Natural-based coagulants from chestnut shells for turbidity and color removal: toward sustainable wastewater treatment

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Abstract

Turbidity and color are critical parameters in industrial wastewater treatment, particularly in textile effluents containing suspended particles and dyes. Conventional coagulation methods are effective but have significant drawbacks. In this context, the development of natural coagulants has emerged as a promising alternative method. This study investigated the production of a natural-based coagulant from chestnut shells (*Castanea sativa*), an abundant agro-industrial residue in Portugal, through tannin extraction followed by chemical modification via the Mannich reaction. The tannin extraction process was statistically optimized using a Box-Behnken Design to evaluate the effects of pH, extraction time, and biomass dosage. The coagulant performance was evaluated using Jar Test assays for turbidity (kaolin) and color (methylene blue) removal. Statistical analysis showed that these factors affected turbidity and color removal differently, with higher extraction times and biomass dosage generally favoring turbidity removal. The results showed high turbidity removal efficiencies, reaching up to 96%, whereas color removal efficiencies were lower, reaching approximately 26%, suggesting that dye removal depends not only on coagulation mechanisms but also on specific interactions between the dye and the coagulant. Overall, the results demonstrate the potential of chestnut shell residues as a sustainable source of natural-based coagulants for wastewater treatment.

Keywords: chestnut shells, natural coagulant, Response Surface Methodology, tannin extraction, wastewater treatment.

Resumo

Turbidez e cor são parâmetros críticos no tratamento de águas residuais industriais, especialmente nos têxteis contendo partículas em suspensão e corantes. Os métodos convencionais de coagulação são efetivos, mas apresentam desvantagens significativas. Neste contexto, o desenvolvimento de coagulantes naturais tem surgido como uma alternativa promissora. Este estudo investigou a produção de um coagulante de base natural a partir de cascas de castanha (*Castanea sativa*), um resíduo agroindustrial abundante em Portugal, através da extração de taninos seguida de modificação química via reação de Mannich. O processo de extração de taninos foi otimizado estatisticamente usando um planeamento Box-Behnken, avaliando os efeitos do pH, tempo de extração e quantidade de biomassa. O desempenho do coagulante foi avaliado através de ensaios Jar Test para remoção de turbidez (caulino) e cor (azul de metileno). A análise estatística mostrou que estes fatores afetaram a remoção de turbidez e cor de forma diferente, tendo tempos de extração e quantidades de biomassa mais elevados geralmente favorecido a remoção de turbidez. Os resultados revelaram elevadas eficiências de remoção de turbidez, atingindo até 96%, enquanto as eficiências de remoção de cor foram mais baixas, alcançando aproximadamente 26%, sugerindo que a remoção do corante depende não apenas dos mecanismos de coagulação, mas também de interações específicas entre o corante e o coagulante. No geral, os resultados demonstram o potencial das cascas de castanha como uma fonte sustentável de coagulantes de base natural para aplicações de tratamento de águas residuais.

Palavras-chave: cascas de castanha, coagulante natural, extração de taninos, Metodologia de Superfície de Resposta, tratamento de águas residuais.

INDEX

Index of Figures

Index of Tables

List of Abbreviations

AdP	Águas de Portugal (Water of Portugal)
ANOVA	Analysis of Variance
APA	Agência Portuguesa do Ambiente (Portuguese Environmental Agency)
BBD	Box–Behnken Design
BOD	Biochemical Oxygen Demand
CE	Circular economy
COD	Chemical Oxygen Demand
IARC	International Agency for Research on Cancer
MS	Mannich solution
NOM	Natural Organic Matter
PGRH	Planos de Gestão de Região Hidrográfica (River Basin Management Plans)
RHs	Regiões Hidrográficas (Hydrological regions)
RSM	Response Surface Methodology
SDG	Sustainable Development Goals
UV	Ultraviolet
UV-Vis	Ultraviolet–Visible
WFD	Water Framework Directive
WHO	World Health Organization

1. Introduction

Water is essential for every aspect of life; therefore, ensuring its purity is crucial (Nzeyimana & Mary, 2024). However, many countries are experiencing severe water scarcity due to population growth, extreme climate events, and cropland expansion (Alshoubaki, 2025; Tong et al., 2025). A major concern in this regard is the excessive withdrawal of water resources and pollution associated with inadequate sanitation practices (Rajendrakumar et al., 2025).

To address this, the United Nations launched the 2030 Agenda for Sustainable Development, which includes 17 Sustainable Development Goals (SDGs), with SDG 6 focusing on ensuring universal access and sustainable management of water and sanitation (*United Nations*, 2015). In this context, improving wastewater treatment technologies has become increasingly important for reducing environmental impact and promoting water reuse.

Among the main parameters used to assess water quality, turbidity and color are of particular concern, as they interfere with light penetration, hinder disinfection processes, and are often associated with the presence of organic and potentially toxic substances (Soros et al., 2019; Collave et al., 2024; Zourif et al., 2023).

Industrial activities, particularly those associated with the textile sector, are major contributors to water pollution. Textile effluents are typically characterized by high color intensity, turbidity, and the presence of persistent dyes, which are often resistant to conventional biological treatment processes (Verma et al., 2011).

Coagulation–flocculation is a widely applied process for removing turbidity and color from drinking water and wastewater treatment systems (Song et al., 2025; Zourif et al., 2023). Conventional inorganic coagulants, such as aluminum sulfate, are highly effective; however, their use presents several drawbacks, including high sludge production, sensitivity to operational conditions, residual metal content, and potential environmental and health concerns (Soros et al., 2019). These limitations have motivated the search for alternative coagulants derived from renewable and environmentally friendly material.

In recent years, natural coagulants obtained from plant-based materials have attracted increasing attention as sustainable alternatives to conventional chemical coagulants (Nzeyimana & Mary, 2024; Tomasi et al., 2025a; Ibrahim et al., 2020). Among these materials, tannins are particularly noteworthy due to their availability, biodegradability, and low cost (Zourif et al., 2023). However, because tannins typically exhibit an anionic nature, similar to most colloidal particles found in wastewater, chemical modification through cationization is generally required to enhance their coagulation performance. The Mannich reaction is a widely reported method for improving tannin efficiency, involving the reaction of the tannin structure with a primary or secondary amine and an aldehyde, typically formaldehyde (Tomasi et al., 2025).

Portugal is one of the main chestnut-producing countries in Europe, generating large quantities of chestnut shells as agroindustrial by-products (Tomasi et al., 2025b). These residues are rich in condensed tannins and represent promising and low-cost raw materials for producing bio-based coagulants (Tomasi et al., 2024; Tomasi et al., 2025b). The valorization of this residue aligns with circular economy principles and contributes to reducing the environmental impact associated with waste disposal and conventional coagulant production.

2. Objectives

2.1 General Objective

The main objective of this study was to develop a natural-based coagulant derived from chestnut shells through tannin extraction and subsequent chemical modification via the Mannich reaction, optimize the extraction process using Response Surface Methodology, and evaluate the coagulant performance in turbidity and color removal using synthetic wastewaters.

2.2 Specific Objectives

The specific objectives of this study are as follows:

- To extract tannins from chestnut shells;
- To optimize the tannin extraction conditions using a Box–Behnken Design;
- To chemically modify the extracted tannins through cationization via the Mannich reaction to improve coagulation performance;
- To assess the efficiency of the synthesized natural-based coagulant in turbidity and color removal using the Jar Test;
- To compare the performance of the chestnut shell–based coagulant with that of the conventional coagulant, aluminum sulfate.

3. State of The Art

3.1 Sustainability: SDGs and Circular Economy

The 2030 Agenda for Sustainable Development, adopted by United Nations Member States in 2015, establishes a global blueprint for ensuring long-term prosperity for both society and the planet (*United Nations*, 2015). Among its 17 Sustainable Development Goals (SDGs), SDG 6 focuses specifically on ensuring universal access to safe water and sanitation and, the sustainable management of water resources (*United Nations*, 2015).

Sustainable wastewater treatment plays an essential role in achieving SDG 6 and contributes to other goals, including SDG 3 (Good Health and Well-being), SDG 7 (Affordable and Clean Energy), SDG 9 (Industry, Innovation and Infrastructure), SDG 11 (Sustainable Cities and Communities), SDG 12 (Responsible Consumption and Production), SDG 13 (Climate Action), SDG 14 (Life Below Water), and SDG 15 (Life on Land) (Wada et al., 2026).

In recent years, wastewater has been increasingly viewed as a valuable source of water, nutrients, and energy rather than merely a waste stream. Consequently, treatment plants are being adapted to enable resource recovery and process optimization, addressing the high energy demand and environmental impact associated with conventional systems, while harnessing the significant energy potential contained in wastewater (Wada et al., 2026).

In this context, the circular economy (CE) has emerged as a strategic framework for addressing environmental challenges and fostering sustainable development. Its core principles involve redesigning systems to eliminate waste and pollution, keeping materials and products in continuous use, and regenerating natural systems (Bermejo-Campos & García-Avila, 2025).

In the water sector, this approach goes beyond water reuse to include nutrient and energy recovery, pollution reduction, and the valorization of treatment by-products, reinforcing wastewater treatment as a key component of sustainable resource management (Bermejo-Campos & García-Avila, 2025).

3.2 Water Quality: Turbidity and Color

Water quality monitoring covers a wide range of parameters, including physical, chemical, and biological parameters (Gao et al., 2025). Among the key physical indicators that demand stringent control are turbidity and color.

Turbidity refers to an optical characteristic of water in which light is scattered and absorbed by particles instead of being transmitted with no change in direction or intensity, and it results from the presence of suspended and colloidal matter, including clay, silt, finely divided organic and inorganic matter, plankton, and other microscopic organisms (Baird et al., 2017). This parameter can hinder water treatment by prematurely clogging the filter, reducing the effectiveness of chemical disinfection through increased oxidant demand, and limiting the efficiency of ultraviolet (UV) irradiation by blocking the light transmission (Soros et al., 2019). In addition, turbidity affects consumer perception, as visible cloudiness in treated water often leads to doubts about its cleanliness and safety for consumption (Soros et al., 2019).

The color parameter refers to the appearance of water and represents one of the main contaminants in wastewater (Zourif et al., 2023). It can be categorized as true color due to only dissolved substances, or apparent color due to both dissolved and suspended materials (Kubinova et al., 2023). This parameter primarily results from the presence of natural organic matter (NOM), specifically aquatic humic matter, such as humic and fluvic acids (Baird et al., 2017). These acids can cause yellow, red, brown, and gray colors in water (Ordonez et al., 2022). It is also important to note that the intensity and hue of the water color are usually dependent on the pH value and must be related to this parameter for accurate evaluation (Kubinova et al., 2023). Similar to turbidity, high levels of color have an adverse impact on aquatic ecology by severely interfering with the transmission of sunlight into water bodies, consequently reducing photosynthetic activity, leading to eutrophication and significant perturbations in aquatic life (Collave et al., 2024; Mane et al., 2006).

For drinking water and specific industrial applications, the presence of turbidity and color impairs water quality, necessitating treatment to enhance its operational efficiency and aesthetic appeal.

3.3 Industrial Wastewater and Textile Sector

While natural organic matter contributes to the color and turbidity of drinking water sources, the most significant and challenging source of both pollutants often comes from industrial effluents, such as those from the paper, rubber, cosmetics, and plastics industries (Ong et al., 2010). The global scale of this challenge is significant, with approximately 10000 different synthetic dyes and pigments used in these industries, resulting in an annual production exceeding 7×10^5 tons worldwide (Ong et al., 2010).

Among these sectors, the textile industry is recognized as one of the largest industrial consumers of water, with an estimated annual consumption of approximately 93 billion m^3 (Tomasi et al., 2025a). In this industry, water consumption is primarily driven by wet processing operations, such as pre-treatment, dyeing, washing, and printing, which are responsible for the most significant environmental impacts and generate substantial volumes of wastewater, estimated at 113 - 151 m^3 per ton of textile produced, particularly during the dyeing and rinsing stages (Verma et al., 2011). During the dyeing process, a significant portion of the dye, estimated to be 15% of the total world dye production, is lost and subsequently released into textile effluents (Mane et al., 2006).

Among the various dyes employed, methylene blue stands out as one of the most extensively used colorants in the textile industry, and is commonly applied to materials such as cotton, wool, silk, linen, and jute (Zourif et al., 2023). Owing to its high toxicity, methylene blue is an environmental and public health concern; because exposure may result in adverse effects, including gastrointestinal disturbances, ocular damage, and methemoglobinemia (Zourif et al., 2023).

The residual materials from textile processes released as wastewater are characterized by high levels of color, biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, temperature, turbidity, and other toxic compounds (Verma et al., 2011). Textile dye wastewaters not only impact aquatic environments, but may also pose risks to human health because of the presence of dye intermediates and other xenobiotic compounds, capable of inducing ecotoxic, mutagenic, and carcinogenic effects (Amato et al., 2025).

Therefore, the treatment of textile wastewater is challenging, as the diverse composition and complex characteristics of dyes create major obstacles to achieving efficient and economical color removal, emphasizing the pressing need to address these environmental issues (Tomasi et al., 2025a).

3.4 Water and Wastewater Management in Portugal

Water management and protection in Portugal follow the European Union’s Water Framework Directive (WFD), which was implemented in 2000. This directive provides a comprehensive legal framework for safeguarding inland, coastal, and groundwater resources using an integrated, ecosystem-based approach. It establishes pollutant regulations and quality standards, organized by river basin districts to promote coordination among neighboring countries (*Water Framework Directive*, 2025).

On the national level, the *Agência Portuguesa do Ambiente* (APA) (Portuguese Environment Agency) acts as the National Water Authority and is the competent entity for the protection and management of water resources, including the protection and conservation of the country's coastal strip and promotion of efficient water use (*Agência Portuguesa Do Ambiente*, 2026). Complementarily, the publicly owned business group *Águas de Portugal* (AdP) (Water of Portugal) is responsible for the integrated management of the urban water cycle in the country. Its operations encompass every stage, from water abstraction, treatment, and distribution for public supply to the collection, transportation, treatment, and discharge of urban and industrial wastewater, as well as activities related to water recycling and reuse (*Águas de Portugal,* n.d.).

For management purposes, continental Portugal is divided into eight hydrological regions (RHs), as legally defined in Decree-Law No. 347/2007 (amended by Decree-Law No. 117/2015) (*Agência Portuguesa Do Ambiente*, 2025). The geographical distribution of this status in Portuguese rivers is shown in Figure 1.

Figure 1. Portugal's hydrological regions (RHs) (Adapted from Agência Portuguesa do Ambiente, 2025).

Data from the APA's River Basin Management Plans (PGRH) reveal that water quality in Portugal faces significant challenges. The status and potential of surface water bodies in these regions were classified into five categories (Figure 2).

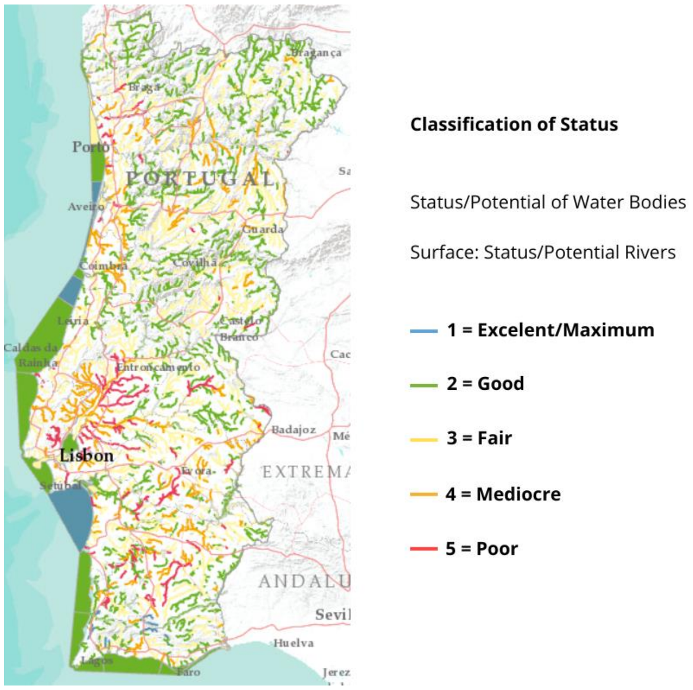


Figure 2. Classification of River Status by Hydrological Region in Continental Portugal *(*Adapted from *Agência Portuguesa do Ambiente*, 2025).

In the 3rd planning cycle, only 47% of continental water bodies achieved "Good Ecological Status/Potential" (*Agência Portuguesa Do Ambiente*, 2025). According to the APA's analysis (2025), of the 999 surface water bodies with a status below good, the primary impacts registered are pollution by nutrients (41% of total impacts), followed by organic pollution (14%), and chemical pollution (13%).

Although some hydrological regions (RH1 and RH8) showed better results (66% and 65% in good status, respectively) (*Agência Portuguesa Do Ambiente*, 2025), a comparison with the 2nd cycle indicated a decrease in river quality across almost all regions.

In the northern coastal region (RH3), the Douro River, the most important freshwater input, is the main contributor of sediments to the Northwestern Iberian coast, generating high levels of turbidity and influencing the biogeochemical features of the coastal area (Mendes et al., 2014). In contrast, the southern hydrological basins, characterized by a Mediterranean climate, are strongly influenced by climatic seasonality and historically high levels of human intervention, including intensive agriculture, damming, and urban development (Silva, 2012). This combination leads to large-scale deterioration of fluvial ecosystems due to hydrological alterations, physical disturbances, and both point and diffuse pollution (Silva, 2012).

Regarding Chemical Status, which assesses the presence of hazardous pollutants, the results are more favorable, with 69% of continental water bodies classified as being in "Good Chemical Status" in the 3rd cycle (*Agência Portuguesa Do Ambiente*, 2025).

3.5 Conventional Coagulation Processes

The coagulation-flocculation process dominates conventional wastewater treatment technologies and is widely applied to reduce turbidity in highly turbid waters because of its operational simplicity, efficiency, and wide availability of coagulant-flocculant agents (Song et al., 2025; Zourif et al., 2023). In practical wastewater treatment, the coagulation-flocculation process is initiated by adding coagulants to destabilize small colloidal particles suspended in water. This is followed by flocculation, in which the destabilized particles aggregate to form larger masses known as flocs, which are then easily separated by sedimentation (Yang et al., 2016). This process is fundamental not only for its effectiveness in removing suspended particles but also for its ability to remove organic content and other contaminants, such as fluoride (Al-Hanaktah et al., 2025).

However, the efficiency of coagulation-flocculation is sensitive to several operational and environmental factors, such as coagulant type and dose, temperature, pH, water quality to be treated, and hydrodynamic conditions, such as mixing speed and time (Karnena et al., 2022).

Traditionally, large-scale wastewater treatment relies on metal salts such as aluminum sulfate, ferric sulfate, and ferric chloride. Although these metal salts are effective, their use has a significant drawback the resultant sludge, which contains residual metals (Soros et al., 2019). Their residues in treated water are associated with neurotoxic and carcinogenic properties, with studies linking them to diseases such as Alzheimer's disease, Parkinson's disease, and various types of cancer (Karnena et al., 2022). Therefore, the presence of these residual metals means that the resulting by-product requires careful management and proper disposal to mitigate the risk of secondary environmental pollution.

3.6 Natural Coagulants and Tannins

To overcome the challenges posed by chemical coagulants, such as sludge management and potential toxicity, attention has shifted towards natural materials from plants, particularly those based on tannins, as organic coagulants (Tomasi et al., 2025a). This intensified focus on natural coagulants is justified by their inherent advantages, as numerous studies have consistently demonstrated their effectiveness (Nzeyimana & Mary, 2024; Tomasi et al., 2025a; Ibrahim et al., 2020). These tannin materials are typically derived from agricultural residue products, ensuring their availability and low cost (Zourif et al., 2023). Additionally, they can yield safer treated water with significantly reduced levels of harmful residual metals (Al-Hanaktah et al., 2025).

In this context, it is essential to acknowledge the strong potential of utilizing agricultural residues as a sustainable source for producing tannin-based coagulants, thereby actively promoting the principles of circular economy (Tomasi et al., 2024).

Tannins, which are water-soluble phenolic compounds, are among the most abundant substances extracted from vegetable cells, following cellulose, hemicellulose, and lignin. They are primarily concentrated in the soft tissues of plants (Ibrahim et al., 2020). These naturally occurring polyphenolic compounds exhibit a broad range of molecular weights and are distinguished by their ability to precipitate proteins from aqueous solutions (Aguilar et al., 2007). Found in bark, seeds, leaves, and comestibles such as grapes and walnuts, tannins act as defensive agents, protecting plants from fungi, pathogens, and herbivores (Das et al., 2020).

Tannins are generally classified into condensed tannins, flavonoid-based compounds without a sugar core, and hydrolyzable tannins, which consist of gallic and ellagic acids linked to a sugar moiety (Das et al., 2020). Hydrolyzable tannins are further subclassified into gallotannins, which are defined by molecules of organic acids, such as gallic acids, esterified to a molecule of glucose, and ellagitannins, which release ellagic acid as the principal hydrolysis product (Aguilar et al., 2007; Arbenz & Avérous, 2015). Another category includes complex tannins, which are structurally characterized by the linkage of an ellagitannin moiety to a flavan-3-ol unit (Arbenz & Avérous, 2015). Figure 3 illustrates the different tannin classes and their representative structural features.

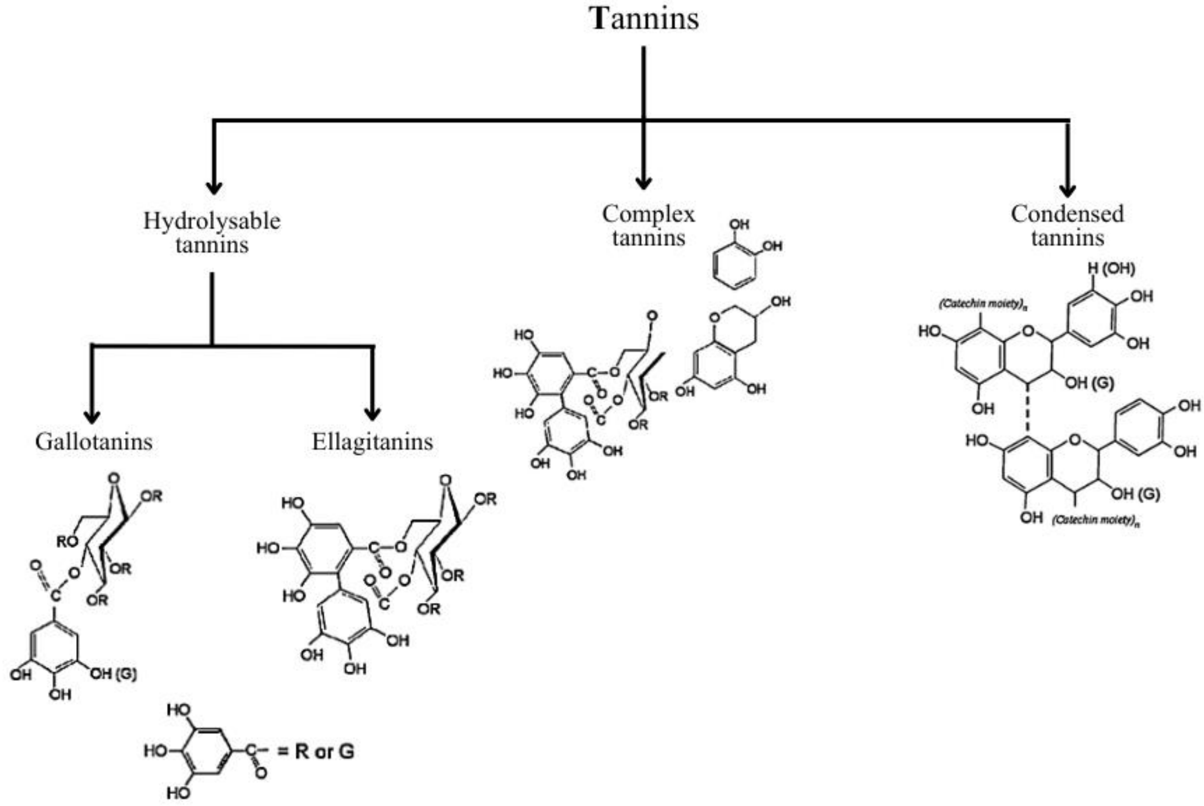


Figure 3. Tannin classes and structural features (Adapted from Aguilar et al., 2007).

The scientific literature documents a wide array of tannin sources that have been investigated for their potential as coagulants, primarily derived from forestry, agriculture, and food industry water (Tomasi et al., 2025b).

Given that agro-industrial residues are a major sustainable source of new biopolymers, chestnut shells (Castanea sativa), a significant by-product of Portuguese agriculture, have a high potential as a raw material for natural coagulants (Tomasi et al., 2025b).

Portugal is among the world's top ten chestnut producers, generating approximately 1.1 thousand tons of chestnut shells annually (Tomasi et al., 2025b). Although these shells are sometimes used as natural soil conditioners or for energy recovery, they are generally discarded, representing an underutilized agricultural byproduct (Tomasi et al., 2025b).

Chestnut shells are a rich source of tannins, which can be chemically modified to introduce positive charges while maintaining their solubility and chelating properties, resulting in a polymer that functions efficiently as a coagulant (Kim et al., 2025; Tomasi et al., 2024).

3.7 Mannich Reaction

The classical Mannich reaction is a three-component condensation in which a structurally diverse substrate bearing at least one active hydrogen atom (X–H) reacts with an aldehyde and an amine to form a family of products known as Mannich bases (Roman, 2014). Despite of the variety of possible substrates, an essential requirement is the presence of an activating functional group that makes the X–H position sufficiently reactive in the Mannich reaction, such as the phenolic hydroxyl group in phenols (Roman, 2014), which is the case for tannins.

Under acid catalysis, the first step of the Mannich reaction involves the interaction between an amine and an aldehyde, forming a hemiaminal intermediate. This intermediate then undergoes dehydration to produce an iminium ion. Subsequently, an enolizable carbonyl compound acts as a nucleophile and attacks the iminium ion, leading to the formation of the corresponding Mannich base (Fernandes, 2016).

When applied to tannins, the resulting tannin Mannich polymer exhibits ampholytic characteristics due to the presence of cationic amines and anionic phenols (Sánchez-Martín & Beltrán-Heredia, 2012). Consequently, Mannich-modified tannins have been investigated as bio-based coagulants for wastewater treatment (Arismendi et al., 2018; Bello & Leiviskä, 2021; Tomasi et al., 2025).

3.8 Current Research on Chestnut Shell Coagulants

The use of chestnut (*Castanea sativa*) as a promising source for the development of natural coagulants has gained attention because of its high tannin content and wide availability as an agro-industrial residue (Kim et al., 2025; Tomasi et al., 2025b).

Recent studies have established that raw chestnut extracts possess limited coagulation efficiency because of their predominantly anionic nature. Therefore, research has shifted toward chemical modifications. Studies have demonstrated that the cationization of chestnut tannins via the Mannich reaction is a critical step in enhancing their performance, as it introduces quaternary ammonium groups that facilitate charge neutralization (Arismendi et al., 2018; Bello & Leiviskä, 2021; Tomasi et al., 2025).

Recently, Tomasi et al. (2024, 2025) expanded the application of these bio-coagulants to real effluents. Their findings indicated that chestnut shell coagulants can effectively reduce pollutants while simultaneously providing value-added applications for agricultural waste.

Similarly, studies using tannin-based coagulants from other biomass sources, such as *Acacia mearnsii*, have reported satisfactory treatment performance after chemical modification (Grenda et al., 2018).

While extraction optimization has been explored in previous studies, including microwave-assisted extraction techniques (Tomasi et al., 2023), few studies have evaluated the effects of extraction parameters, such as pH, extraction time, and biomass dosage, on the coagulation performance of chemically modified chestnut shell extracts.

Current research trends suggest that variables such as the biomass-to-solvent ratio, pH, and extraction time significantly influence the final tannin yield (Hoyos-Martínez et al., 2018; Zedan et al., 2022) and its subsequent performance. This study addresses this gap by employing a Box-Behnken Design (BBD) to determine the optimal extraction equilibrium, ensuring maximum efficiency off the chestnut shell residue before its conversion into a coagulant.

Furthermore, while the removal of some dyes using bio-based coagulants is well documented (Tomasi et al., 2025; Shamsnejati et al., 2015), there is a scarcity of studies addressing the removal of methylene blue using chestnut shell coagulants. Such investigations may contribute to improving the efficiency and applicability of chestnut shell coagulants as sustainable alternatives for water treatment applications.

4. Methodology

4.1 Tannin Extraction

First, the chestnuts (Castanea sativa) were manually peeled to remove the edible kernel. The shell fraction (Figure 4). a), including both the outer woody shell and the inner fibrous layer naturally attached to it, was used for tannin extraction. The material was then oven-dried at 60 °C for 24 h to remove the moisture. Subsequently, the dried shells were ground to obtain a fine particulate material (Figure 4). b) and placed in an oven at 60 °C for 24 h.

(a)

(b)

Figure 4. (a) Chestnut shells after peeling; (b) ground chestnut shells.

Most extraction techniques rely on the use of solvents such as water alone, organic solvents, alkaline solutions, or acidic solvents (Arbenz & Avérous, 2015).

In this study, tannins were extracted using an alkaline solution, as alkaline pH conditions are reported to increase extraction yields due to the partial breakage of pyran rings in phlobaphenes, which are tannin-derived compounds (Hoyos-Martínez et al., 2018). In addition, alkaline solutions such as NaOH can promote tannin depolymerization and increase their solubility, enhancing the extraction efficiency (Rodrigues et al., 2026) Different dosages of ground shells were mixed with 100 mL of a 0.05 M sodium hydroxide (NaOH) solution (Banwan et al., 2024). The mixture was maintained under constant agitation at room temperature.

After extraction, the solid residue was separated from the liquid via gravimetric filtration. The tannin extract was stored under refrigeration for subsequent applications.

4.1.1 Experimental Design and Process Optimization

Response Surface Methodology (RSM) was applied to optimize the extraction conditions of natural-based coagulants obtained from chestnut shells for wastewater treatment, as this approach allows the evaluation of linear, interaction, and quadratic effects of the studied variables on the response, providing information on the shape of the response surface within the experimental domain (Tomasi et al., 2023).

For this purpose, a Box–Behnken Design with three factors at three levels was employed, which is considered a simple and efficient alternative among three-level designs, as it reduces the number of experimental runs while maintaining the ability to model the curvature and interactions between variables (Tomasi et al., 2023).

The effects of extraction pH (x1), chestnut shell mass (x2), and extraction time (x3) on the coagulation performance of the modified coagulant were evaluated. The extraction pH was adjusted to the desired values (9, 10, or 11) using 0.1 M HCl solutions prior to the extraction process. The response variables (turbidity and color removal efficiencies) were determined by Jar Test experiments using the coagulants produced after Mannich modification of the extracts obtained under each experimental condition, as shown in Table 1.

Table 1. Factors, codes and levels used in the Box–Behnken Design for tannin extraction optimization.

Factor	**Code**	**Levels**		
		Low (-1)	**Mid (0)**	**High (1)**
pH	x_1	9	10	11
Chestnut shell Mass (g)	x_2	5	6	7
Extraction Time (h)	x_3	12	24	36

Statistical analysis was performed to evaluate the influence of the studied extraction variables and their interactions on turbidity and color removal efficiencies. Analysis of variance (ANOVA) was used to identify the factors that most significantly affected coagulation performance.

4.2 Cationization via Mannich Reaction

Tannins are inherently anionic due to the presence of phenolic functional groups. However, these compounds can be converted into cationic polymers through chemical modification, while maintaining their solubility and metal-chelating properties (Tomasi et al., 2024).

Among the available modification routes, the Mannich Reaction, shown in Figure 5, is a widely employed cationization method that involves the reaction between tannins, an amine source, and an aldehyde (Tomasi et al., 2025b). This modification increases the molecular weight by introducing positively charged functional groups into the tannin structure, enabling the effective destabilization and neutralization of negatively charged colloidal particles that are commonly found in water. Consequently, the newly formed water-soluble compound exhibits flocculating properties suitable for removing suspended particles from wastewater (Tomasi et al., 2024; Arbenz & Avérous, 2015).

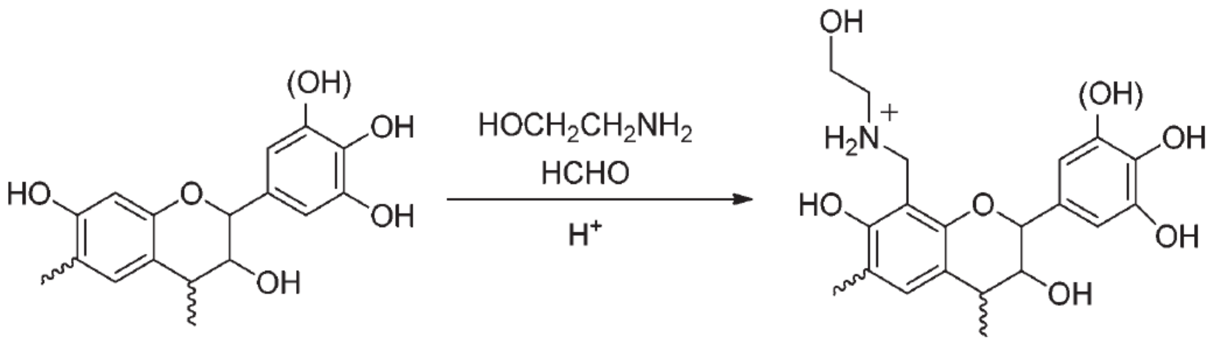


Figure 5. Mannich Reaction with tannins (Arbenz & Avérous, 2015).

Initially, the pH of the alkaline extract was adjusted to 6 using an HCl solution. The Mannich reaction involving tannins is known to occur under acidic conditions, leading to the formation of water-soluble amphoteric tannins with flocculating properties (Arbenz & Avérous, 2015).

The Mannich solution (MS) was prepared by mixing Ammonium Chloride (NH_4Cl) and formaldehyde (37%), following the proportions reported by Tomasi et al. (2025a), maintaining a Tannin: NH_4Cl: Formaldehyde ratio of 1:1.25:3.75. MS was heated at 120 °C for 2 h under continuous mixing to form the iminium ion required for tannin cationization (Bello & Leiviskä, 2021).

Ammonium chloride (NH_4Cl) is frequently selected as an amine source because of its capacity to form highly reactive electrophiles that contribute to improved coagulation performance (Tomasi et al., 2024). Additionally, NH_4Cl is considered a more environmentally friendly option among amine sources, as it presents lower overall environmental and toxicity impacts than alternatives such as ethanolamine and melamine (Tomasi et al., 2025b).

Formaldehyde is essential in the Mannich reaction because it forms an iminium ion intermediate (Tomasi et al., 2025a). However, it is a known human carcinogen according to the International Agency for Research on Cancer (IARC) (Bello & Leiviskä, 2021). Despite these risks, formaldehyde is not inherently detrimental when used in controlled environments to mitigate health hazards (Tomasi et al., 2025a). In fact, researches observed that an excess of this reagent can inhibit iminium ion formation, potentially leading to product gelation or a significantly reduced shelf life (Tomasi et al., 2025a; Bello & Leiviskä, 2021). Therefore, both environmental and health risks can be effectively managed using optimized synthesis methods.

Simultaneously, the tannin extract was heated to 55°C under continuous stirring to ensure complete homogenization of the mixture. Moderate heating is necessary because low temperatures may hinder reaction efficiency, whereas excessively high temperatures can promote tannin degradation and reduce the coagulant performance (Tomasi et al., 2025a). The Mannich reagent was then slowly added to the extract, and the temperature was increased to 85 °C (Tomasi et al., 2025a). The system was maintained under these conditions until a noticeable increase in viscosity was visually observed, indicating the successful cationization of the tannin structure (Tomasi et al., 2025a). Finally, distilled water was added to the reaction mixture to quench the reaction.

The synthesized coagulant was stored at 4 °C to preserve its stability and ensure its validity for subsequent experiments.

4.3 Coagulation-Flocculation Assay

The coagulant performance was evaluated using synthetic wastewater to ensure a controlled analysis of the turbidity and color removal mechanisms. This approach allowed for a controlled assessment of individual removal mechanisms while minimizing the variability typically associated with real industrial effluents.

For the turbidity removal experiments, synthetic wastewater samples were prepared using 1000 mL of distilled water and 5 g of kaolin clay as a model suspended solid (Zourif et al., 2023). The kaolin suspension was thoroughly mixed at 50 rpm for 2 h and subsequently allowed to rest overnight to ensure complete hydration and stabilization of the particulate material (Zourif et al., 2023; Tomasi et al., 2024).

Methylene blue was used as a model dye compound to determine the color removal efficiency. A stock solution of methylene blue (50 mg L^{-1}) was prepared, from which standard solutions were derived to construct a calibration curve (Zourif et al., 2023). The standards included 0, 1, 2, 4, 6, 8, and 10 mg L^{-1}. The absorbance of each standard solution was measured using UV–Vis spectrophotometry at 664 nm. The resulting calibration curve (Figure 6) exhibited linearity (R^2 = 0.9975) and was used to quantify the residual dye concentration after the coagulation–flocculation treatment.

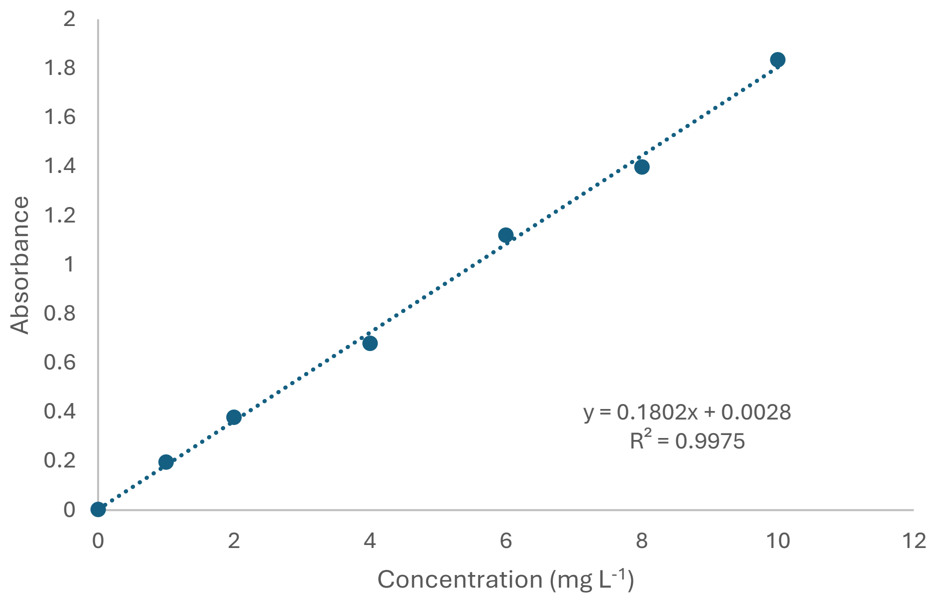


Figure 6. Calibration curve for methylene blue at 664 nm.

Coagulation–flocculation experiments were performed using a standardized Jar Test (Paralab - Equipamentos industriais de Laboratório, S.A., Portugal) (Figure 7). For turbidity removal assays, 300 mL of kaolin suspension was placed in each jar, the pH was adjusted to 6, and different dosages of the synthesized liquid coagulant (1.5, 3 and 4.5 mL) were added. Since the synthesized coagulant was applied as a liquid extract, the dosage was expressed as volume (mL) rather than mass concentration (mg L^{-1}), as the concentration of active tannin compounds in the extract was not determined. The samples were subjected to rapid mixing (300 rpm for 3 min) to ensure uniform dispersion of the coagulant, followed by slow mixing (60 rpm for 20 min) to promote floc formation, and a subsequent settling period of one hour to allow floc sedimentation (Zourif et al., 2023). After mixing, the suspensions were allowed to settle, and turbidity measurements were performed every 30 min during sedimentation.

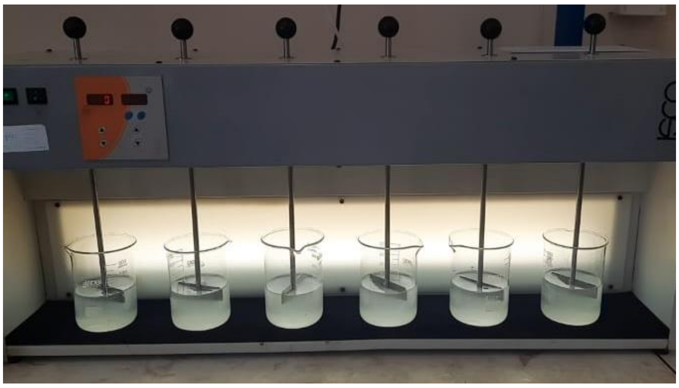


Figure 7. Jar Test apparatus used in coagulation-flocculation experiments.

For the color removal experiments, 50 mL of methylene blue solution (10 mg L^{-1}) was used for each assay. Coagulant dosages of 0.5, 1.0, and 1.5 mL of the modified tannin coagulant were evaluated under the same rapid and slow mixing conditions as those used in the turbidity assays. After a settling period of 1 h, supernatant samples were collected and analyzed using UV–Vis spectrophotometry at 664 nm wavelength.

The turbidity removal efficiency was calculated using Eq. (1) using measurements from a portable turbidimeter (TIR210, VWR International, Germany), while the percentage removal of methylene blue was determined using Eq. (2) based on absorbance measurements obtained using UV–Vis spectrophotometry (Jasco, V-530, Japan) (Zourif et al., 2023).

$$Turbidity\ Removal\ (\%) = \frac{T_i - T_f}{T_i} \cdot 100 \quad (1)$$

$$Color\ Removal\ (\%) = \frac{C_i - C_f}{C_i} \cdot 100 \quad (2)$$

where:

T_i corresponds to the initial turbidity of the water sample

T_f final turbidity measured after treatment

C_i initial absorbance of the solution

C_f absorbance after coagulation–flocculation treatment

For performance comparison, aluminum sulfate ($Al_2(SO_4)_3$) 50 g L^{-1} was used as a reference conventional coagulant under the same experimental conditions. Jar Tests was conducted using aluminum sulfate at comparable dosage ranges, and the results was evaluated in terms of turbidity and color removal efficiency.

5. Results and Discussion

This chapter presents and discusses the results regarding the production, optimization, and application of chestnut shell coagulants. The discussion begins with the analysis of tannin extract statistical optimization through a Box-Behnken Design, followed by the evaluation of the coagulant performance in terms of turbidity and color removal, comparing it with the literature and aluminum sulfate. Finally, sustainability and economic viability are briefly discussed.

5.1 Statistical Analysis and Process Optimization (Box-Behnken)

The alkaline extraction of tannins from chestnut shells was optimized using a Box–Behnken Design to analyze the influence of pH (x1), chestnut shell mass (x2), and extraction time (x3) on the coagulation performance of the final coagulant. For each experimental condition, the extracted tannins were subsequently modified via the Mannich reaction before being evaluated by Jar Test. Turbidity and color removal efficiencies (%) were used as the response variables. The adequacy of the model was evaluated using an analysis of variance (ANOVA). The complete Box-Behnken design matrix for the turbidity and color experiments is presented in Tables 2 and 3, respectively.

Table 2. Experimental matrix used in the Box-Behnken Design for turbidity removal.

Run	pH [A]	Chestnut shell Mass (g) [B]	Extraction Time (h) [C]	Coded Level [A, B, C]	Turbidity Removal (%)
1	9	6	12	[-1, 0 -1]	65
2	11	6	12	[1, 0, -1]	70
3	10	5	12	[0, -1, -1]	74
4	10	7	12	[0, 1, -1]	92
5	9	5	24	[-1, -1, 0]	62
6	11	5	24	[1, -1, 0]	61
7	9	7	24	[-1, 1, 0]	64
8	11	7	24	[1, 1, 0]	48
9	10	6	24	[0, 0, 0]	51
10	10	6	24	[0, 0, 0]	64
11	10	6	24	[0, 0, 0]	64
12	10	6	24	[0, 0, 0]	72
13	10	6	24	[0, 0, 0]	65
14	9	6	36	[-1, 0, 1]	96
15	11	6	36	[1, 0, 1]	96
16	10	5	36	[0, -1, 1]	61
17	10	7	36	[0, 1, 1]	95

Table 3. Experimental matrix used in the Box-Behnken Design for color removal.

Run	pH [A]	Chestnut shell Mass (g) [B]	Extraction Time (h) [C]	Coded Level [A, B, C]	Color Removal (%)
1	9	6	12	[-1, 0 -1]	14
2	11	6	12	[1, 0, -1]	18
3	10	5	12	[0, -1, -1]	26
4	10	7	12	[0, 1, -1]	19
5	9	5	24	[-1, -1, 0]	23
6	11	5	24	[1, -1, 0]	28
7	9	7	24	[-1, 1, 0]	14
8	11	7	24	[1, 1, 0]	12
9	10	6	24	[0, 0, 0]	23
10	10	6	24	[0, 0, 0]	15
11	10	6	24	[0, 0, 0]	13
12	10	6	24	[0, 0, 0]	13
13	10	6	24	[0, 0, 0]	13
14	9	6	36	[-1, 0, 1]	13
15	11	6	36	[1, 0, 1]	14
16	10	5	36	[0, -1, 1]	17
17	10	7	36	[0, 1, 1]	16

5.1.1 Turbidity Removal Optimization

To evaluate the influence of the extraction parameters on turbidity removal, a statistical analysis was performed using ANOVA. Table 4 summarizes the corresponding statistical results.

Table 4. ANOVA results for turbidity removal using the Box-Behnken Design.

Source	Degrees of Freedom	F- value	p-value
Model	9	1	0.531
Time	1	1.19	0.325
pH	1	0.08	0.792
Mass	1	0.91	0.385
$Time^2$	1	5.4	0.068
pH^2	1	0.18	0.692
$Mass^2$	1	0.33	0.588
pH*Mass	1	0.24	0.643
pH*Time	1	0.03	0.876
Mass*Time	1	0.28	0.622
Lack of Fit	3	17.46	0.055

The results indicated that the model was not statistically significant ($p = 0.531$) suggesting that the extraction variables did not significantly explain turbidity removal within the evaluated experimental domain. Although the model presented a moderate coefficient of determination ($R^2 = 64.26\%$), the adjusted R^2 values was close to zero, suggesting limited predictive capability. Among the quadratic terms, $time^2$ exhibited the lowest p-value ($p = 0.068$), suggesting a possible nonlinear effect of extraction time on turbidity removal, and that there may be an optimal extraction time.

This lack of overall model significance may be attributed to the narrow range of extraction parameters evaluated and/or high experimental variability. Additionally, the number of experimental runs (17 runs) may not have been sufficient to fully capture moderate nonlinear effects and interactions among variables. This indicates that coagulation performance may be governed by factors outside the selected domain, such as extract composition, or reaction efficiency during the Mannich reaction. In this sense, the observed trends should be interpreted as indicative of system behavior rather than as definitive optimization boundaries, and future studies could refine the experimental window to better identify the conditions that maximize coagulant performance.

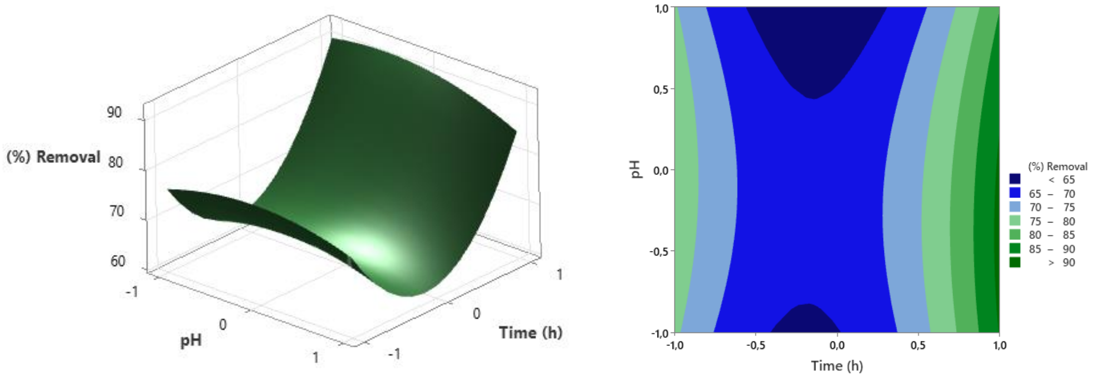
Table 5 presents the coded coefficients for the turbidity removal model, which help identify the influence of each evaluated extraction parameter on turbidity removal.

Table 5. Coded coefficients for turbidity removal using the Box-Behnken Design.

Source	Coded Coefficient	Standard Error	t-value	p-value
Time	5.87	5.39	1.09	0.325
pH	-1.5	5.39	-0.28	0.792
Mass	5.13	5.39	0.95	0.385
$Time^2$	18.42	7.93	2.32	0.068
pH^2	-3.33	7.93	-0.42	0.692
$Mass^2$	-4.58	7.93	-0.58	0.588
pH*Mass	-3.75	7.62	-0.49	0.643
pH*Time	-1.25	7.62	-0.16	0.876
Mass*Time	4	7.62	0.53	0.622

Although the model was not statistically significant, the positive coded coefficients for time (5.87) and mass (5.13) indicate a tendency for higher levels of these factors to be associated with improved turbidity removal within the studied range. In contrast, the negative coefficient for pH (-1.50) suggests that lower pH values may favor turbidity removal under the tested conditions.

The response surface and contour plots for pH and extraction time (Figure 8) shows a curvature that indicates a nonlinear relation between these variables.

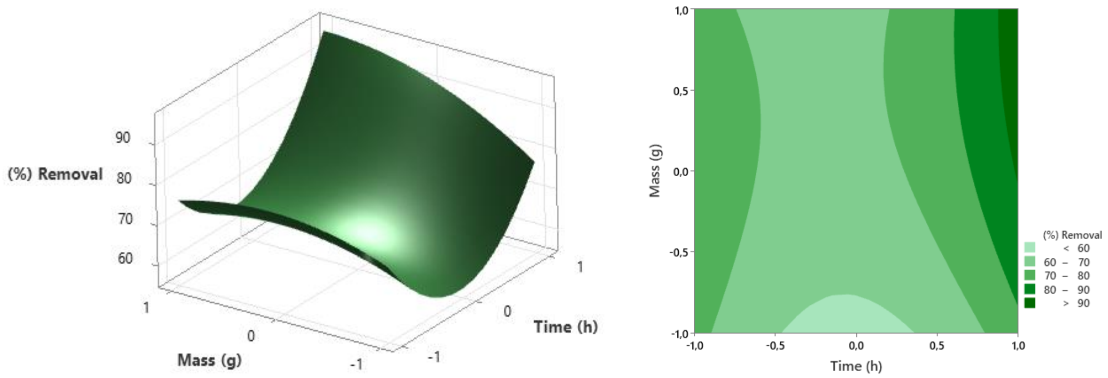


(a) (b)

Figure 8. Response surface (a) and contour plot (b): pH vs. extraction time for turbidity removal.

A minimum region was observed at intermediate values of pH and extraction time, where the removal efficiency decreased to 60%. In contrast, higher removal efficiencies (> 90%) were achieved at the extreme regions, especially at the higher extraction time.

The response surface and contour plots for the chestnut shell mass and extraction time (Figure 9) exhibited nonlinear behavior.

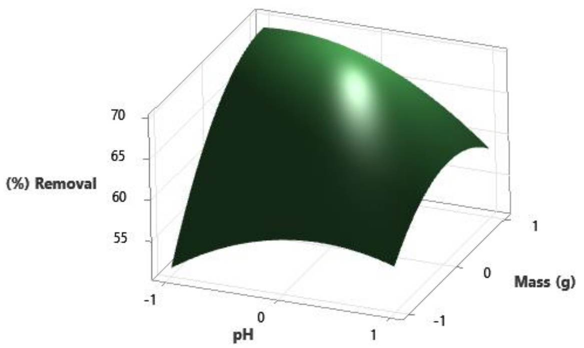


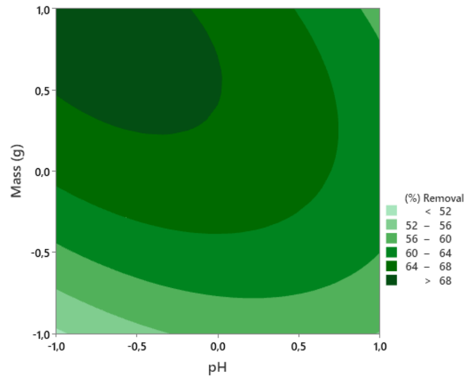
(a) (b)

Figure 9. Response surface (a) and contour plot (b): mass vs. extraction time for turbidity removal.

A region of lower turbidity removal (< 60%) was observed at intermediate values for both variables. In contrast, high turbidity removal (> 90%) was achieved at a greater mass combined with a longer extraction time. This behavior may be attributed to the extended time between the solute and solvent, favoring mass transfer (Hoyos-Martínez et al., 2018).

The response surface and contour plots for the pH and chestnut shell mass (Figure 10) show a curved surface, indicating a nonlinear relationship between these variables.





(a) (b)

Figure 10. Response surface (a) and contour plot (b): mass vs. pH for turbidity removal.

Higher turbidity removal (> 68%) was observed at higher quantities combined with lower pH values. The influence of pH is associated with its effect on tannin extraction and chemical stability. Slightly alkaline conditions are reported to enhance extraction yields due to increased solubility, while highly acidic conditions may promote the formation of insoluble compounds (Hoyos-Martínez et al., 2018). On the other hand, a high alkaline pH may cause a degradation of the coagulant (Benalia et al., 2024). Therefore, the observed behavior suggests that the extraction of tannins depends on pH, with slightly alkaline conditions potentially providing higher extraction yields.

When evaluating these behaviors statistically, it is important to note that a lack of statistical significance limits the model's predictive ability for strict mathematical optimization. However, this study provides a crucial exploratory baseline. The clear experimental trends observed—such as the positive effects of biomass dosage and extraction time on turbidity removal—offer valuable insights into the process dynamics and serve as a guideline for future optimization studies.

5.1.2 Color Removal Optimization

The effect of the extraction parameters on color removal was examined using ANOVA and regression analysis for the quadratic model. The corresponding statistical results are presented in Table 6.

Table 6. ANOVA results for color removal using the Box-Behnken Design.

Source	Degrees of Freedom	F- value	p-value
Model	9	1.13	0.47
Time	1	1.16	0.33
pH	1	0.26	0.634
Mass	1	4.38	0.091
$Time^2$	1	1.75	0.244
pH^2	1	1.98	0.218
$Mass^2$	1	0.05	0.827
pH*Mass	1	0.39	0.558
pH*Time	1	0.07	0.799
Mass*Time	1	0.29	0.614
Lack of Fit	3	0.25	0.856

Regarding the color removal efficiency, the results indicated that the model was not statistically significant ($p = 0.47$), suggesting that the selected extraction parameters did not significantly affect methylene blue removal under the studied conditions.

The model presented an R^2 value of 67.10%. However, the adjusted R^2 was considerably lower (7.88%), suggesting limited predictive capability and indicating that a substantial portion of the variability may be associated with experimental noise or factors not included in the model.

Table 7 presents the coded coefficients for the color removal model, which help identify the influence of each evaluated extraction parameter on methylene blue removal.

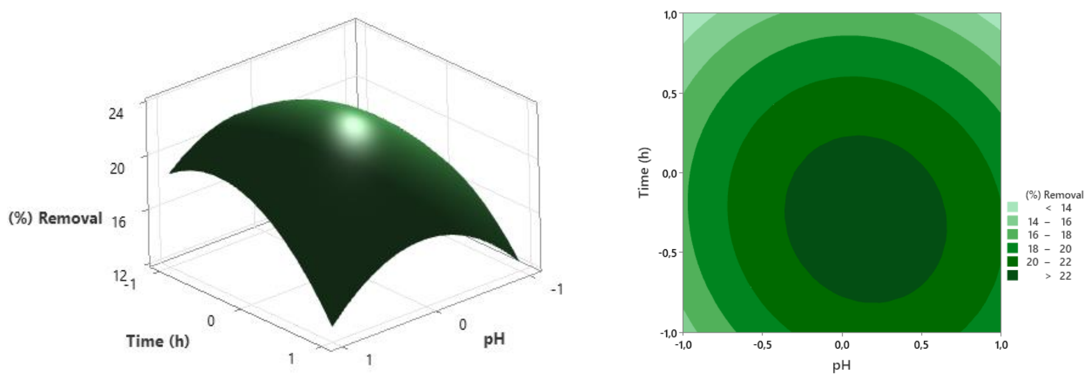
Table 7. Coded coefficients for color removal using the Box-Behnken Design.

Term	Coded Coefficient	Standard Error	t-value	p-value
Time	-2.13	1.97	-1.08	0.33
pH	1	1.97	0.51	0.634
Mass	-4.13	1.97	-2.09	0.091
$Time^2$	-3.83	2.9	-1.32	0.244
pH^2	-4.08	2.9	-1.41	0.218
$Mass^2$	0.67	2.9	0.23	0.827
pH*Mass	-1.75	2.79	-0.63	0.558
pH*Time	-0.75	2.79	-0.27	0.799
Mass*Time	1.5	2.79	0.54	0.614

Among the evaluated variables, mass exhibited the most relevant effect ($p = 0.091$), suggesting a possible tendency. The negative coefficient for this variable (-4.13) suggests that increasing the mass of chestnut shells may decrease the color removal efficiency.

The pH variable showed a positive effect ($p = 0.634$), indicating a slight improvement in the color removal at higher pH values. The extraction time showed a negative coefficient (-2.13), suggesting that longer extraction times may not necessarily enhance color removal.

The response surface and contour plot of pH and extraction time (Figure 11) exhibited a convex profile, indicating a maximum at the intermediate values of both variables.

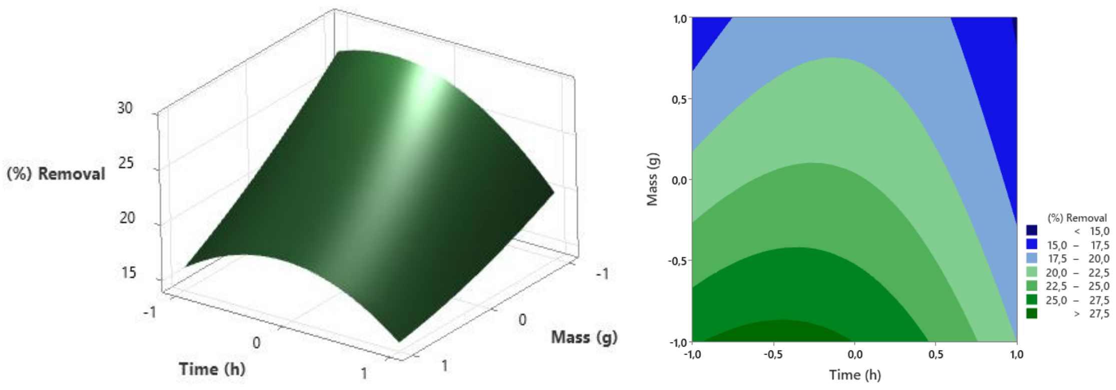


(a) (b)

Figure 11. Response surface (a) and contour plot (b): pH vs. extraction time for color removal.

As the variables move away from the central region, a decrease in color removal is observed, indicating that both insufficient or excessive extraction conditions may affect negatively the performance of the coagulant.

The response surface and contour plot of the mass and extraction time (Figure 12), exhibited an inclined surface.

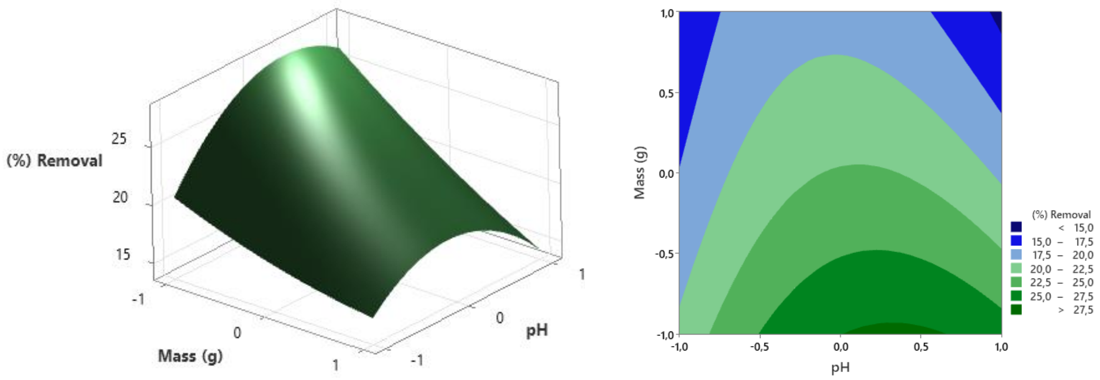


(a) (b)

Figure 12. Response surface (a) and contour plot (b): mass vs. extraction time for color removal.

The results indicated an increase in response towards lower values of mass and extraction time. This may be because a higher waste mass and extraction time can also extract other impurities, such as pigments.

The response surface and contour plots for the pH and chestnut shell mass (Figure 13) show an inclined surface with a moderate curvature.



(a) (b)

Figure 13. Response surface (a) and contour plot (b): mass vs. pH for color removal.

The highest color removal (~26%) seems to occur at lower mass of chestnut shells combined with lower to intermediate pH values.

The response surface and contour plots demonstrated relatively smooth profiles without pronounced curvature, indicating limited interactions among the evaluated variables. The limited statistical significance of the model may be partly explained by the nature of the Box-Behnken Design, which reduces the number of experimental runs while still allowing response surface modeling. However, this also limits the degrees of freedom available to estimate experimental error. As a result, if the true effect of the variables is small or the experimental variability is relatively high, the model may not have sufficient statistical power to achieve significance.

Overall, the results suggest that the chestnut shell–based coagulant modified via the Mannich reaction was more effective for turbidity removal than for the removal of dissolved dyes. The different responses to turbidity and color removal highlight the distinct mechanisms involved in the two processes and indicate that each response should be interpreted separately.

5.2 Coagulation and Flocculation Performance

The coagulation–flocculation performance of the chestnut shell–based coagulant was evaluated through Jar Test experiments. The coagulant was produced by extracting tannins from chestnut shells, followed by chemical modification via the Mannich reaction. Its efficiency was assessed based on turbidity removal from kaolin suspension and color removal from methylene blue solution.

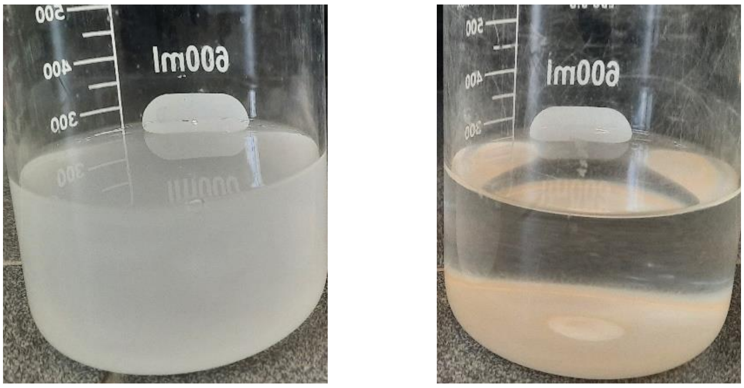
5.2.1 Turbidity Removal Performance

The removal of turbidity can be explained by a combination of charge neutralization, in which the coagulant neutralizes the charged particles; sweep flocculation, where larger aggregates capture smaller particles during sedimentation; and interparticle bridging, in which polymeric chains establish physical links between particles, thereby promoting the formation of larger settling flocs (Tomasi et al., 2025).

Overall, the chestnut shell coagulant demonstrated high turbidity removal, with the best results reaching values between 93-96% under optimal experimental conditions.

The Mannich reaction introduces positively charged groups into the chestnut shell tannin structure, destabilizing anionic compounds suspended in water (Sánchez-Martín et al., 2013). Therefore, the Mannich modification was important for improving the coagulation-flocculation performance of the natural extract, making it more effective for turbidity removal.

Visual observations during the Jar Test indicated the formation of larger and denser flocs at higher coagulant dosages, consistent with the measured turbidity reduction efficiencies, as shown in Figure 14.



(a) (b)

Figure 14. Visual aspect of the wastewater before (a) and after (b) the coagulation-flocculation treatment.

In general, a direct relation was observed between the coagulant dosage and turbidity removal efficiency, whereas higher volumes of coagulant led to superior turbidity reduction.

For instance, as illustrated in Figure 15, after 90 min of settling time, samples 4 and 15 showed removal efficiencies of 79 and 87%, respectively, when using 1.5 mL of coagulant. In contrast, with a coagulant dosage of 4.5 mL, the removal efficiencies increased to 92 and 96%, respectively.

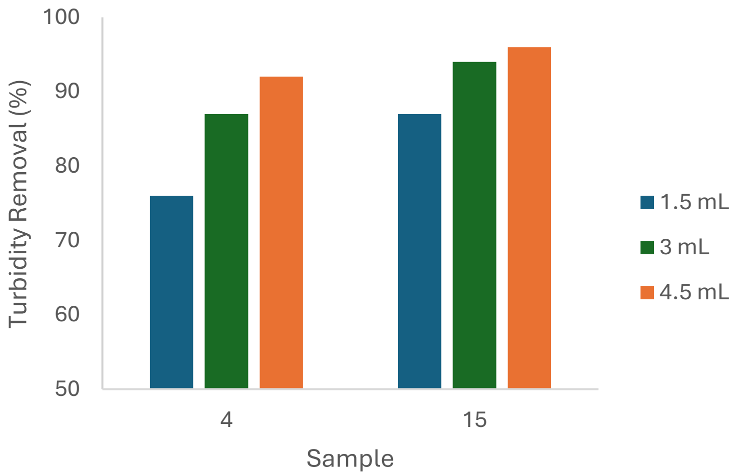


Figure 15. Turbidity removal for samples 4 and 15 for different coagulant dosages.

This behavior is illustrated in Figure 16, which shows the Jar Test of sample 17, where a noticeable reduction in turbidity can be observed with increasing coagulant dosage.

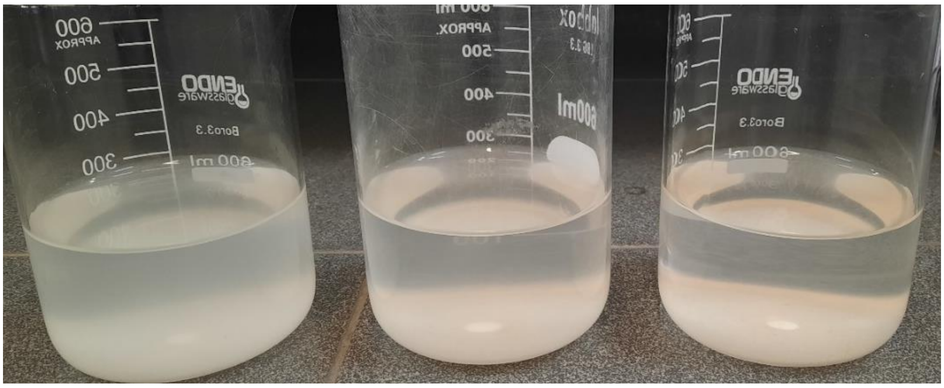


Figure 16. Jar Test results for sample 17 showing turbidity removal at different coagulant dosages: 1.5 mL (left), 3.0 mL (center), and 4.5 mL (right).

The observed improvement in turbidity removal with increasing coagulant dosage is consistent with the literature, which reports that the dose directly influences the interaction between the coagulant and colloidal particles, floc growth, and sedimentation rate (Zhang et al., 2024; Zedan et al., 2022).

Within the evaluated dosage range, no signs of particle re-stabilization were observed, indicating that the applied dosages did not exceed the optimal range for coagulation. As reported by Benalia et al. (2024), an overdose of coagulant can re-stabilize colloidal particles and reduce floc formation, leading to a lower clarification efficiency.

Settling time also has a considerable effect on coagulation efficiency because suspended materials in water differ in size, weight, and charge after coagulation (Zedan et al., 2022).

The results demonstrate that extending the settling time further improves turbidity removal, with most samples showing increased removal efficiency from 60 to 90 min. Representative samples are presented in Figure 17 to illustrate the general settling behavior observed during the experiments.

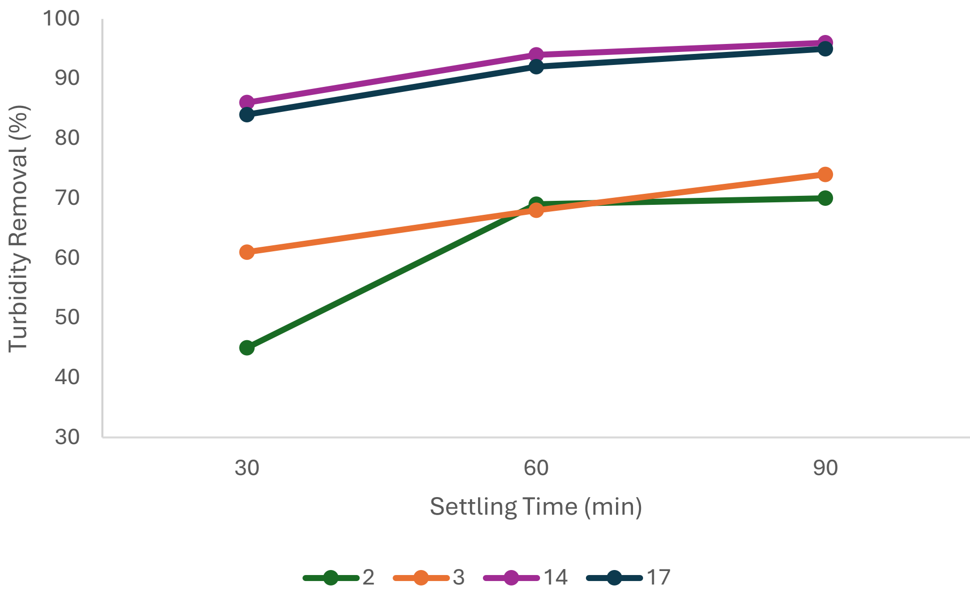


Figure 17. Turbidity removal of samples 2, 3, 14 and 17 for 4.5 mL of dosage.

This behavior is in agreement with the literature, which reports that settling time affects the coagulation efficiency. In a study using walnut seed extract by Zedan et al. (2022), turbidity removal increased when the settling time was extended from 30 to 60 min. Another study by Pathiranage et al. (2026), who used calcined oyster shell powder, also showed an increase in turbidity removal from 30 to 60 min.

5.2.2 Color Removal Performance

The methylene blue removal efficiency was also investigated. In contrast to turbidity, the removal of methylene blue is a greater challenge because it is a dissolved dye.

Similarly to turbidity removal, increasing the coagulant dosage resulted in higher color removal efficiencies, with the best performances reaching values between 20 and 26%. Representative samples are presented in Figure 18 to illustrate the effect of coagulant dosage on methylene blue removal.

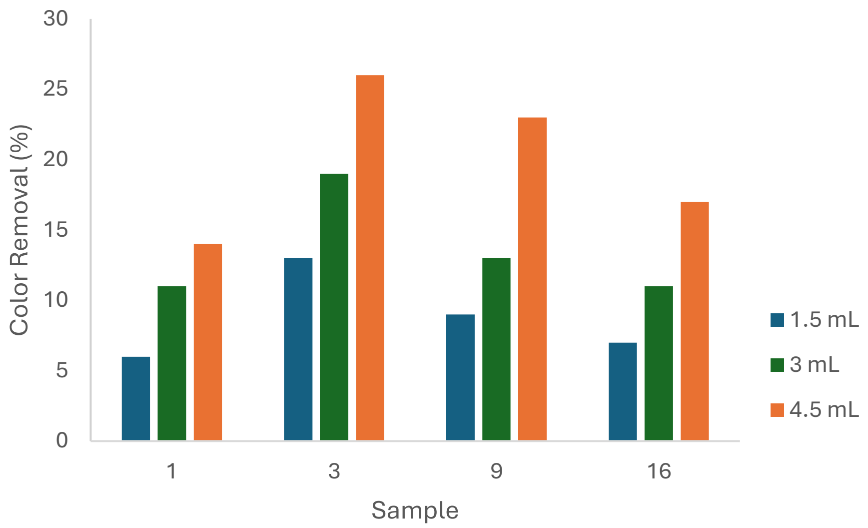


Figure 18. Color removal of samples 1, 3, 9 and 16 at different coagulant dosage levels.

The color removal results were lower than those achieved for turbidity, which can be explained by the different removal mechanisms involved. In the literature, decoloration using modified tannin-based materials is often described as an adsorption-like coagulation process, in which the dye must interact with cationic biocoagulant species and form dye–coagulant aggregates (Grenda et al., 2018).

In the specific case of methylene blue, a cationic dye, the removal process faces electrostatic repulsion from the predominantly cationic modified tannin, which hinders the charge-neutralization mechanism. According to Beltrán-Heredia et al. (2010), in such scenarios, polymeric bridging becomes the predominant removal pathway, as the high molecular weight of the tannin chains allows them to physically entrap dye molecules despite the lack of electrostatic attraction.

This proposed mechanism is supported by the visible precipitate observed during the color removal experiments (Figure 19), suggesting interactions between methylene blue molecules and the natural-based coagulant. Although the exact composition was not characterized, this precipitate may indicate the formation of dye–coagulant aggregates through polymeric bridging.

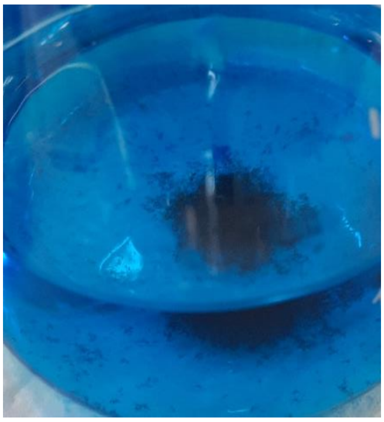


Figure 19. Precipitate formation during color removal using chestnut shell coagulant.

Furthermore, in a study on tannin-based coagulant from *Acacia mearnsii* extract by Grenda et al. (2018), it was reported that the tannin coagulant alone was ineffective for color removal in some cases, while performance improved only when a tertiary system including bentonite and a flocculant was applied.

Although these results were lower than those achieved for turbidity, the observed results confirm that the natural-based coagulant can interact with the dye, highlighting its potential as a decolorizing agent and suggesting further process optimization.

5.3 Comparative Analysis with Literature Results

To contextualize the performance of the natural coagulant from chestnut shells developed in this study, the results were compared with those reported in the literature for tannin-based coagulants applied to turbidity and color removal.

The comparison was carried out considering the tannin source, chemical modification applied, type of water or effluent treated, settling time, and removal efficiency obtained.

5.3.1 Comparative Analysis for Turbidity Removal

For turbidity removal, the best result obtained in this study was 96% using 4.5 mL of coagulant in kaolin clay water. This performance is comparable to that reported in the literature for tannin-based coagulants (Table 8).

For comparison, chemically modified tannins from spruce trees showed high turbidity removal (> 90%) at a dosage of 15 mg L^{-1} (Bello & Leiviskä, 2021). In another study, Tomasi et al. (2024) reported 80% turbidity removal using chestnut shells modified via the Mannich reaction.

In contrast, Arismendi et al. (2018) reported lower or even negative turbidity removal in more complex matrices such as washing machine wastewater. This variability can be attributed primarily to differences in wastewater composition and matrix complexity, as synthetic kaolin suspensions provide a more controlled environment, whereas real wastewaters contain a broader range of dissolved and suspended substances that may interfere with coagulation performance.

Table 8. Comparison of turbidity removal efficiencies reported in the literature for natural and tannin-based coagulants

Tannin Extract	Extraction Method	Chemical Modification	Coagulant dosage	Water/effluent	Settling Period	Turbidity Removal (%)	Author
Castanea sativa	Solid-liquid extraction	Mannich reaction (formaldehyde and NH_4Cl)	15 mL L^{-1}	Kaolin clay (5 g L^{-1})	90 minutes	96	This study
Castanea sativa	Shredded and Dried	-	0.3 g L^{-1}	Gray clay (1 g L^{-1})	2 hours	-19.4 ± 1.3	Santos et al., 2024
Castanea sativa	Microwave-assisted	Mannich reaction (formaldehyde and NH_4Cl)	2 mg L^{-1}	Kaolin clay (65 mg L^{-1})	15 minutes	~80	Tomasi et al., 2024
Bark of spruce trees (*Picea abies*)	Hot water extraction	Mannich reaction (formaldehyde and melamine)	50 mg L^{-1}	River water + kaolin clay (250 mg L^{-1})	30 minutes	>90	Bello & Leiviskä, 2021
Castanea Sativa Tree Leaves	Solid-liquid extraction	-	0.5 mL L^{-1}	Kaolin clay (0.05 g L^{-1})	1 hour	76	Hasan et al., 2024
Castanea sativa	-	Mannich reaction (formaldehyde and NH_4Cl)	Between 500 mg L^{-1} and 1250 mg L^{-1}	Washing-machine wastewater and river sample	30 minutes	Between -27 and -470	Arismendi et al., 2018
			Between 32.5 mg L^{-1} and 40 mg L^{-1}	Simulated water		~80	

Additionally, the results emphasize the importance of chemical modification, as unmodified tannin exhibited lower performance, likely because of its anionic characteristics.

In general, the results obtained in this study are consistent with those reported in the literature while providing additional contributions to the development of chestnut shell–based coagulants. Although direct numerical comparisons should be made carefully because of differences in dosage, settling time, and suspension characteristics, the present results confirm the potential of chestnut shell coagulants as an alternative for turbidity removal.

5.3.2 Comparative Analysis for Color Removal

For color removal, the best results obtained in this study (~26%) were lower than those observed for turbidity. Even so, the results are relevant compared with the literature (Table 9), since color removal is dependent on diverse variables, such as tannin source, dosage applied, and the nature of the dye.

For comparison, Tomasi et al. (2025a) reported a higher color removal (82%) in real textile wastewater using a chestnut shell coagulant, showing that tannin-based systems can achieve strong performance in real effluents. This may be because real effluents, owing to their higher ionic strength and conductivity, can promote the compression of the electrical double layer surrounding suspended particles, enhancing colloidal destabilization and improving the overall coagulation efficiency (Tomasi et al., 2026). Therefore, although synthetic solutions provide controlled experimental conditions, some characteristics of real effluents may favor coagulation performance depending on the contaminant type and the removal mechanism involved.

Table 9. Comparison of color removal efficiencies reported in the literature for natural and tannin-based coagulants.

Tannin Extract	Extraction Method	Chemical Modification	Coagulant dosage	Water/effluent	Color Removal (%)	Author
Castanea sativa	Solid-liquid extraction	Mannich reaction (formaldehyde and NH_4Cl)	15 mL L^{-1}	Synthetic water (Methylene blue 10 mg L^{-1})	26	This study
Castanea sativa	Solid-liquid extraction	Mannich reaction (formaldehyde and ethanolamine)	100 mg L^{-1}	Synthetic water (Sirius Blue K-CFN)	~40	Tomasi et al., 2025
Castanea sativa	Solid-liquid extraction	Mannich reaction (formaldehyde and ammonium chloride)	150 mg L^{-1}	Real textile wastewater (190 Scale Pt-Co)	82	Tomasi et al., 2025a
Castanea sativa	Solid-liquid extraction	Mannich reaction (glyoxal and ammonium chloride)	20 g L^{-1}	Synthetic water (Sirius Blue K-CFN)	Between 10 and 70	Tomasi et al., 2026
Moringa Oleifera	Solid-liquid extraction (with water)	-	80 mL	Synthetic water (Methylene blue 1 g L^{-1})	10	Azura et al., 2012
	Solid-liquid extraction (with 1M NaCl)	-			70	
Ocimum basilicum	Solid-liquid extraction	-	1.6 mg L^{-1}	Synthetic water (Congo red - C.I. 22120)	69	Shamsnejati et al., 2015
		-	9.6 mg L^{-1}	Real textile wastewater (Abs. 0.208, wavelength 442 nm)	51	

The relatively low methylene blue removal observed in this study is also consistent with the findings of Sánchez-Martín & Beltrán-Heredia (2012), who reported that no methylene blue removal was achieved using *Moringa oleifera* seed extract, attributing this behavior to the cationic nature of the dye, which limits destabilization by cationic biopolymers and protein-based flocculants. However, Azura et al. (2012) successfully achieved methylene blue removal using *Moringa oleifera*, indicating that dye removal efficiency depends on experimental conditions, extraction procedures, and coagulant characteristics.

Therefore, it is important to note that most studies evaluate color removal using different dyes or complex textile effluents, making direct comparisons difficult. In addition, there are few reports specifically addressing methylene blue removal using chestnut shell coagulants, which reinforces the exploratory nature of this study.

5.4 Comparison with the Conventional Coagulant Aluminum Sulfate

To evaluate the practical relevance of the developed natural-based coagulant, its performance was compared with a 50 g L^{-1} aluminum sulfate ($Al_2(SO_4)_3$) solution under the same experimental conditions (water, settling time, and coagulant dosage).

As illustrated in Figure 20, aluminum sulfate removed 88–96% of the turbidity within 30–90-minute interval. Comparatively, the chestnut shell coagulant, specifically at a dosage of 4.5 mL, achieved turbidity removal rates between 90 and 96% between 60 and 90 min of settling time.

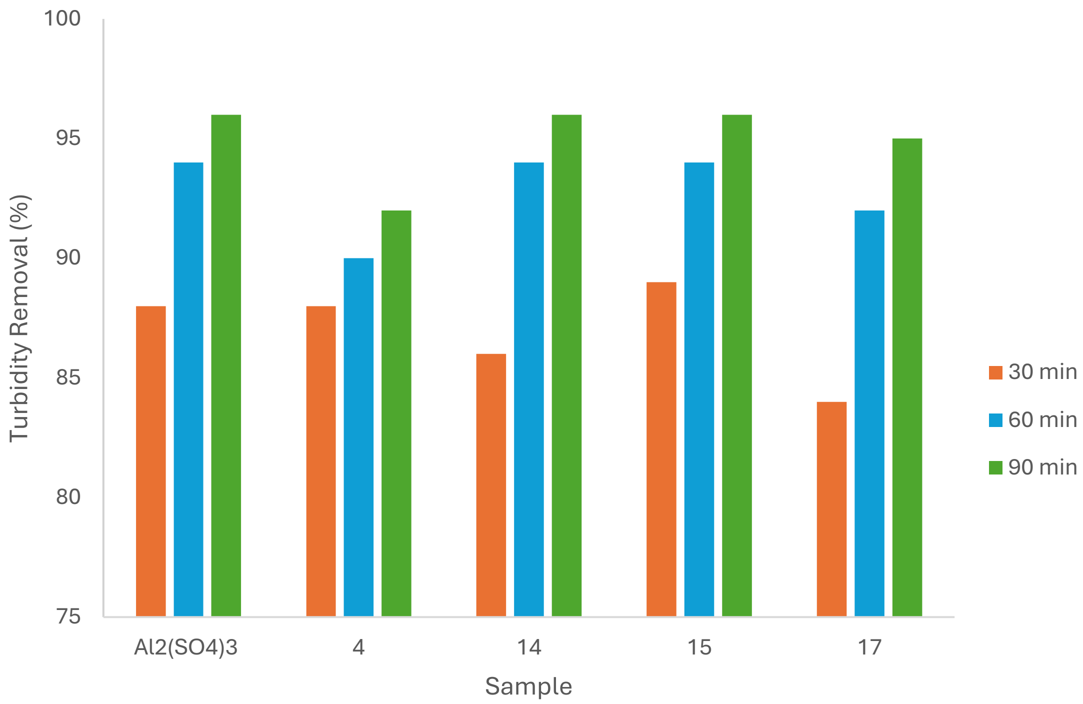


Figure 20. Turbidity removal for $Al_2(SO_4)_3$, and samples 4, 14, 15 and 17 for 4.5 mL of coagulant.

Although $Al_2(SO_4)_3$ showed a faster removal, the natural-based coagulant reached a similar efficiency, indicating that it can be considered a viable sustainable alternative.

Regarding color removal, $Al_2(SO_4)_3$ showed very low efficiency, with a removal rate between 1.2 and 2.5%. These results suggest that $Al_2(SO_4)_3$ was not effective in removing methylene blue under the experimental conditions studied. In contrast, the chestnut shell coagulant showed a higher color reduction, indicating a better interaction with methylene blue molecules.

Compared with aluminum sulfate, the chestnut shell-based coagulant showed promising performance, particularly in turbidity removal. Although conventional coagulants remain highly effective, natural alternatives offer advantages related to renewability, residue valorization, and reduction of dependence on metal-based chemicals.

5.5 Sustainability and Economic Outlook

From a sustainability perspective, the use of chestnut shell coagulant aligns with the principles of a circular economy, representing a sustainable alternative to conventional inorganic coagulants.

Unlike aluminum sulfate, which is produced from bauxite mining and reacts with sulfuric acid (Vaz, 2019), the proposed chestnut shell coagulant is produced from agro-industrial residues, reducing waste generation and supporting circular economy principles.

One of the main advantages of the chestnut shell coagulant is the generated sludge. Aluminum sulfate causes sludge contamination, which is difficult for companies to handle properly (Jesus et al., 2024). In addition, natural-based coagulants produce less sludge, and the sludge produced is biodegradable (Ibrahim et al., 2020). According to Ibrahim et al. (2020), this biodegradable sludge may have potential for agricultural applications, such as use as fertilizer, although its suitability depends on proper characterization and safety assessment. Therefore, further studies on sludge characterization, disposal, and potential reuse are necessary to confirm these possibilities for the coagulant developed in this study.

Although the chestnut shell coagulant is derived from a renewable source, the Mannich reaction introduces formaldehyde, which must be considered in sustainability assessments. In a recent study, Tomasi et al. (2025b) evaluated the formaldehyde concentration in water treated with a Mannich-modified chestnut shell coagulant (1.05 mg L^{-1}) and reported that it was significantly below the 2.6 mg L^{-1} limit considered acceptable by the World Health Organization (WHO). Furthermore, the residual formaldehyde concentration in the sludge was notably low (46 ± 2 μg g^{-1}) (Tomasi et al., 2025b).

Nevertheless, finding safer alternatives to formaldehyde remains a priority for developing these coagulants. Glyoxal, for instance, was studied by Tomasi et al. (2026) as a substitute for formaldehyde because of its high reactivity, low volatility, biodegradability, and cost-effectiveness.

Aluminum sulfate is widely used because of its high efficiency and relatively low industrial-scale cost. Commercial industrial-grade aluminum sulfate typically presents estimated prices ranging from approximately 0.20 to 0.40 US$ per kilogram (Kexing Water Treatment Co., Ltd.), values that are considerably lower than those associated with laboratory-grade reagents. However, its application may involve additional costs associated with transportation, pH correction, sludge handling, and disposal after water treatment (Jesus et al., 2024).

In contrast, natural-based coagulants may reduce the initial cost associated with raw materials, as they are often obtained from discarded residues. An estimated laboratory-scale production cost of approximately $1.59 per liter of coagulant was calculated considering the consumption of NaOH, NH_4Cl and 37% formaldehyde. Reagent prices were estimated based on the commercial laboratory supplier Lab Alley Essential Chemicals, using values available in May 2026. Therefore, direct economic comparisons with commercial aluminum sulfate should be interpreted with caution, since the present estimate refers exclusively to laboratory-scale production.

Although the estimated laboratory-scale production cost is currently higher than the commercial price of aluminum sulfate, natural-based coagulants may offer important environmental and operational advantages. Besides being produced from renewable agro-industrial residues, they are generally associated with lower sludge production and biodegradable residues, which may reduce sludge management and disposal costs (Jesus et al., 2024; Ibrahim et al., 2020). In addition, substantial cost reductions are also expected for industrial-scale production owing to bulk reagent acquisition and process optimization.

6. Conclusions

In this study, a natural coagulant derived from chestnut shells was developed through tannin extraction followed by chemical modification via the Mannich reaction. The extraction conditions were optimized using a Box–Behnken Design, considering pH, extraction time, and biomass dosage as the main variables affecting coagulation performance.

Although the quadratic model did not reach statistical significance, the observed trends demonstrated that the evaluated extraction parameters influenced the efficiency of turbidity and color removal, although different behaviors were observed for each response. In general, longer extraction times and higher biomass dosages improved turbidity removal, whereas color removal was more sensitive to the nature of the dye and to the physicochemical interactions between the dye molecules and the modified coagulant.

Regarding coagulation–flocculation performance, the developed coagulant exhibited high turbidity removal efficiencies, reaching 96% under the best experimental conditions. The results also revealed a direct relationship between coagulant dosage and turbidity reduction, indicating that higher doses promoted better floc formation and sedimentation. These findings highlight the potential of chestnut shell–based coagulants as sustainable alternatives for suspended particle removal.

In contrast, the color removal efficiencies were lower than those obtained for turbidity, reaching approximately 26% for methylene blue removal. This suggests that dye removal depends not only on coagulation mechanisms, but also on specific physicochemical interactions between the dye molecules and the coagulant structure. Nevertheless, these results are relevant given the limited number of studies focusing on methylene blue removal using chestnut shell–based coagulants.

Overall, this study reinforces the potential valorization of agro-industrial residues within the context of a circular economy and sustainable wastewater treatment. Using chestnut shells as a raw material for coagulant production offers a promising route to reduce dependence on conventional chemical coagulants while promoting waste reutilization.

Future studies should focus on replacing formaldehyde with greener alternatives in the Mannich modification process and applying the developed coagulant to real industrial effluents. In addition, evaluating mixed synthetic suspensions containing both kaolin and methylene blue could provide a more representative model of industrial wastewater by allowing the simultaneous assessment of turbidity and color removal. Additional investigations on sludge characterization, disposal, and potential reuse are also necessary to better assess the environmental sustainability of the proposed treatment. Furthermore, pilot-scale studies are recommended to evaluate the feasibility of scaling up the extraction and coagulant-production processes. Finally, life cycle assessment studies could provide a more comprehensive evaluation of the environmental benefits associated with replacing conventional coagulants, such as aluminum sulfate, with chestnut shell–based coagulants.

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