turbidity at the coagulation pH 4 and 5, JcPc extracted in an alkaline media demonstrated an improved coagulation activity with a 5- and 10-fold reductions in the required coagulant dosage compared with JcPc extracted in distilled water, respectively. The experimental results indicate the efficiency of the optimized extraction conditions in the active component extraction from JcPc. The protein content of pH10/60°C-JcPc was 8.2 times higher than that of the DWE-JcPc. The pH10/60°C-JcPc could efficiently coagulate more than 91% of the initial 200-NTU kaolin turbidity using only a dosage of  $20 \text{ mg L}^{-1}$  at a coagulation pH of 5. However,  $200 \text{ mg L}^{-1}$  of the DWE-JcPc was required to remove only 80% of the same kaolin water. With an alkaline solution as the extractant, a coagulation activity at pH 6 corresponding to more than 85% turbidity removal was observed. However, distilled water extract did not show any coagulation activity at this pH.
- (2) As confirmed via the elemental/proximate analyses as well as the FTIR spectra, the effective coagulant components were protein molecules. This was further confirmed through enzymatic hydrolysis. The water-extract solution

demonstrated at least 20 protein bands in SDS-PAGE gel. Comparing the two SDS-PAGE gels, it was realized that the active proteins had a molecular weight between 20 and 35 kDa.

This study has showed that the pH10/60°C-JcPc can be a promising coagulant to be used in water treatment plants, especially in tropical countries such as Malaysia with an easy access to Jc. It was realized that the Jc seeds could be used as an efficient coagulant in water and wastewater treatment upon an initial purification of the active component. It is mentionable that the ultimate application of this coagulant in pilot and full-scale plants requires appropriate cost-benefit and a full techno-economic analyses for a viable commercial application of pH10/60°C-JcPc. Therefore, the authors would see it critical and with key benefits to perform such calculations in future works.

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