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CATIONIC AMINO ACIDS FROM KRATOK BEAN (*PHASEOLUS LUNATUS* L.) AS A NATURAL COAGULANT FOR TURBIDITY REMOVAL: EFFECTS OF CONCENTRATION AND PH

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ABSTRACT
Chemical water treatment usually uses coagulation with alum and chlorine. In contrast, natural coagulants, such as proteins, are highly potent due to their amino acid composition. Specifically, cationic amino acids, such as lysine, arginine, and histidine, have side chains with positively charged amine groups. These groups play an important role in destabilizing colloids, thereby removing impurities and other pollutants and facilitating coagulation. Building on this, the present study aims to determine the effects of concentration and pH on reducing water turbidity using kratok bean powder as a coagulant, tested at concentrations of 0.5%, 1.0%, and 1.5% and pH values of 5.0, 5.5, 6.0, and 7.0. The results showed that krotok bean coagulant reduced water turbidity optimally at a concentration of 1% (70.05 ± 0.23) and an optimum pH of 5.5 (73.0 ± 0.18) at 60℃ for 50 minutes. Finally, LCMS analysis showed that the types and levels of cationic amino acids in kratok beans (Phaseoulus lunatus) were arginine (1.453%), lysine (1.261%), and histidine (0.822%).

KEYWORDS: cationic Amino acids, coagulation, kratok bean (*Phaseoulus lunatus*), water.

INTRODUCTION

The most commonly used chemical method for clean water treatment is coagulation, typically employing alum and chlorine. However, their use in drinking water can negatively affect health, potentially causing throat irritation, skin diseases, and kidney disorders. To reduce these effects, plant-derived natural coagulants are needed. Cationic amino acids with positively charged amine side chains destabilize colloidal impurities, facilitating floc formation. Their activity enables adsorption and bonding to turbid water particles, leading to stable precipitate aggregates. Proteins are promising natural coagulants due to their amino acid content; lysine, arginine, and histidine are particularly effective. The kratok bean (Phaseolus lunatus) is a potential coagulant due to its high protein content (19.93–21.40%) and its cationic amino acids: lysine (6.42–7.41%), arginine (7.51–8.37%), and histidine (5.01–5.49%).[1] Both coagulant concentration and pH are critical in the coagulation process. pH influences aggregation into larger, easily removed flocs, while coagulant concentration aids sedimentation. Coagulation outside the optimal pH range or with incorrect concentration may hinder floc formation and degrade water quality.[2] Research is thus needed to determine the optimal concentration and pH, and to identify amino acid types and levels in kratok beans.

MATERIALS AND METHODS

Materials

The materials utilized in this study included mature kratok beans (*Pisum sativum* L.) sourced from the Bangli region, alum (aluminum sulfate), n-hexane, H_2PO_4 buffer, hydrochloric acid (HCl), turbid water, orthophthaldialdehyde (OPA), sodium hydroxide, boric acid, 30% Brij-30 solution, 2-mercaptoethanol, standard amino acid solution, sodium ethylenediaminetetraacetic acid (Na-EDTA), methanol, tetrahydrofuran (THF), sodium acetate, and distilled water.

The equipment comprised laboratory glassware, sealed plastic containers, basins, sprayers, water buckets, plastic spoons, thermometers, petri dishes, filter paper, spray bottles, amber glass bottles, volumetric pipettes, pipette fillers, droppers, plastic funnels, a freezer, an oven, a blender, a 100-mesh sieve, a desiccator, electronic and analytical balances, a hotplate, magnetic stirrers, volumetric flasks, sealed sample vials, a stopwatch, a set of High-Performance Liquid Chromatography (HPLC) apparatus, a set of jar test apparatus, a turbidimeter, and a pH meter.

METHODS

Preparation of Coagulant Samples

Kratok bean samples were initially dried for three days, followed by sun-drying for 24 hours and oven-drying at 60°C for an additional 24 hours. Subsequently, the beans were ground using a blender and sieved through a 100-mesh sieve.

The kratok bean powder was macerated by immersion in amber glass bottles using n-hexane as the solvent for five days to reduce lipid content. The mixture was stirred every 12 hours to enhance extraction efficiency. After five days, the extract was filtered, the filtrate was discarded, and the residue was dried.

Assessment of Coagulation Capacity at Different Coagulant Concentrations (0.5%, 1.0%, 1.5%) Using the Jar Test Method (Martin et al., 2022)

Turbid water collected from the Tukad Badung River served as the water source for this study. Samples were placed in 500 mL glass beakers, and initial turbidity and pH were measured. Kratok bean powder coagulant was added in amounts of 2.5 g, 5 g, and 7.5 g to achieve concentrations of 0.5%, 1.0%, and 1.5%, respectively. The mixtures underwent rapid mixing at 180 rpm for 2 minutes, followed by slow mixing at 40 rpm for 15 minutes. Subsequently, the mixtures were allowed to stand for 60 minutes to facilitate floc settlement. Each treatment was conducted in triplicate.[3]

Assessment of Coagulation Capacity at Different pH Levels Using the Jar Test Method

A 500 mL sample of turbid water was placed in a 500 mL glass beaker, and initial turbidity and pH were measured. Coagulant powder was added at the optimum concentration. The pH was adjusted to 5.0, 5.5, 6.0, and 7.0. For alkaline samples, HCl solution was used to lower the pH; for acidic samples, NaOH solution was added to reach the target pH. Phosphate buffer solution was employed to maintain pH stability. The mixtures were stirred at 180 rpm for 2 minutes, followed by 40 rpm for 15 minutes, and then allowed to stand for 60 minutes to facilitate floc settlement. Each treatment was conducted in triplicate.[4]

Measurement of Turbidity Using a Turbidimeter

Coagulated water samples were analyzed using a turbidimeter to determine turbidity reduction (NTU) following the addition of the natural coagulant. Comparative turbidimetric tests were conducted on water treated with 1% alum. The percentage reduction in turbidity was calculated using the following formula.

% Reduction = (Initial value – Final value) / Initial value × 100%

Note

Initial value = NTU value of raw water (NTU)
Final value = NTU value after treatment (NTU)

Determination of Amino Acid Content in Kratok Beans Using the HPLC Method

Amino acid content was analyzed using HPLC to quantify the levels of histidine, lysine, and arginine, which are classified as cationic amino acids.

RESULTS AND DISCUSSION

Coagulation Performance at Coagulant Concentrations of 0.5%, 1%, and 1.5%

The optimal coagulant concentration for the coagulation process was determined to be 1% (Table 1). This effectiveness is due to the water-soluble proteins in the coagulant, which provide abundant positive charges that neutralize negatively charged colloids and reduce the solution's zeta potential to zero. Four primary mechanisms of colloid destabilization are recognized: double-layer compression, charge neutralization, interparticle bridging, and sweep flocculation. Double-layer compression occurs when a high concentration of ions reduces the double-layer thickness. Charge neutralization results from the addition of a coagulant that neutralizes the colloid surface charge, decreasing electrostatic repulsion among colloids. Interparticle bridging involves polymer chains adsorbing onto colloid surfaces and forming aggregates. Sweep flocculation typically arises from excessive use of inorganic coagulants, which generate hydroxide precipitates. Protein-based coagulants primarily function through charge neutralization. As amphoteric compounds, proteins can carry either a positive or negative charge depending on their isoelectric point. The amino groups within the protein structure serve as cationic centers, which are essential for neutralizing the typically negatively charged colloids. Additionally, proteins act as polyelectrolytes and can adhere to colloid surfaces via the electrostatic patch mechanism.[5]

Table 1: Coagulation results with varying concentrations.

Concentration (%)	Initial NTU	Final NTU			Turbidity Reduction (%)			Average Reduction (%)
		1	2	3	1	2	3	
0.5	16.800	6.64	6.70	6.70	60.43	60.10	60.10	60.21±0.19
1.0	16.800	4.99	5.02	5.07	70.25	70.11	69.80	70.05±0.23
1.5	16.800	9.22	9.25	9.30	45.12	44.89	44.64	58.38±0.24

A 1.5% coagulant dosage constitutes an excessive amount, resulting in colloid restabilization. Excess cations hinder the settling of unreacted coagulant, causing improperly formed flocs to remain suspended and increasing turbidity.[6] This outcome underscores the necessity of identifying the optimal coagulant concentration.

Increasing the coagulant concentration can promote precipitate formation and enhance particle collisions, resulting in larger flocs.[7,8] However, excessive coagulant leads to colloid restabilization, as surplus coagulant on the colloid surface induces electrostatic repulsion and reduces turbidity removal efficiency.[9] Conversely, a 0.5% coagulant concentration is insufficient for effective coagulation. At this suboptimal level, the coagulant cannot adequately neutralize the colloid surface, resulting in a zeta potential below zero and poor binding of organic substances, which hinders floc formation.[10]

Coagulation Performance at Different pH Levels

The coagulation process is most effective at an optimal pH (Table 2)

Table 2: Coagulation results with varying pH.

pH	Initial NTU	Final NTU			Turbidity Reduction (%)			Average Reduction (%)
		1	2	3	1	2	3	
5.0	16.800	4.791	4.754	4.791	71.48	71.70	71,.8	71.55±0.13
5.5	16.800	4.501	4.554	4.555	73.21	72.89	72.89	73.0±0.18
6.0	16,.00	8.271	8.38	8.334	50.77	50.55	50.39	50.57±0.19
7.0	16.800	10.194	10.055	10.263	39.32	40.15	38.91	39.46±0.63

At this pH, colloid particles serve as nuclei for aggregate formation, facilitating sweep flocculation.[11] The results indicate that the optimum pH for the kratok bean coagulant is 5.5. Proteins dissolved from kratok beans contain -NH_3^+ groups that bind to negatively charged colloids, destabilizing them and forming settleable particles. These groups function as the active sites of the coagulant. Martina et al. (2018) reported that at low pH, protein amine groups (-NH_2) ionize to -NH_3^+, enhancing coagulant activity. At higher pH, amine groups do not ionize to -NH_3^+; instead, carboxyl groups (COOH) ionize to COO^-, reducing available coagulant sites.[12] Colloids bind to single polymer molecules, forming chemical bridge-like bonds, and increased bonding leads to greater aggregation and floc formation.[13] Coagulation and flocculation are interdependent, with flocculation success relying on effective coagulation. Both processes require a coagulant. Under acidic conditions, proteins carry a positive charge, which enhances their ability to neutralize colloid particles.[14] This explains the reduced effectiveness of the coagulant at pH 6-7, where proteins are negatively charged. Acidic conditions enable optimal destabilization of colloid particles, allowing the charge-neutralization mechanism to proceed efficiently and produce flocs that can be separated from the waste.

Analysis of Amino Acid Content

High-performance liquid chromatography (HPLC) analysis of the coagulant identified three cationic amino acids: histidine (0.82%), arginine (1.45%), and lysine (1.26%). These amino acids possess -NH_3^+ groups that facilitate binding to negatively charged particles in turbid water, promoting destabilization and aggregation into larger, settleable particles. Lysine's positive charge results from the ionization of amino groups on its aliphatic chain, while arginine's charge is due to its guanidino group. Histidine contains an imidazole ring that ionizes at this pH and is unique among amino acids for having a pKa near pH 7.[15] The amine groups attached to the Cα atom do not participate in coagulation, as they form peptide bonds with other amino acids and cannot ionize to produce positively charged NH_3^+.[16,17,18]

CONCLUSION

Kratok beans (Phaseolus lunatus) contain the cationic amino acids arginine (1.453%), lysine (1.261%), and histidine (0.822%). The kratok bean coagulant demonstrated optimal turbidity reduction at a concentration of 1.0% and pH 5.5.

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