Synthesis and Characterization of a Coagulating Agent From Plantain Peel Starch (Musa Paradisiaca), As Adjuvant in Water Treatment

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Research

Keywords: plantain peel waste, plantain starch, acetylation, water treatment, turbidity removal, coagulation

DOI: https://doi.org/10.21203/rs.3.rs-686997/v1

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Abstract

Coagulation processes are widely used for water treatment, mainly with chemical coagulants. In this research, starch derived from a residue (plantain peel, *Musa paradisiaca*) was used as a starting point for a chemical modification. Through acetylation, its chemical structure was modified and characterized by Infrared Spectrophotometry, for its evaluation as a coadjuvant in coagulation operations to reduce the turbidity of raw water. Two experimental designs were developed to evaluate the incidence of modified starch as the main coagulant or in conjunction with a conventional coagulant ($\text{Al}_2(\text{SO}_4)_3$), at different $\text{Al}_2(\text{SO}_4)_3$ / acetylated starch ratios, in jar-test experiments. In the first experimental design, with the acetylated starch as the main coagulant, turbidity removal percentages reached 47.93% (average value, 41.18%). For the $\text{Al}_2(\text{SO}_4)_3$ / acetylated starch coagulation process, 98.91% turbidity removal was reached in the second experimental design (average value, 97.16%). The incidence on turbidity in a jar-test of starch chemical substitution degree and the $\text{Al}_2(\text{SO}_4)_3$ / Acetylated starch ratio was investigated using ANOVA analysis. There was a great incidence of the chemical substitution degree and the concentration of acetylated starch used, when modified starch was used as the main coagulant. For the second experimental design, the $\text{Al}_2(\text{SO}_4)_3$ / Acetylated starch relationship had a greater incidence on the turbidity removal. Thus, modified starch obtained from plantain peel waste is a promising coadjuvant material for water coagulation processes.

1. Introduction

The contamination of water sources is a widespread situation of international interest in agreement to the Sustainable Development Goals (SDG). The targets proposed for SDG 14 “Life below water” aim to protect water ecosystems from pollution while SDG 6 “Clean water and sanitation” targets aim to protect and restore water ecosystem from untreated wastewater disposal, and to attack the lack of drinking water problem suffered by about 30% population, due to water stress conditions and lack of treatment and distribution infrastructure [1]. Thus, the efforts made for water treatment improvement are of great importance considering that water is one of the most essential substances for life, and it is necessary for both the economic and social development of populations and industries.

Coagulation and flocculation processes are necessary in various fields of human development for the removal or suspended organic or inorganic particles in colloidal forms in water. Coagulant reagents are used to destabilize the colloids that will further add forming flocs and settling. Since ancient times, for the treatment of drinking water, clarification processes for turbidity reduction by means of coagulation and flocculation practices have been applied. For example, the Egyptians around 2000 BC used smeared almond fruits to cover their boats external surface as a way to clear the river water. It is also reported that around 77 AD the Romans used to apply aluminum compounds as coagulant for water clarification, while municipal water treatment plants in English have used aluminum coagulant compounds sin the 18th century [2].
Since the demand for innovative and environmentally friendly water treatment technologies is growing, there is currently certain interest in finding alternative and natural compounds, such as starch obtained from organic waste, as a substitute or a coadjuvant for chemical coagulants and flocculants in water treatment, not only for domestic water production but also for the treatment of industrial waters, and as a reagent for processes within the food and beverage industry, biotechnology, and medicine applications. Due to their biodegradability, natural polysaccharides have proved to be useful as coagulation and flocculation reagents, since they are considered to be environmentally friendly when compared to inorganic and organic synthetic coagulants [3, 4].

Starch is one of the most important commercially available biopolymer sources, since it is used by plants as an energy reserve mechanism, and is abundantly found in plant seeds, roots, and fruits, in many vegetable types [5, 6]. Starch is chemically composed of two main structures: Amylose, which is a straight-chain biopolymer with 99% 1-4-alpha bonds and 1% 1-6-alpha bonds, and Amylopectin, which is a branched-chain biopolymer with 95% 1-4-alpha bonds and 5% 1-6-alpha bonds [7]. Since starch is highly hydrophilic, has a low cost, and is biodegradable, it is considered a naturally renewable biomaterial, which plays an important role in the food industry and is also used as a raw material to prepare different products in industries such as plastics, cosmetics, textiles, paper, pharmaceutical, among others [6, 8, 9].

Due to its energy content in the form of carbohydrates, starch is used as a food basis for people in many countries. Therefore, most industrial starch applications are mainly limited by its need as a food source as well as its physical properties [10]. In order to avoid using a food source as an industrial raw material, it has been proposed to use agro-industrial waste as a starch source, thus contributing to the circular economy. An example of starch source are the plantain peels (Musa paradisiaca) that can contain a significant amount of it and are widely cultivated in Latin American and African countries [5, 6, 11, 12]. However, the applications of native starch are limited by its solubility properties and its tendency to retrograde. One strategy that shows promising results is the chemical modification of the native starch, to introduce new functional groups [8, 9, 13].

In this research, the starch obtained from green plantain peel (Musa paradisiaca) waste was chemically modified using acetylation in order to evaluate its effectiveness as a coadjuvant in the coagulation and water clarification processes with the aim of reducing the use of synthetic coagulants such as Aluminum Sulfate (Al₂(SO₄)₃).

2. Materials And Methods

2.1 Materials

The raw material selected for starch extraction was green plantain (Musa paradisiaca) peel, obtained from the local food public market in Cartagena (Colombia). The original sample contained 5 kg plantain peels, which were processed immediately after peeling the fruits and separated in 150 g samples [6].
The reagents used to develop the starch chemical modification and water treatment experiments were: Sodium hypochlorite, Hydrochloric Acid, and Ascorbic Acid (AppliChem-Panreac®); Sodium Hydroxide (Merck®); Aluminum Sulfate and Acetic Anhydride (J.T. Baker®).

2.2 Native starch isolation method

The method used to extract the starch from green plantain peels with the best starch yield conditions was optimized and described in detail in a previous work of Hernández-Carmona, et al. [6], where it was evaluated how the extraction parameters, antioxidant concentration, and immersion time affect the starch production yield and purity. The method is a dry extraction process that starts with the crushing of the green plantain peel wastes in acidic solution (ascorbic acid, 5% w/v) with 5 min contact time, to promote the starch separation from the lignocellulosic fraction. The settled material is smashed to obtain a paste that is washed and filter before decanting the starch product. Finally, the starch is dehydrated at 40 °C during at least 10 h obtaining a dry starch powder.

2.3 Starch chemical modification

To chemically modify the starch, 40 g of native starch were weighed, adding 100 mL distilled water, and homogenizing the suspension with a magnetic stirrer platform in order to maintain a uniform suspension. The initial pH was adjusted to 8.0-8.5 adding a few drops of 3% NaOH, which acts as a catalyst. The acetic anhydride was slowly added (dropwise), simultaneously adjusting the pH between 8.0-8.5 until the required volume of acetic anhydride was added (volumes of 3.0 and 6.0 mL were used to obtain different degrees of substitution (DS)). Then the system was left to react for 10 minutes on the magnet stirring platform. Subsequently, the reaction was stopped by adding 0.5 N HCl solution, washing the starch 3 times with water and centrifuging the solution at 2500 rpm for 10 minutes. The last washing stage was performed with absolute alcohol and centrifuged at 2500 rpm for 10 minutes, and then dried in a tray oven at 40 °C for 12 hours [14, 15].

2.4 Physicochemical characterization

The native starch and the modified starch were characterized to determine their main physicochemical properties including moisture and ash content, starch detection, purity degree, iodine test, Fourier Transform Infrared (FTIR) spectroscopy, gelatinization temperature, acetylation percentage (acetyl %), and degree of substitution (DS).

Moisture content in plantain peels and starch (native and acetylated) was determined with a MB 45 OHAUS moisture analyzer. Ashes content was measured by incineration at 550°C during 3.5 h (according to AOAC 2000 method). Both moisture and ash content were expressed as % of the sample.

The Lugol test was performed with Lugol iodine, also known as Lugol solution, which was used as a reagent for starch detection in routine laboratory. The Lugol solution was prepared with 5 g of I₂ and 10 g
of KI diluted with 100 mL distilled water, giving a brown solution with total iodine concentration of 150 mg mL\(^{-1}\) [5, 16].

The native starch purity was characterized to determine the purity degree using method AOAC 920.44, for starch determination in baking powders by means of acid hydrolysis [17] and method AOAC 906.03, for invert sugar determination in sugars and syrups by Munson-walker general method. The amylose-amylopectin ratio was then calculated using the colorimetric method described by Morrison and Laignelet [18–20].

Samples of native and acetylated starch were analyzed in a FTIR SHIMATZU 8400S spectrophotometer, using the KBr pellet method according to the ASTM-E168 and ASTM-E1252 standards, with the objective of obtaining information on the characteristic functional groups. Each spectrum was analyzed in the resolution range from 400 to 4000 cm\(^{-1}\) [21].

To determine the gelatinization temperature, 10 g of starch were dissolved in 100 mL distilled water and the suspension was heated to 85 °C. Then, 50 mL of the suspension were taken and introduced into an 85 °C water bath. The starch slurry was constantly stirred until a paste was formed and the temperature was stable for a few seconds. Finally, the gelatinization temperature was read with a thermocouple [22].

The acetyl % and the DS for both native and acetylated starch, were determined titrimetrically following the methods described in Sodhi et al., Bello-Perez et al., and Lee Phillips et al. [23, 24]. Initially, 1.0 g sample was placed in a 250 mL flask with 50 mL of distilled water, and the suspension was stirred for 60 min at 25 °C. Phenolphthalein was added as an indicator, the solution was neutralized with NaOH 0.45 N until equilibrium was reached and stirred for 30 min. Then the samples were titrated with HCl 0.2 N to the end point. A blank sample consisting of native starch was also used for comparison. The acetyl % was determined with Eq. (1).

\[
\text{Acetyl} \% = \frac{[\text{mL Blank mL Sample}] \times [\text{HCl} 0.043 \text{ mL}]}{\text{Sample weight(g)}} \times 100
\]  

Finally, the DS is defined as the average number of sites per glucose unit that possess a substituent group [25] and calculated with Eq. (2).

\[
\text{DS} = \frac{162 \times \text{Acetyl} \%}{4300 - (42 \times \text{Acetyl} \%)}
\]  

2.5 Coagulation-flocculation tests

To evaluate the coagulant behavior of the modified starch and the hybrid coagulation agent (Al\(_2\)(SO\(_4\))\(_3\) + acetylated starch), we performed coagulation-flocculation tests. The raw water samples for the evaluation were taken from the Canal del Dique, a 118 km artificial water channel that connects Cartagena’s Bay to the Magdalena River (Bolivar Department, northern Colombia), since the Canal del
Dique is the surface water used by the water company in Cartagena (serving a population over 1 million inhabitants), and several smaller towns and population settlements located along its course.

The turbidity value for the raw water was measured with a VELP® TB1 model turbidimeter, using ASTM method D7315-07a (Standard Test Method for Determination of Turbidity above 1 Turbidity Unit (TU) in Static Mode), with and average value of 385.9 NTU. The turbidity removal efficiency of the acetylated starch and the hybrid coagulant agent was calculated with the difference between the initial and final turbidities as a percentage respect to the initial turbidity. The pH values were also measured for each experiment.

The flocculator used to perform the coagulation-flocculation experiments was a 6-jar apparatus from VELP® Scientica (Figure 1), following the procedure indicated in ASTM method D2035-13. Initially, 6 samples of 1 L of the raw water were put into the coagulation flasks and rapidly stirred at 200 rpm. Then, the acetylated or the hybrid coagulant were added to each flask, and the samples were stirred for another 5 min at the same speed to ensure the coagulation process under rapid mixing conditions. After that, the samples were stirred at 45 rpm for the next 15 min to perform the flocculation or slow mixing step. Finally, the samples were allowed to settle for 20 min. After sedimentation, the supernatant liquid was collected and analyzed for pH and turbidity. The effect of the acetylated starch was investigated using 100 and 350 mg L\(^{-1}\) doses and two different DS values (Low and High), that correspond to the different acetyl % obtained. Also, the effect of the hybrid coagulant was investigated using a mixture of acetylated / Al\(_2\)(SO\(_4\))\(_3\) coagulant at different ratios with two different DS. All experiments were performed at room temperature (25 ± 1 °C), and each experimental test was performed in four replicates [4, 26–28].

Experimental design was applied in order to investigate the effects of acetylated starch dose, the DS, and Al\(_2\)(SO\(_4\))\(_3\) / acetylated starch relationships. Two different experimental design tests, with two levels and two factors each (2x2), were proposed and 4 independent replications were taken at each of the 2x2 treatment combinations. The first experimental design test was applied to the experiments with acetylated starch as the main coagulant, while the second one was applied to the hybrid coagulant Al\(_2\)(SO\(_4\))\(_3\) / acetylated starch. The details of the first and second experimental designs are presented in Table 1 and Table 2, respectively. The design size was N=2×2×4=16 and was carried out randomly. The response variable for the two designs was the turbidity removal percentage [29].

Table 1 Experimental design test 1

| Factor | Factor definition     | Levels       | Symbol |
|--------|-----------------------|--------------|--------|
| A      | Acetylated starch dose| 100 mg/L Low | -      |
|        |                       | 350 mg/L High| +      |
| B      | Degree of Subs. (DS)  | 0.361 Low    | -      |
|        |                       | 0.562 High   | +      |
### Table 2 Experimental design test 2

| Factor | Factor definition                        | Levels | Symbol |
|--------|-----------------------------------------|--------|--------|
| C      | Wight ratio: Aluminum Sulfate/Acetylated starch | 3:1    | Low    |
|        |                                          | 1:1    | High   |
| D      | Degree of Subs. (DS)                     | 0.361  | Low    |
|        |                                          | 0.562  | High   |

#### 2.6 Statistical Analysis

A statistical significance analysis was performed to test the hypothesis for the means difference. In order to demonstrate whether there was a significant difference in the means of the response values (turbidity) of the experiment, they were divided into two groups characterized by the proportion of acetylated starch as an adjuvant, then a test of difference of means (two-sample t-Test) was accomplished [30].

Based on the data obtained, an analysis of variance (ANOVA) was performed to determine which factors have the greatest influence on the final turbidity degrees. The results were later analyzed with PAST v3.14® and STATGRAPHICS CENTURION XVI ® software for statistical evaluation. A two-way ANOVA test was performed, to evaluate whether the chemical acetylation, the DS, and Al$_2$(SO$_4$)$_3$ / acetylated starch mixtures relationships affect the turbidity in raw water, and if there are interactions between them [31].

### 3. Results And Discussion

#### 3.1. Native starch characterization

The organic plantain peel waste used as a raw material for starch extraction had an average humidity of 86%. From the analysis of the extracted powder, the Lugol test resulted positive for starch confirming the presence of the component in the extracted material. The test for purity determined that the extracted dry starch had a purity of 66.87 ± 1.04 %, and an ash content of 0.1127 ± 0.001 %. The amylose / amylopectin ratio values for the starch extracted using this methodology are 23.62/76.28 (%ratio).
Table 3

Typical bands in infrared spectroscopy of native starch

| Wavenumbers (cm⁻¹) | Bond Type                                                                 |
|--------------------|---------------------------------------------------------------------------|
| 3500               | Stretching of OH groups                                                   |
| 2929.97            | Stretching of CH bonds                                                    |
| 2150 – 1370        | Modes of atmospheric CO₂ present at the time of analysis                  |
| 1645               | Modes of vibration of H₂O present in the sample (Humidity)               |
| 1462               | Bending CH from CH₃                                                       |
| 1167               | Stretching and deformation of the C-O-C bond of the molecule              |

The chemical identification of native starch by FTIR-spectrophotometry can be found in a previous work by Ferreira-Villadiego et al. [13], who used the same extraction methodology. The FTIR spectrum of native starch produced from this study corresponds to the characteristic absorption peaks previously reported [13, 32, 33]. The absorption bands belonging to the acetylated sample are detailed in the Table 3. Some variations in the intensity of the peaks may differ from the standards due to the concentration of the constituent polymers (Amylose and Amylopectin).

The gelatinization temperature values for the native plantain peel starch used in this research (Musa paradisiaca), showed an average value of 72.6 ºC, which is similar to the values reported by Tribess et al. [34] from banana species Musa cavendischii with gelatinization values around 69ºC, which is the average gelatinization temperature in most native starches. The green plantain peel starch obtained, gelatinizes in a close temperature range to other starches obtained from the Musaceae family, such as that of the export banana from Ecuador (Musa sapientum) with a range between 77.7–80°C.

### 3.2. Modified starch characterization

The starch chemical modification or acetylation was carried out by varying the volume of acetic anhydride (3.0 and 6.0 mL) to obtain different DS, obtaining acetylated starch as a result. From the analysis if the acetyl % we obtained that the modified starches presented acetylation values of 8.77 ± 0.12% and 13.02 ± 0.79% (Low and High), and the DS calculation results were 0.361 ± 0.013 and 0.562 ± 0.06 (Low and High), respectively. The values of the acetylation percentages are in agreement with those reported by Vargas et al. [35], for the modification of potato-based starch. Additionally, Rendon-Villalobos et al. [36] report values for acetylated starch based also on green plantain (Musa paradisiaca), but on the pulp section, which were also in accordance with this study.

The analysis of the modified starch resulted in a gelatinization temperature for the acetylated starch samples with average 67ºC and 60ºC for the Low and High DS samples, respectively. If compared to the native starch results, we observe a reduction in the gelatinization temperature when the starch undergoes
a chemical modification, as reported in by Sandoval et al. [37], while both the native and the modified starch present gelatinization temperatures in the typical range reported for the starch specie.

Finally, the FTIR analysis for the acetylated starch (Fig. 2) confirms the effectivity of the chemical modification since the bands in the spectrum are in the region between 1580–1800 cm\(^{-1}\) which is typical for starch that was modified by acetylation [38]. These signals correspond to the vibration modes associated to the acetyl groups added to the starch structure. When compared to the native starch FTIR, the main variation in the spectra is caused by to the appearance of new bands at 1740, 1375 and 1240 cm\(^{-1}\), that are characteristic to the acetate group which is formed during the acetylation process [39]. The decrease in the 1605 cm\(^{-1}\) band in the modified starch can be attributed to the lower water affinity in comparison to that of the native sample. The reduction in water affinity due to the introduction of acetyl groups in the starch molecule gives them a hydrophobic character, as has previously been reported by Luo and Shi [40].

### 3.3. Coagulation test results

The results of the jar test experiments for turbidity removal performed under the different conditions described in the two experimental designs are shown in Table 4 and Table 5 for the experimental design test 1 and 2 respectively.

When the modified starch was used as the main coagulant reagent (Table 4), the turbidity removal average % increased from 34.2–37.5% with the coagulant dose applied, when the Low DS starch sample was used. The effect on pH was the opposite with a reduction from 7.4 to 7.2 for the same situation. For the experiments conducted with the high DS starch samples, the turbidity removal % reached 46.5% average while the pH was reduced to 6.9 average, with no significative differences caused by the increase in the coagulants dose. These results indicate that the samples with high DS are more efficient for turbidity removal, with no significative effect caused by the coagulant dose applied. However, the turbidity removal efficiency reached with the modified starch as a main coagulant is not enough to consider it a promising coagulant in this situation, since the final turbidity reached is still too high for a water coagulation process to be considered as successful (turbidity should be < 8 NTU if the water continues to a filtration stage as the final clarification step).
Table 4
Coagulation-flocculation test results for the experimental design test 1

| Acetylated starch dose (mg L\(^{-1}\)) | Experimental design test 1 |          |          |          |          |          |          |
|---------------------------------------|-----------------------------|----------|----------|----------|----------|----------|----------|
|                                       | Low DS                      | High DS  |          |          |          |          |          |
|                                       | pH  | NTU  | Turb. Removal (%) | pH  | NTU  | Turb. Removal (%) |
| 100                                   | 7.47 | 249  | 35.49      | 6.87 | 201  | 47.93    |
| 100                                   | 7.34 | 260  | 32.64      | 7.01 | 208  | 46.11    |
| 100                                   | 7.36 | 259  | 32.90      | 7.03 | 203  | 47.41    |
| 100                                   | 7.41 | 248  | 35.75      | 6.97 | 210  | 45.60    |
| 350                                   | 7.22 | 241  | 37.56      | 6.76 | 206  | 46.63    |
| 350                                   | 7.25 | 242  | 37.31      | 6.87 | 208  | 46.11    |
| 350                                   | 7.26 | 240  | 37.82      | 6.93 | 207  | 46.37    |
| 350                                   | 7.21 | 242  | 37.31      | 6.90 | 209  | 45.85    |

Table 5
Coagulation-flocculation test results for the experimental design test 2

| Al\(_2\)(SO\(_4\))\(_3\) / Acetylated Starch ratio | Experimental design test 2 |          |          |          |          |          |          |
|--------------------------------------------------|-----------------------------|----------|----------|----------|----------|----------|----------|
|                                                  | Low DS                      | High DS  |          |          |          |          |          |
|                                                  | pH  | NTU  | Turb. Removal (%) | pH  | NTU  | Turb. Removal (%) |
| 3:1                                              | 7.47 | 6.66 | 98.27      | 6.87 | 4.92 | 98.73    |
| 3:1                                              | 7.34 | 6.33 | 98.36      | 7.01 | 4.21 | 98.91    |
| 3:1                                              | 7.36 | 5.80 | 98.50      | 7.03 | 5.15 | 98.67    |
| 3:1                                              | 7.41 | 5.91 | 98.47      | 6.97 | 5.30 | 98.63    |
| 1:1                                              | 7.22 | 16.07| 95.84      | 6.76 | 15.79| 95.91    |
| 1:1                                              | 7.25 | 17.02| 95.59      | 6.87 | 16.02| 95.85    |
| 1:1                                              | 7.26 | 18.21| 95.28      | 6.93 | 16.10| 95.83    |
| 1:1                                              | 7.21 | 16.34| 95.77      | 6.90 | 15.80| 95.91    |

The experiments performed with the hybrid coagulant Al\(_2\)(SO\(_4\))\(_3\) / acetylated starch (Table 5), when the modified starch is acting as a coadjuvant reagent, present promising results adequate for a coagulation industrial process since the turbidity removal rates reach up to 98.91\% and turbidity minimum at 4.21
NTU, which is adequate for water that is later undergoing a filtration step. When the Low and High DS samples are compared in this case, there is no significative difference in the removal rate, although there is a slight pH reduction for the High DS case. On the other hand, the $\text{Al}_2(\text{SO}_4)_3 / \text{acetylated starch}$ ratio showed to affect the removal rate, since there was a significant reduction in the final turbidity for the ratio experiments that were low in starch content (3:1) when compared to the high starch relationship (1:1) ones. These results indicate that the samples with the hybrid coagulant $\text{Al}_2(\text{SO}_4)_3 / \text{acetylated starch}$ are efficient for water treatment processes reaching adequate removal rates and final turbidity values, mainly for the lower starch relationship, with no significant changes due to the DS.

3.4. Statistical analysis

Finally, to verify if there is a significant difference between the two groups of data derived from the experimental work, they were divided into two groups, Experimental Design 1 and Experimental Design 2, and subjected to two statistical tests. Firstly, the t-test was applied to determine if there was a difference between the means of the two groups of data, obtaining a p-value < 0.05. The results were then subjected to the Kolmogorov-Smirnov test to determine if each set of data came from different distributions, obtaining a p-value < 0.05. With these results, it can be stated with a 95% certainty that the two groups of data reflect phenomena influenced by different factors.

The ANOVA variance test was performed determine the statistical significance of each effect by comparing its mean square against an estimate of the experimental error. In this way, we analyzed the values of the significant parameters in the turbidity minimization process with the acetylated starch dose and the DS used in the coagulation experiments performed with the modified starch as the main coagulant (experimental design test 1). The results obtained from the ANOVA variance analysis are presented in Table 6 which shows that all factors (acetylated starch dose, DS value and the combination of them) have a high relevance (p-value < 0.05) in the turbidity removal efficiency.

The estimated response variable as a function of each experimental factor in experimental design test 1 is presented in Fig. 3 where it is clear that the combination of the effects minimizes the response variable to some extent, however the DS values play the most influential and important role for this design. The first part of the graph shows the response variable (turbidity) when factor the acetylated starch dose goes from a low level (100 mg L$^{-1}$) to a high level (350 mg L$^{-1}$), while the second shows its response for the DS value going from low level (0.36) to high (0.56). The two factors give negative slope lines which means that the turbidity response improves if the factor remains at a high level but, with a clear advantage, the DS value is the one that shows the highest effect.
Table 6
ANOVA Variance Analysis for the experimental design test 1

| Source                  | Sum of Squares | DF | Mean Square | F-ratio | p-value |
|-------------------------|----------------|----|-------------|---------|---------|
| A: Acetylated Starch    | 115.563        | 1  | 115.563     | 8.19    | 0.0187  |
| B: DS                   | 6765.06        | 1  | 6765.06     | 479.18  | 0.0000  |
| A*B                     | 217.563        | 1  | 217.563     | 15.41   | 0.0035  |
| Block                   | 55.6875        | 3  | 18.5625     | 1.31    | 0.3287  |
| Residual                | 127.063        | 9  | 14.1181     |         |         |
| Total (corr.)           | 7280.94        | 15 |             |         |         |

On the other hand, the results obtained from the ANOVA variance analysis in the experimental design test 2 are presented in Table 7 showing that both the Al$_2$(SO$_4$)$_3$ / acetylated starch ratio and the DS value have a high relevance (p-value < 0.05) in the turbidity removal efficiency, but there is no influence of the combination of both factors.

Table 7
ANOVA Variance Analysis for the experimental design test 2

| Source                   | Sum of Squares | DF | Mean Square | F-ratio | p-value |
|--------------------------|----------------|----|-------------|---------|---------|
| C: Al$_2$(SO$_4$)$_3$ / Acetylated Starch ratio | 473.824 | 1  | 473.824     | 1269.70 | 0.0000  |
| D: DS                    | 5.11891        | 1  | 5.11891     | 13.72   | 0.0049  |
| C*D                     | 0.0885063      | 1  | 0.0885063   | 0.24    | 0.6379  |
| Block                   | 0.616469       | 3  | 0.20549     | 0.55    | 0.6603  |
| Residual                | 3.35861        | 9  | 0.373178    |         |         |
| Total (corr.)           | 483.007        | 15 |             |         |         |

The estimated response variable as a function of each experimental factor in experimental design test 2 is presented in Fig. 4 where it is clear that the combination of the effects minimizes the response variable, however the DS values do not have a high influence being the most important in this case for the Al$_2$(SO$_4$)$_3$ / acetylated starch ratio. The first part of the graph shows the response variable (turbidity) when factor the starch content in the Al$_2$(SO$_4$)$_3$ / acetylated starch coagulant goes from a low starch level (3:1 ratio) to a high starch level (1:1 ratio), while the second shows its response for the DS value going from low level (0.36) to high (0.56). The DS factors gives a negative slope lines which means that the turbidity response improves if the factor remains at a high level but, since the slope is low, the influence can be considered minimum. On the other hand, the Al$_2$(SO$_4$)$_3$ / acetylated starch ratio presents a positive
and high slope confirming that this factor has the most important influence in the turbidity results with the best removal efficiency when the starch content in the hybrid coagulant is low.

4. Conclusions

Green plantain (Musa paradisiaca) peel starch was extracted from the residual biomass confirming that the product obtained is rich in starch (66.87% purity) and presents an FTIR response that corresponds to the values previously reported. After modifying the starch by means of an acetylation reaction, the FTIR spectra confirmed the effect of the acetylation procedure due to the changes in the spectra and the analysis if the degrees of substitution.

The coagulation-flocculation experiments performed with the acetylated starch as the main coagulant under the conditions of the experimental design test 1 indicate that the highest turbidity removal rates were obtained for the starch with the highest degrees of substitution with no significant influence from the coagulant dose. However, the turbidity removal efficiency reached for these experiments is low for a coagulation-flocculation process to be considered successful for a water treatment plant, since the minimum turbidity value reached was 201 NTU, which is far from the recommended 8 NTU required for a plant with filtration stage as the final clarification step. On the other hand, the experiments conducted using the modified starch in a hybrid coagulant with Al₂(SO₄)₃, that correspond to the experimental design test 2, confirm that the modified starch is a promising coadjuvant reagent for the coagulation-flocculation water treatment, since the turbidity removal was considerably higher, reaching final turbidity values as low as 4.21 NTU.

The statistical analysis helped to identify the dependence of the turbidity response with the coagulant dose, the degrees of substitution obtained from the starch modification and the starch relationship in the hybrid coagulant. All the factors presented an incidence on the turbidity removal rate, however there were some differences in their intensity. When the modified starch was used as the main coagulant, the most important factor was the degree of substitution, meaning that the chemical modification process has a significant effect on the behavior of the coagulant, resulting in higher efficiency for the higher acetylation effect. On the other hand, when the modified starch acts as a coadjuvant in a Al₂(SO₄)₃ / acetylated starch hybrid coagulant, the turbidity responses are not dependent on the degrees of substitution but is mainly affected by the modified starch proportion in the hybrid coagulant.

Finally, it can be concluded that the best experimental conditions for the use of green plantain (Musa paradisiaca) peel modified starch as a reagent for coagulation-flocculation processes are found for a hybrid Al₂(SO₄)₃ / acetylated starch coagulant reagent with high degrees of substitution levels, reaching turbidity removal efficiencies adequate for water treatment plants.

Declarations

Availability of data and materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors’ contributions

Mr. Cortes-Perez and Mr. Perez-Montalvo participated in the design and interpretation of the data and performed the experiments and analysis. Mr. Puello-Silva provided technical support, participated in the design and interpretation of the data, supervised the research, and revised the manuscript. Dr. Pasqualino provided technical support, participated in the design and interpretation of the data, wrote the paper, and participated in the revisions of it. Mr Lambis conceived this research and designed experiments, participated in the design and interpretation of the data, supervised the research, wrote the paper, and participated in the revisions of it. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank the support given by Fundación Universitaria Tecnológico Comfenalco – Cartagena (Colombia), the members of the CIPTEC Research Group, and Universidad Tecnológica de Bolívar for their support during the development of the research.

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**Figures**

![Figure 1](image_url)

**Figure 1**

Coagulation-flocculation 6-jar apparatus
Figure 2

FTIR spectra from acetylated plantain peel starch
Figure 3

Main Effects for Turbidity Response (Experimental Design 1)

Figure 4

Main Effects for Turbidity Response (Experimental Design 2)