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Cellulose nanocrystals: Synthesis procedures and characterization techniques

Selüloz Nanokristaller: Sentez prosedürleri, karakterizasyon teknikleri

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Abstract

The production of renewable materials from lignocellulosic materials through various processes has become a significant research area for scientists, leading to the emergence of novel materials such as cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs). The development of these bio-based materials not only contributes to the expansion of fundamental knowledge and the creation of innovative processes but also facilitates the widespread application of cellulosic nanomaterials across various engineering disciplines. In addition to being biodegradable and biocompatible, their production from agricultural and industrial waste makes cellulosic nanomaterials environmentally sustainable, thereby increasing researchers' interest in the field. This review article aims to provide a comprehensive analysis of the latest developments in the production processes, raw material sources, and characterization methods of cellulosic nanomaterials, with a particular focus on cellulose nanocrystals (CNCs).

Keywords: Cellulose nanocrystals (CNCs), Cellulose nanofibrils (CNFs), Lignocellulosic materials, Sustainable nanomaterials, Characterization techniques

Öz

Lignoselülozik materyallerden çeşitli proseslerle yenilenebilir malzemeler üretmek, bilim insanları için önemli bir araştırma alanı haline gelmiş ve bu süreç, selüloz nanokristal (SNK) ve selüloz nanofibril (SNF) gibi yeni malzemelerin ortaya çıkmasını sağlamıştır. Bahsi geçen biyo-bazlı malzemelerin geliştirilmesi, temel bilgi birikiminin artmasına ve yenilikçi proseslerin oluşturulmasına katkıda bulunurken, selülozik nano malzemelerin çeşitli mühendislik disiplinlerinde yaygın kullanımına da zemin hazırlamıştır. Biyobozunur ve biyouyumlu olmalarının yanı sıra, tarımsal ve endüstriyel atıklardan dahi üretilebilmeleri, selülozik nanomalzemeleri çevresel açıdan sürdürülebilir hale getirmekte ve bu nedenle araştırmacıların konuya ilişkin ilgisi giderek artırmaktadır. Bu derleme makalesi, selüloz nanokristaller (SNK) başta olmak üzere selülozik nanomalzemelerin (SNF) üretim süreçleri, elde edildikleri kaynaklar, karakterizasyon yöntemleri ilişkin güncel gelişmeleri ayrıntılı bir şekilde incelemeyi amaçlamaktadır.

Anahtar kelimeler: Selüloz nanokristaller (SNK), Selüloz nanofibriller (SNF), Lignoselülozik malzemeler, Sürdürülebilir nanomalzemeler, Karakterizasyon teknikleri

1 Introduction

Concerns about the future of the planet have driven consumers to demand sustainable and environmentally friendly products. In response, the European Union has enacted legislation aimed at reducing the use of harmful products and materials that pose a threat to ecological balance. Additionally, the EU has launched public awareness initiatives and actively supports multidisciplinary research to promote the production and widespread adoption of eco-friendly materials derived from natural resource [1]. The increasing awareness of global warming, along with published reports and the rising environmental consciousness of the public, is steering the chemical industry—alongside other industrial sectors—toward the production of bio-based products. Green chemistry aims to minimize or entirely eliminate the use of environmentally harmful substances, such as petroleum and its derivatives, while maximizing the efficiency of bio-based raw materials and reducing waste emissions. Indeed, recent literature also emphasizes the chemical industry's transition toward bio-based products. This approach plays a crucial role in the development of sustainable chemical processes and products [2].

The production of all bio-based "renewable" and "sustainable" materials relies on annually renewable plants and agricultural waste as natural resources [3]. A significant portion of the waste generated during the processing of agricultural products worldwide is utilized for energy production through combustion or as animal feed. However, the remaining waste often remains unutilized due to factors such as storage, transportation, and labor costs. Yet, the organic agricultural waste produced in these processes represents a renewable biomass resource and can be converted into industrial materials with commercial value.

Cellulose, classified under the category of biodegradable natural polymeric materials, is the most abundant renewable, biodegradable, and environmentally friendly polymer in the world. According to the literature, cellulose constitutes up to 40–50% of wood and approximately 90% of other plant-based materials [4], [5]. Cellulose, an insoluble polymer in water,

plays a critical role in maintaining the structural integrity of plant cell walls. Traditionally, cellulose has been used in industries such as paper, textiles, and construction; however, it can also be derived from annual plants and agricultural waste. Wood and cotton, however, are considered the primary raw material sources. Recent literature confirms that cotton and wood are the primary raw materials for cellulose extraction.

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Wood pulp, obtained from various tree species, contains approximately 40–50% cellulose, while cotton fibers, which contain approximately 90% cellulose, are a pure natural polysaccharide source. These raw material sources are primarily used in industries such as paper, textiles, and construction. Additionally, agricultural residues and other plant-based materials are also used for cellulose extraction, expanding the range of raw materials used in various application [6], [7].

Cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs), which fall under the category of chemically modified cellulose derivatives, are obtained through processes such as purification and hydrolysis of cellulose using various chemical treatments. Nanocellulosic materials have garnered significant attention due to their superior mechanical properties, ability to enhance the strength of composite structures, biodegradability, and cost-effectiveness compared to petroleum-based alternatives. These advantages make cellulose-based nanomaterials a key product group in the context of sustainable development.[3].

Cellulose-based nanomaterials, which can be efficiently obtained from cellulosic sources through sustainable processes, are utilized in various innovative technologies and applications, including antimicrobial materials and adsorbents for heavy metals and dyes [8], [9], [10]. Additionally, these materials can be utilized as drug delivery systems in the pharmaceutical field and are also employed in the production of various products such as composite materials, films, medical devices, adhesives, membranes, food additives, and packaging materials [2],[11], [12], [13], [14], [15]. Comprehensive reviews in the literature reveal that, in addition to being biodegradable, nanocellulose derivatives exhibit rheological and gas barrier properties, which provide a significant advantage in terms of product diversity. These properties position them as competitive alternatives to petroleum-based plastics.

This review focuses on the production processes of cellulose nanocrystals, a nanocellulose derivative characterized by high crystallinity and superior mechanical strength. Additionally, it examines recent research on characterization techniques for this material. Furthermore, it aims to contribute to innovative production strategies that facilitate the development of bio-based, high-performance, and compostable nanofiber textile structures, offering a sustainable alternative to petroleum-derived counterparts widely used in various industries.

2 Cellulosic nanomaterials

Cellulose, which constitutes approximately 50% of lignocellulosic biomass, is a polysaccharide composed of linear glucose chains and exhibits a flat, ribbon-like structure. Two anhydrous glucose rings are covalently linked via oxygen atoms, forming repeating cellulose units through 1,4-glycosidic bonds. Each glucose unit contains hydroxyl (OH) groups on the C2, C3, and C6 carbon atoms, which can establish hydrogen bonds both within the molecule and with other cellulose macromolecules. These hydrogen bonds play a fundamental role in the formation of the crystalline structure and in determining the physicochemical properties of cellulose [16].
Cellulose, one of the most common natural polymers, has gained a new structural and functional dimension with the advancements in nanotechnology. In the literature, the term "nanoselulose" is used to describe cellulose-based materials with particle sizes smaller than 100 nm. Nanoselulose materials attract significant
attention in materials science for the development of functional products, due to the renewable and non-toxic nature of cellulose, as well as their fine diameters, low densities, and superior rheological and colloidal properties. Furthermore, compared to other materials, they exhibit specific physicochemical, optical, magnetic, and biological characteristics, addressing a wide range of applications [12], [15], [17], [18], [19], [20], [21], [22], [23].
Although there is no complete terminological consensus in the literature regarding the classification of nanoselulose materials, they are generally categorized into three main

groups: cellulose nanocrystals, cellulose nanofibrils, and bacterial cellulose [24]. These materials are categorized based on the cellulose source from which they are derived, the extraction method applied, and their physical properties [24], [25], [26], [27]. Table 1 provides a detailed overview of the fundamental properties of cellulose-based nanomaterials [24], [25], [28], [29], [30], [31], [32].

Tablo 1. Nanocellulose Derivatives and Properties.

Properties	Cellulose Nanofibers (CNF)	Cellulose Nanocrystals (CNC)	Bacterial Nanocellulose (BNC)
Raw Material Source	Wood, agricultural residues, lignocellulosic biomass	Wood, cotton linters, agricultural residues	Produced via bacterial fermentation (Gluconacetobacter spp.)
Production Process	Mechanical grinding, TEMPO oxidation, enzymatic treatments	Acid hydrolysis (typically sulfuric acid, hydrochloric acid)	Biosynthesis (produced by bacteria)
Crystallinity Level	Medium (contains both crystalline and amorphous regions)	High (typically 70–90% crystallinity)	Very high (due to cellular arrangement)
Physical Dimensions	Diameter: 5–100 nm, Length: several microns	Diameter: 2–20 nm, Length: 100–500 nm	Diameter: 20–100 nm, Length: several microns
Surface Charge	Generally neutral or slightly negative	Negative (due to sulfate groups)	Slightly negative or neutral
Mechanical Strength	High flexibility and mechanical strength	Rigid, brittle structure	Flexible, highly durable, and elastic (exists in hydrogel form)
Hydrophilicity	Medium-high	Low	Very high

In recent years, in order to prevent the overconsumption of forest resources and in line with sustainability approaches, the raw material sources used for nanocellulose production have been diversified. Biomass waste derived from agricultural and industrial by-products [33], [34], [35], [36] nd algae-based cellulose have been explored as alternative raw materials [37], [38], [39]. The utilization of biomass waste from agricultural and industrial by-products as raw materials offers both economic and ecological advantages, contributing to the reduction of environmental problems. Additionally, these wastes contain lower levels of lignin and impurities compared to tree-based raw materials, allowing for the use of lower concentrations of chemicals during purification processes [35].

In the process of obtaining nanocellulose from lignocellulosic biomass, pre-purification steps are applied to facilitate the transformation of the raw material into the final product. These processes, carried out before nanocellulose extraction, aim to remove undesirable components such as fats, waxes, pectin, hemicellulose, lignin, and ash, as well as complex carbohydrate structures and branched hydrocarbons from the environment. The effects of the relevant pre-purification processes, which can be performed under acidic or alkaline conditions (such as bleaching procedures), on the properties of the resulting nanocellulose have been extensively studied in various research Works [40], [41].

Bacterial cellulose (BC), one of the nanocellulose derivatives, is a biopolymer produced by specific bacterial species, characterized by high purity and a nanofibrillar structure. It exhibits remarkable differences when compared to its plant-based counterparts. The first distinguishing feature is its high purity, as bacterial cellulose does not contain impurities such as lignin, hemicellulose, and pectin. Additionally, BC has a nanofiber structure that forms a three-dimensional network, which contributes to its exceptional mechanical strength [42], [43]. Bacterial cellulose produced at the nanoscale typically has a diameter ranging from 20 to 100 nm and a length of several micrometers. These dimensions can vary depending on the production conditions and the bacterial species used. Furthermore, the size of the nanofibrils may differ based on factors such as the culture medium and fermentation conditions [44]. Bacterial cellulose, similar to cellulose nanocrystals, stands out with its high crystallinity and mechanical strength properties. Its collagen-like structure and 99% water retention capacity enhance cellular adhesion and immobilization abilities, providing significant advantages in biomedical applications such as tissue engineering and cellular therapy [45]. These properties classify bacterial cellulose as a biomaterial with a wide range of applications and contribute to its recognition as a Generally Recognized As Safe (GRAS) material.

In conclusion, cellulose-based nanomaterial derivatives stand out as high-value, multifunctional materials due to their ability to undergo physical and chemical modifications and the integration of functional groups into their structure [46]. hese characteristics have attracted interest from researchers across various disciplines. As a significant category within biomaterials, these materials also establish a strong connection with key research areas such as sustainable economy and life cycle assessment [47], [48].

Recent studies have demonstrated the integration of CNCs into fully renewable composite systems, offering improved mechanical and thermal properties. For instance, all-cellulose biocomposites produced using water-based vacuum filtration systems have shown promising results for sustainable material applications [49].

2.1 Pre-Treatments

The initial step in the preparation of lignocellulosic biomass through pre-treatment involves creating suitable channels to enhance the penetration of chemicals in subsequent processes. Therefore, the cell walls are fragmented according to the selected mechanical methods. Throughout this process, cellulose sources may undergo modifications such as size reduction, structural transformation, increased crystallinity, and enhanced hydrophilicity [47], [50], [51], [52], [53]. Pre-treatment methods are generally classified into physical, biological, and chemical approaches.

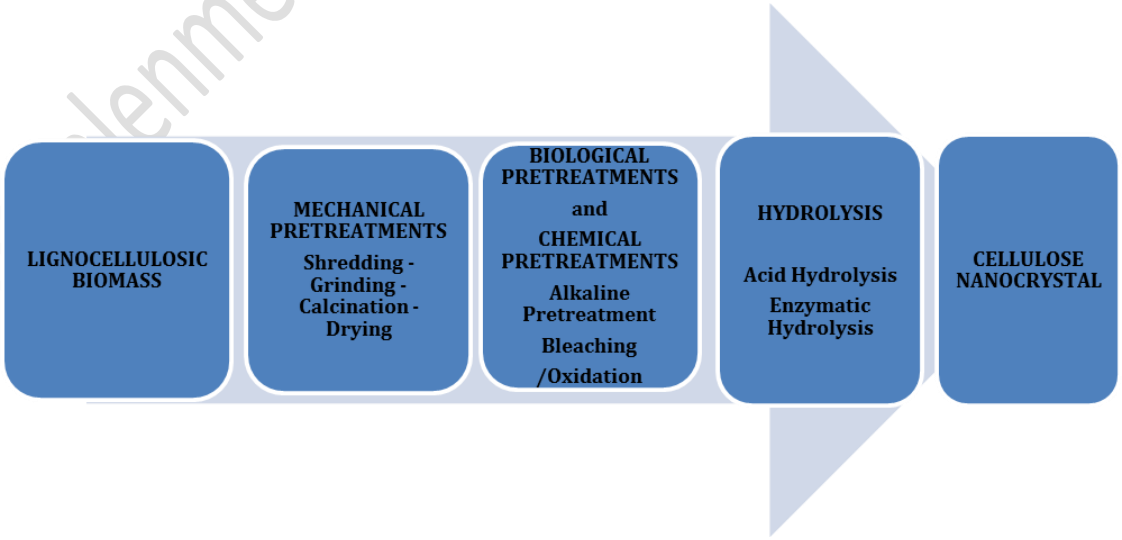

Figure 1. Cellulose Nanocrystal Production Steps

2.1.1 Physical and Biological Pre-Treatments

Physical pretreatment methods involve a range of techniques such as mechanical grinding, fragmentation, pulverization, pyrolysis, ultrasonication, microwave irradiation, and spray drying [54]. Grinding primarily targets the reduction of particle size down to a few millimeters, with minimum sizes reaching approximately 0.2 mm, leading to a more homogeneous particle distribution. Characteristics such as specific surface area, degree of polymerization, and crystallinity of nanocellulose are influenced by the grinding technique employed and the processing time [50], [54]. Mechanical pretreatments are generally economical and have the advantage of not generating hazardous by-products. In contrast, biological pretreatment strategies—including bacterial, enzymatic, and fungal methods—support the selective degradation of hemicellulose and lignin, thereby aiding in cellulose purification [54].

2.1.2. Chemical Pretreatments

2.1.2.1. Alkaline Treatment

The chemical pretreatment steps commonly employed in cellulose extraction generally include alkaline treatment and bleaching [50], [54]. Alkaline and subsequent bleaching treatments remove a portion of the lignin, wax, hemicellulose, and pectin surrounding the α-cellulose structure [54], [55]. The alkaline treatment facilitates the ionization of hydroxyl groups into alkoxide, reducing the number of hydroxyl groups on the cellulose surface. This process leads to a smoother surface morphology and an increase in hydrophobicity [56].

Hemicellulose is a carbohydrate matrix that, unlike the crystalline regions of α-cellulose, is not arranged in an orderly manner. Consequently, alkaline solutions can more easily penetrate and dissolve hemicellulose, facilitating its removal from the structure [57]. The elimination of the amorphous hemicellulose component increases the degree of crystallinity [58]. Since hemicellulose functions as a binding agent that interconnects cellulose fibrils, its removal results in a looser fiber arrangement and a decrease in density. The reduction in binding components promotes the reorganization of fibers under tensile deformation, leading to a more uniform alignment [59].

The alkaline treatment performed at room temperature offers advantages in terms of lower energy consumption and minimal degradation of cellulose sources [54], [60], [61], [62]. The most commonly used reagent in this process is sodium hydroxide, although treatments involving potassium, calcium, and ammonium hydroxides are also used [63], [64]. The reagents and application conditions used in the alkaline pretreatment are presented in Table 2.

Hassan and colleagues (2024) subjected sugarcane leaf sheaths, used as a cellulose nanocrystal source, to an alkaline treatment by immersing them in a NaOH solution with a concentration of 17%. This process was carried out at 80°C with mild agitation for 4 hours, maintaining a 1:18 fiber-to-solution ratio. In their study, it was noted that the intense alkaline treatment effectively dissolved non-cellulosic components, such as highly branched hemicellulose, through strong base-induced hydrolysis [65] . In another study utilizing sugarcane bagasse as the raw material, Fathana and colleagues (2022) applied a method that included ultrasound technology for producing cellulose nanocrystals (CNC) with a high crystallinity index. In this study, the raw material, pretreated with a toluene-ethanol mixture, was subjected to an alkaline treatment in a 5% (w/v) NaOH solution at 50°C for 4 hours, using a magnetic stirrer at 500 rpm. After the alkaline treatment, the sample was filtered, washed with distilled water until a neutral pH was achieved, and then dried at 50°C. It was reported that this pretreatment increased the crystallinity index in the final product and positively affected thermal stability [66].

In a different study conducted in the same year, cellulose nanocrystals were produced from the rachis (the stem part of the fruit attaching it to the tree) fibers of banana (*M. oranta*) trees. After a pre-cleaning process, the biomass was treated with a 16% aqueous sodium hydroxide solution under continuous stirring at 80.0 ± 1 °C for 3 hours. In the study, the fiber-to-solution ratio was set at 1:15, and the achieved purity level was found to be sufficient for subsequent steps, with the process being considered efficient [67]. In another study using sugarcane straw as the raw material, Lu and colleagues (2022) performed the alkaline treatment with a 10% NaOH solution at a solid-to-solution ratio of 1:10 [34]. In another study using corn stalks as lignocellulosic raw material, Costa and colleagues set the NaOH concentration of the alkaline solution at 2%, with processing conditions at 80°C for 4 hours, aiming to remove impurities and waxy substances from the fiber's outer surface. The alkaline treatment swelled the fiber structure, increasing the surface area, and thus enhancing the hydrolysis of cellulose polymer chains. It was noted that following the bleaching process, the cellulose content and crystallinity increased [68].

In a study conducted by Kasapoğlu and colleagues on cellulose nanocrystal production from apricot pulp, the alkaline treatment conditions were set as follows: 4% (w/v) NaOH concentration, 120°C temperature, 2 hours of processing time, and a solid-to-solution ratio of 1:20 under mechanical stirring. The study reported that the alkaline treatment significantly removed lignin and hemicellulose, and this played a critical role in enhancing the crystallinity of the final product [69].

Table 2. Reagents Used in Alkaline Pretreatment and Their Chemical Effects

Reagents	Chemical Effects
Sodium Hydroxide (NaOH)	Removal of lignin, hemicellulose, and other impurities, swelling of cellulose fibers
Potassium Hydroxide (KOH)	Dissolution of lignin and hemicellulose, activation of cellulose fibers (Improvement of the surface properties of cellulose and activation of the fibers, or making them capable of greater interaction)
Ammonium Hydroxide (NH_4OH)	Creates a milder basic environment to dissolve lignin and impurities

Hancock and colleagues, who proposed an unexplored opportunity for the utilization of hemp's green waste, composed of vegetative components such as plant stalks, through biomass optimization, performed an alkaline pre-treatment for cellulose nanocrystal production. In this study, they treated 2 g of ground fiber with 50 mL of 4 M sodium hydroxide solution at a 1:25 (solid/liquid) ratio for 3 hours at 50°C to remove non-cellulosic components. After the treatment, the obtained solid was washed with deionized water and filtered using filter paper [70].

The primary aim of the alkaline treatments mentioned in these studies is to prepare lignocellulosic biomass for bleaching and subsequent acidic hydrolysis, while removing a certain degree of impurities. However, it is also known that after the alkaline treatment, the binder component, lignin, is largely preserved in the lignocellulosic biomass, and the associated impurities must be removed through subsequent acidic treatments [71], [72].

2.1.2.2. Bleaching/Oxidation Process

As stated earlier, an alkaline medium facilitates the partial removal of lignin, making each phase of the delignification process essential for achieving efficient cellulose fiber defibrillation [73], [74]. Residual lignin within the fibers decreases the interfacial wettability between the polymer matrix and the natural fibers. Throughout the delignification process, lignin residues induce a pale brown discoloration in the cellulose. As the treatment progresses, the bleaching solution shifts to a vivid yellow, and the cellulose gradually acquires a white coloration [75].

The bleaching process may consist of one or multiple stages, depending on the desired final product. Wood-based sources, which have a higher lignin content, undergo more intensive bleaching treatments with longer exposure times and more concentrated solutions. In contrast, low-lignin raw materials, such as cotton linters, bacterial cellulose, and certain agricultural residues, require milder bleaching procedures. Commonly used bleaching agents include sodium chlorite ($NaClO_2$), sodium hypochlorite (NaClO), hydrogen peroxide (H_2O_2), sodium sulfite (Na_2SO_3), oxygen, and ozone [76], [77], [78]. Oxidizing chemicals used in bleaching interact with fibers and may induce surface modifications. To prevent reactive instability—such as uncontrolled oxidation or undesirable side reactions—the process is supplemented with alkaline washing steps. The primary goal of this process is to enhance solubility and transform the lignin within the structure. The bleaching effect observed in the solid material during the treatment serves as a significant indicator of lignin removal [79]. In cellulose nanocrystal (CNC) production, the bleaching procedure is typically performed following alkaline pre-treatment and is carried out using peroxide- or chlorine-based reagents. The bleaching agents and the corresponding application conditions are summarized in Table 3.

Farias *et al.* (2024) conducted a study on the extraction of cellulose nanocrystals from Acacia mearnsii brown kraft pulp. In their bleaching and delignification process, a completely dried 25 g sample was treated in an Erlenmeyer flask containing 3.5 g sodium chlorite, 3.5 g sodium acetate, 25 drops of anhydrous acetic acid, and 400 mL of distilled water. The bleaching process was performed in a water bath at 80°C for 1 hour, with manual stirring every 10 minutes. Upon completion of the reaction, the material was washed with distilled water to remove unreacted chemicals and subsequently dried at ambient conditions for 12 hours. The study reported that this procedure was repeated twice to achieve a high level of whiteness, ensuring the effective removal of non-cellulosic components [84].

Similarly, Makanda and colleagues (2024) focused on the production of cellulose nanocrystals from the bagasse of Zhombwe, Neorautanenia brachypus (Harms) C.A. Sm., a locally available plant. For the bleaching process, they employed an equal mixture of sodium chlorite (1.7% w/w), acetate buffer solution, and water. This solution was mixed with the alkali-treated solid material at a mass ratio of 1:20 and processed under reflux conditions for 4 hours. The bleaching treatment was repeated twice. The material was then filtered and washed with distilled water to completely remove the bleaching solution, ensuring that the desired level of whiteness was achieved [85].

Table 3. Reagents Used in the Bleaching-Oxidation Process and Their Chemical Effects

Reagents Used in the Oxidation/Bleaching Procedure	Chemical Effect and Application
Sodium Chlorite ($NaClO_2$) + Acetic Acid (CH_3COOH) (acidic medium, pH 3-4) [80]	➢ Oxidizes and removes lignin and impurities. ➢ Can be applied at room temperature or 70-80°C. ➢ Commonly used for wood-based cellulose sources.
Hydrogen Peroxide (H_2O_2) in Alkaline Medium (with NaOH) [77]	➢ Suitable for raw materials with low lignin content. ➢ An environmentally friendly and chlorine-free method.
Sodium Hypochlorite (NaOCl) [81]	➢ Decomposes lignin, acting as a strong oxidizing agent. ➢ Less preferred environmentally due to the formation of chlorine-containing byproducts.
Ozone (O_3) and Oxygen Bleaching [82], [83]	➢ Considered modern and eco-friendly methods. ➢ Typically used for raw materials with high lignin content (e.g., in kraft cellulose production).

The same bleaching reagents were used in another study, where 10 g of fibers obtained from Water Hyacinth (*Eichhornia crassipes)* underwent a bleaching process following an alkali treatment. The fibers were treated with 400 mL of deionized water, 1.5 g of sodium chlorite ($NaClO_2$), and 15 mL of acetic acid. The process was conducted in a water bath at 100 °C for 2 hours, and the bleaching procedure was repeated 10 times until perfectly white fibers were obtained [86].

Chlorine-free processes incorporating hydrogen peroxide (H_2O_2) have been frequently utilized in the literature for lignin removal during cellulose purification. Kim *et al.* investigated cellulose isolation from sugarcane bagasse, employing a $NaOH/H_2O_2$ mixture in the bleaching step. In this process, the pretreated sample was treated with 1 M NaOH solution, adjusting the pH to 11. Subsequently, 30% H_2O_2 solution, equivalent to 40% of the solid mass, was gradually added, and the mixture was stirred at 80°C for 1 hour to complete the bleaching process [87]. Similarly, in another study utilizing corncob and sugarcane bagasse as raw materials, sequential chemical treatments, including alkaline treatment and hydrogen peroxide bleaching, were applied for cellulose isolation. In this approach, the fibrous material was treated with a 38% H_2O_2 solution, followed by pH adjustment using 2 M citric acid. After the bleaching process was completed, the mixture was cooled, filtered, and dried [88]. Another study investigated the production of cellulose nanocrystals from jute, employing a hydrogen peroxide-based bleaching procedure. In this research, the material obtained after microwave-assisted sodium hydroxide treatment was bleached with a 30% H_2O_2 solution. Subsequent steps involved hydrolysis of the raw cellulose using ultrasonication and sulfuric acid [89].

Studies specifically addressing ozone-based bleaching processes for cellulose nanocrystal production remain limited in the literature. However, extensive research exists on the use of ozone (O_3) in bleaching cellulose-based materials. As a strong oxidizing agent, ozone effectively removes lignin and other chromophoric components, enhancing the whiteness of cellulose [82]. Recent studies highlight the effectiveness and environmental sustainability of ozone-based bleaching. Eren et al. examined the efficiency of ozonation in bleaching towel fabrics at various pH levels (3, 5, 7, 9, 11), conducting ozonation at a gas flow rate of 5 L/min for 15 and 45 minutes. Another study explored the potential of combining ozone and hydrogen peroxide at room temperature for bleaching and dyeing with selected natural dyes [90]. Additionally, a 2024 study proposed a novel method for environmentally friendly gas-phase bleaching of cellulose fiber materials using ozone and hydrogen peroxide[91]. Lastly, another recent study compared ozone-bleached cellulose pulp with conventional chlorine-based bleaching in paper-grade cellulose production. The findings indicated that ozone, when combined with hydrogen peroxide, achieved comparable brightness and durability while offering superior brightness stabilization compared to chlorine dioxide and peroxide mixtures [83].

2.2 Hydrolysis

2.2.1 Acid Hydrolysis

In the production of cellulose nanocrystals, the selective removal of amorphous regions from alpha-cellulose chains is required. Among the various chemical and biological processes reported in the literature, acid hydrolysis is considered the most suitable procedure for cellulose nanocrystal production [92], [93]. During the hydrolysis process, cellulose fibers are treated with selected concentrations of organic or mineral acids under specific temperature and time conditions. During acid hydrolysis, the amorphous domains of cellulose are preferentially broken down, while the crystalline regions largely remain unaffected. Due to their less ordered structure, the amorphous portions are more vulnerable to acid attack, resulting in a faster cleavage of glycosidic bonds compared to the crystalline areas. The success of the hydrolysis process is closely linked to factors such as reaction time, temperature, and acid concentration. Gaining a comprehensive understanding of the hydrolysis mechanism is crucial for tailoring the physical and chemical characteristics of cellulose nanocrystals [54], [94]. Typically, effective acid hydrolysis requires elevated temperatures and pressures; however, temperatures exceeding 110°C can promote the generation of toxic by-products, including furfural and 5-hydroxymethylfurfural [54]. The mineral and organic acids used in acid hydrolysis, along with their chemical effects, are presented in Table 4.

Table 4.Mineral and Organic Acids Used in Acid Hydrolysis and Their Chemical Effects

Acids Used in the Hydrolysis Process	Chemical Effects
Sulfuric Acid (H_2SO_4)	The most commonly preferred acid. It introduces sulfate groups onto the CNC surface, enhancing suspension stability. However, sulfate groups may reduce thermal stability.
Hydrochloric Acid (HCl)	Less reactive than sulfuric acid; it increases the thermal stability of CNCs while leaving no surface charge. However, the suspension stability is lower.
Phosphoric Acid (H_3PO_4)	A less commonly used method that enables the binding of phosphate groups to the CNC surface.
Nitric Acid (HNO_3)	Rarely used due to its strong oxidative nature, which may degrade the cellulose structure.
Oxalic Acid ($C_2H_2O_4$) Maleic Acid ($C_4H_4O_4$) Citric Acid ($C_6H_8O_7$)	Organic acids are generally considered more environmentally friendly alternatives, but they may not be as effective as mineral acids.

The most commonly preferred acid hydrolysis methods for CNC production involve the use of sulfuric acid and hydrochloric acid. This section aims to provide a comprehensive evaluation of the relative significance of various acid hydrolysis parameters and their effects on CNC morphology.

The type of acid used in acid hydrolysis has a direct influence on the surface properties, thermal stability, and suspension stability of CNCs. Notably, sulfuric acid hydrolysis is often chosen to introduce negatively charged sulfate ester groups ($-OSO_3^-$) onto the CNC surface, ensuring high colloidal stability in aqueous solutions. In a study investigating the production of cellulose nanocrystals from eucalyptus pulp using this approach, the hydrolysis parameters were set as follows: H_2SO_4 concentration (30, 40, and 50 wt%), hydrolysis time (30, 60, and 90 minutes), and hydrolysis temperature (60, 70, and 80°C). The optimal conditions were identified as 50 wt% H_2SO_4 concentration, 60 minutes of hydrolysis time, and a hydrolysis temperature of 60°C. Under these conditions, the final material exhibited a crystallinity index of 75.51 ± 1.51% and a crystal size of 4.03 ± 0.10 nm. Additionally, it was observed that as the H_2SO_4 concentration, hydrolysis duration, or temperature increased, the crystallinity percentage decreased while the crystal size became smaller. Moreover, CNCs demonstrated higher thermal stability compared to the raw eucalyptus pulp [95].

In another study investigating the production of cellulose nanocrystals (CNCs) from cotton linters, the hydrolysis conditions using sulfuric acid were determined as follows: 64 wt% acid concentration, 48 g solid content, a temperature of 50°C, and a reaction time of 30 minutes. The process was carried out under continuous magnetic stirring. At the end of the hydrolysis, the CNC suspension, which appeared dark yellow, was diluted tenfold with cold distilled water to halt the reaction and then centrifuged until the suspension reached a pH of approximately 5. In the final stage, the CNC dispersion was frozen in liquid nitrogen and subsequently subjected to freeze-drying at -50°C for 24 hours to ensure complete removal of water. The study reported that the crystallinity index of CNCs derived from cotton linters increased compared to waste cotton fibers. According to the Segal method, the crystallinity index of the raw sample and CNCs was reported as 79.87% and 88.37%, respectively [96].

Wang *et al.* (2021) proposed a sustainable and highly efficient method for preparing dual-functional cellulose nanocrystals using a mixed acid system composed of sulfuric acid and formic acid (FA) in their study exploring the feasibility of CNC production from eucalyptus kraft pulp. It was found that low concentrations (5–10 wt%) of sulfuric acid could significantly enhance the hydrolysis efficiency of formic acid (65–80 wt%), thereby enabling high-yield CNC production. The maximum yield of the obtained CNCs reached 70.65%. The most noteworthy aspect of this study was the approximately 20-fold reduction in the amount of sulfuric acid used compared to conventional sulfuric acid hydrolysis for CNC production. Furthermore, more than 90% of the formic acid used in the hydrolysis process could be recovered through a simple vacuum evaporation process, while the hydrolyzed sugars collected as byproducts had potential applications in the production of platform chemicals. As a result, compared to conventional sulfuric acid hydrolysis, the mixed acid system generated significantly less waste while allowing nearly all raw materials to be converted into value-added products [97].

As an alternative to the high-concentration hydrolysis process commonly employed for cellulose nanocrystal (CNC) synthesis, a study explored the use of controlled cellulose alcoholysis in simple alcohol media. In this approach, CNCs were synthesized via alcoholysis of sulfated cellulose in the presence of sulfuric acid as a catalyst, using four different aliphatic alcohols (methanol, ethanol, propanol, and 1-butanol). The study demonstrated that, compared to hydrolysis, the alcoholysis method enables CNC synthesis under milder reaction conditions, with lower acid concentrations and higher yields. While sulfuric acid hydrolysis was performed following a previously reported method [98] the alcoholysis process was based on the same approach but replaced water with methanol, ethanol, propanol, or 1-butanol. After the alcoholysis process, the resulting suspension was purified by washing with deionized water.

This study, which examined the effects of the two different methods on the physicochemical properties of CNCs, found that alcoholysis increased CNC yield and reduced the required acid concentration compared to hydrolysis. The structural properties of CNCs synthesized via hydrolysis and alcoholysis were generally similar; however, CNCs produced through alcoholysis exhibited a higher sulfate group content and greater surface charge. Additionally, the study highlighted that under the defined experimental conditions (0.025 g/mL sulfated cellulose suspension, 50°C reaction temperature, and 2-hour reaction time), 1-butanol yielded the highest CNC production efficiency at 60% [99] .

When sulfuric acid hydrolysis is employed as the primary process for controlling the morphology of cellulose nanocrystals, understanding the key factors governing the reaction is of critical importance. Knowledge of these parameters allows for the optimization of CNC morphology while minimizing acid, water, and energy consumption, thereby promoting a more environmentally friendly approach. Although sulfuric acid hydrolysis remains a widely used method, alternative approaches such as HCl hydrolysis have also been explored, offering certain advantages, including enhanced thermal stability.

A study focused on the production of cellulose nanocrystals (CNCs) from oil palm empty fruit bunches (OPEFB) using hydrochloric acid (HCl) hydrolysis under sonication and hydrothermal conditions examined the effects of variations in acid concentration, reaction time, and acid-to-cellulose ratio on yield, crystallinity, microstructure, and thermal stability. The optimal process conditions were determined to be hydrolysis with 3 M HCl at 110°C for 1 hour. Under these conditions, the highest CNC yield reached 74.82%, the crystallinity index was 78.59%, and the maximum decomposition temperature (Tmax) was recorded at 339.82°C [100].

Vaezi and colleagues conducted a study in which waste paper was selected as the source material, emphasizing an environmentally friendly approach to CNC production. The researchers performed HCl hydrolysis by treating cellulose fibers with a 3 M HCl solution (40 mL per gram of cellulose fiber) at 100°C for 180 minutes under continuous magnetic stirring. After the reaction, the resulting suspension was washed through sequential centrifugation (approximately five cycles, each at 6000 rpm for 15 minutes) until neutralized with distilled water. The supernatant was collected and subjected to dialysis in deionized water for 4–5 days to remove residual acid and other chemicals. The hydrolysis yield was determined to be 65%, with a crystallinity index of 79.6%. The study highlighted

the successful production of CNCs via HCl hydrolysis following an alkaline treatment and de-inking process of cellulose fibers [101].

Another study investigating CNC production via HCl hydrolysis explored the role of a transition metal complex in enhancing hydrolysis efficiency. In this work, CNCs were isolated from date seeds by incorporating a copper metal complex during HCl hydrolysis. Unlike conventional HCl hydrolysis, which typically yields only microcrystalline cellulose, this approach resulted in CNC production with a 10% higher yield [102].

Based on the evaluation of current studies, it has been observed that the sulfuric acid-based hydrolysis process in CNC production leads to the formation of sulfate ester groups on the material's surface. These groups contribute to maintaining the CNCs in a stable suspension in aqueous environments. However, the presence of sulfate groups can negatively impact the thermal stability of the CNCs due to their heat sensitivity, potentially causing degradation at lower temperatures.

On the other hand, in the hydrolysis process with hydrochloric acid, unlike sulfuric acid, chloride groups do not form on the cellulose surface. This absence of additional functional groups may allow the CNCs to exhibit higher decomposition temperatures and, therefore, increased thermal stability. However, the lack of charged groups on the surface of CNCs synthesized with HCl may result in lower stability in aqueous environments compared to those produced with sulfuric acid [92], [103].

Furthermore, studies have also reported the use of environmentally friendly and sustainable organic acid hydrolysis as an alternative for CNC production. Compared to conventional mineral acid hydrolysis, this process, which is less toxic, includes the recovery of organic acids in some experimental setups [104]. The lower acidic strength (higher pKa values) of organic acids allows for the production of longer CNCs with higher crystallinity than mineral acids, thereby improving the mechanical properties of the resulting CNCs and expanding their potential applications[105].

In another study, ıt was reported the production of cellulose nanomaterials from bleached kraft eucalyptus pulp using recyclable solid organic acids (phosphoric, anhydrous oxalic and anhydrous maleic acids, p-toluenesulfonic acid, and benzenesulfonic acid) in a high-pressure acid hydrolysis process, which resulted in excellent thermal stability. The hydrolysis conditions for the process were determined for hydroxalic (O), maleic (M), and p-toluenesulfonic (T) acids with varying concentrations (30–80 wt%), hydrolysis temperatures ranging from 80 to 120 °C, and reaction times from 30 to 240 minutes. The study emphasized that cellulose could be converted into carboxylated cellulose nanomaterials through esterification with the carboxyl groups of specific dicarboxylic acids, highlighting the importance of this process for material functionalization. Moreover, the recovery of acids through simple crystallization, functionalization of the surface via carboxylation, and the enhanced thermal stability of the obtained cellulose nanocrystals (CNC) and cellulose nanofibrils (CNF) make this process green and sustainable. However, it was also noted that dicarboxylic acids are weaker than mineral acids such as sulfuric acid, leading to lower CNC yields, with a maximum yield of approximately 25%. Materials that underwent no hydrolysis were referred to as cellulose nanofibrils, and the study suggested that the integrated production of both materials could be achieved using the presented method [105].

2.2.2 Enzymatic Hydrolysis

Enzymatic hydrolysis in cellulose nanocrystal (CNC) production is an energy-efficient method as it is performed at low pressure and temperature. Therefore, CNCs produced using enzymes are preferred in fields such as biomedical, pharmaceutical, and advanced material science, where high purity and superior performance are required [106]. Enzymes can break down the amorphous regions of cellulose fibers while largely preserving the crystalline regions, thereby maintaining the hydroxyl groups on the surface and making the material more suitable for direct chemical modification.

Enzymes catalyze the hydrolysis of cellulose fibers, facilitating the fibrillation process [107]. Cellulase enzymes consist of a complex system made up of subcomponents such as endoglucanases (EG), cellobiohydrolases (CBH), and β-glucosidases (GB). EG acts randomly on the amorphous regions of cellulose, breaking the β-1,4-glycosidic bonds and converting long-chain cellulose molecules into smaller structures. CBH targets the ends of linear cellulose molecules, leading to the degradation of the crystalline regions, while GB hydrolyzes cellulose into glucose [108].

When enzymatic hydrolysis is used in CNC production, it is crucial to minimize the damage caused by CBH to the crystalline regions. Therefore, careful separation of the three main components of cellulase is necessary to ensure that each enzyme component acts on its specific target regions. EG selectively breaks down the amorphous regions, allowing the crystalline structures to remain intact, thereby enabling a more controlled and efficient process [41], [109]. The selective hydrolysis of the amorphous structures contributes to the preservation of the crystalline regions, ensuring that the CNC retains its desired structural properties [109].

Dias *et al.* (2022) investigated the effect of using endoxylanase as a helper enzyme in combination with endoglucanase for the isolation of cellulose nanocrystals (CNCs) with improved properties from bleached eucalyptus kraft pulp. In this context, the helper enzymes were selected to create an enzyme combination with high selectivity for irregular regions. When the xylanase activity matched or exceeded that of endoglucanase, the resultant CNCs exhibited enhanced features such as higher crystallinity, improved thermal stability, greater homogeneity, better suspension stability, and optimized aspect ratio. The beneficial role of auxiliary enzymes was attributed not only to their hydrolytic actions on xylan and cellulose but also to non-hydrolytic effects, including increased fiber swelling and enhanced porosity. Furthermore, variations in enzyme ratios played a crucial role in fine-tuning CNC characteristics. Therefore, in contrast to conventional sulfuric acid hydrolysis, the application of supportive enzymatic strategies allows for the isolation of cellulose nanomaterials with tailored dimensions, shapes, and morphologies, potentially broadening their range of applications [110].

In another study, Zhang *et al.* developed a modified enzymatic hydrolysis method to increase the yield of cellulose nanocrystals (CNCs). A one-step mechanical-enzymatic hydrolysis method utilizing the synergy of enzymatic hydrolysis with wet grinding was developed. The proposed method integrates mechanical activation with enzymatic hydrolysis in a single step under suspension or wet conditions, without the need for volumetric solution use or complex procedures. By optimizing ball milling conditions, liquid addition, enzyme loading, and enzyme types, an efficient and cost-effective procedure for CNC production was established.

Under optimal reaction conditions, a maximum CNC yield of 49.3% was achieved, and a 76.7% increase in thermal stability and crystallinity index was observed. This study presents an alternative method for CNC production that significantly reduces water usage, increases production efficiency, and thus lowers production costs [111].

Ren and colleagues (2022) employed a complex enzymatic hydrolysis method to isolate cellulose nanocrystals (CNCs) with a size of approximately 40 nm from microcrystalline cellulose. The pH of the enzymatic hydrolysate was adjusted within the range of 2 to 12 using 1.0 mol/mL HCl and 0.1 mol/mL NaOH solutions. Half a gram of xylanase and cellulase were separately dissolved in 500 mL of deionized water and adjusted to pH levels of 1–6. pH adjustments were made to ensure system stability, large particles were removed in an alkaline environment (pH = 9) by centrifugation at 3000 rpm, and subsequently, spherical CNCs with particle sizes ranging from 24 to 76 nm were precipitated and purified using the flocculation method in an acidic environment (pH = 4). After a three-stage washing process, enzymatic proteins and reducing sugars were completely removed, resulting in high-purity spherical CNCs. Structural analyses revealed that the obtained spherical CNCs retained the natural cellulose Iβ crystal structure, although the crystallinity was reduced. Thermal analyses indicated that the initial pyrolysis temperature of the spherical CNCs was 211 °C, and the maximum pyrolysis temperature was 309 °C [112]. In another study, Meyabadi and colleagues developed a method combining enzymatic hydrolysis with sonication for the production of spherical cellulose nanocrystals (CNCs) from waste cotton [96]. The CNCs produced using this method were reported to have particle sizes below 100 nm.

In general, enzymatic hydrolysis offers a more environmentally friendly alternative compared to hydrolysis methods using inorganic acids. However, this method has certain limitations, including experimental conditions that require careful control, low CNC yield, and long processing times. Essentially, although enzymatic hydrolysis of cellulosic materials is a promising approach for the commercial production of CNCs, further research is needed to improve process efficiency for its industrial-scale applicability.

2.3 Final Processes

After the acidic hydrolysis process, which is commonly used in CNC production, an additional purification step is required to remove the excess acid remaining in the structure. This section discusses various purification methods applied in CNC production.

2.3.1 Centrifugation

Centrifugation is essentially a separation method based on the principle of sedimentation, allowing the components of a mixture to be separated. Therefore, it is commonly used to remove the acidic solution from CNCs. This process, based on gradually centrifuging the acidic supernatant from the CNC suspension, is repeated until the pH balance is achieved. The centrifugation is typically performed at speeds of 8000–10,000 rpm for 10–20 minutes. After removing the supernatant, deionized water is added back to the centrifuge tubes and the process continues[68], [113], [114]. The process continues until the suspension becomes colloidal, and it can be terminated when the supernatant appears cloudy, indicating that the CNCs have begun to migrate into the supernatant phase.

2.3.2 Dialysis Process

Dialysis is another purification method used to remove excess acid remaining in CNCs during acid hydrolysis. The centrifuged cloudy suspension is dialyzed using dialysis tubes with deionized or distilled water. The dialysis tubes used in the literature are cellulose membrane tubes with a molecular weight cutoff of 14,000 Da [33], [115].

The dialysis process is continued for 2-3 days until the pH reaches levels between 6 and 7 [116], [117]. However, this process is not recommended for mechanically produced cellulose nanofibrils.

2.3.3 Sonication Process

Various mechanical techniques, including ultrasonication, high-pressure homogenization, and ball milling, have been employed to obtain CNCs either independently or coupled with other processing methods [118]. Among these, sonication stands out as an intensive mechanical approach, producing hydrodynamic effects by harnessing the energy transmitted through sound waves [119]. The main purpose of sonication is to break down α-cellulose into nano-sized particles. In the literature, it is commonly noted that cellulose nanocrystal suspensions are subjected to sonication for approximately 10–15 minutes with an amplitude of 65–70%[112], [120], [121], [122].

2.3.4 Freeze Drying

Freeze drying, also known as lyophilization, is a method in which the water inside a substance is frozen at low temperatures and then evaporated under low pressure. This process relies on the principle of water transitioning directly from solid to gas phase via sublimation. It is considered one of the effective, modern, and practical techniques for removing liquids or solvents from nanocellulose.

In this method, the nanoselulose suspension is first frozen to form ice crystals. Then, the ice crystals are processed under sublimation conditions, where they directly transition into gas without passing through the liquid phase. One of the major advantages of this technique is that it prevents cellulose particles from clumping together, thus preserving the structural integrity of the nanocellulose. As a result, the dried nanocellulose shows a more homogeneous distribution within the final material's structural matrix.

3 Characterization Methods for Cellulose Nanocrystals (CNCs)

Precisely controlling the production parameters of CNCs and, consequently, their morphology is one of the challenges faced by researchers in this field. The main reasons for this issue are the incomplete understanding of process variables and the insufficient sensitivity of existing characterization techniques. Overcoming the current challenges related to morphological control will enable CNCs to be more effectively utilized in a wide range of morphology-dependent applications. In this regard, developing a systematic approach to the production process will contribute to enabling industrial-scale production and creating environmentally friendly, sustainable manufacturing methods.

The characterization of cellulose nanocrystals (CNCs) is carried out using various analytical techniques to determine their structural, chemical, physical, and morphological properties. The morphologies, crystallinity degrees, dispersion stabilities, thermal stabilities, and functional groups of CNCs are elucidated using the analytical methods summarized below.

In the literature, spectroscopic techniques that stand out in the characterization of CNCs' surface chemistry, crystalline structure, and other properties include solid-state Nuclear Magnetic Resonance (SSNMR) spectroscopy, FTIR spectroscopy, XPS, and Raman spectroscopy. Data related to the crystal structure are obtained through X-ray Diffraction (XRD), while the dispersion stability of CNCs is evaluated using Dynamic Light Scattering (DLS) and zeta potential measurements. Additionally, their thermal stability is investigated using Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). This section provides a comprehensive summary of the relevant analytical techniques.

3.1 Solid-State Nuclear Magnetic Resonance (SSNMR) Spectroscopy

Solid-State Nuclear Magnetic Resonance (SSNMR) spectroscopy stands out as a highly effective analytical method for the structural characterization of solid materials. Through high-resolution spectra obtained under controlled analytical conditions, it provides comprehensive information about both the quantitative and qualitative properties of the material being examined. The SSNMR technique is advantageous due to its rapid and non-destructive nature, as well as its requirement for only a small amount of sample. This technique plays a crucial role, especially in determining phase structures, and is critical in the characterization of cellulose-based materials with different polymorphic forms and their relative abundances [123].

SSNMR spectroscopy has the ability to distinguish between the crystalline and amorphous regions of cellulose nanocrystals. The crystalline regions exhibit different chemical environments compared to the amorphous portions. Depending on the source of cellulose, signals in the 87–92 ppm range correspond to the crystalline regions, while signals in the 80–87 ppm range represent the amorphous structures [124]. In addition, SSNMR spectroscopy is widely used to identify the different allotropes of cellulose (e.g., the Iα/Iβ ratio) and crystalline polymorphic transformations. In the analysis of various cellulose polymorphs (such as Iα, Iβ, and II), signals observed around 89 ppm are considered as key indicators [124].

SSNMR spectroscopy is also a powerful tool for examining the conformational features and crystalline modifications of hydrogen bonds in cellulose nanocrystals (CNCs). High-resolution 13C CP-MAS NMR spectroscopy is a widely used method for determining the degree of chemical modifications of cellulose and its derivatives, and it is frequently employed in the characterization of cellulose nanocrystals (CNCs) and their derivatives due to its ability to detect the degree of substitution in cellulose derivatives [125], [126], [127].

3.2 X-ray Photoelectron Spectroscopy (XPS)

X-ray Photoelectron Spectroscopy (XPS), also known as electron spectroscopy for chemical analysis (ESCA), is a powerful analytical technique widely used to investigate the surface chemical composition of materials. XPS operates based on the photoelectric effect principle. In this method, X-rays with a specific energy are directed onto the surface of the sample, providing information about the electronic structures of the atoms beneath the surface. One of the major limitations of XPS is its inability to precisely distinguish functional groups with similar binding energies. Therefore, complementary analytical techniques, such as Fourier Transform Infrared Spectroscopy (FTIR), are often required to determine the chemical nature of functional groups. Another significant limitation of XPS is the necessity for measurements to be carried out under ultra-high vacuum conditions.

XPS analysis is commonly used to determine elemental composition, oxidation states, binding energies, and to investigate chemical functionality on solid surfaces [128]. In recent years, XPS has been applied to qualitatively analyze the chemical modifications of cellulose nanocrystals (CNCs), particularly based on binding energies related to carbon bonds. Additionally, it plays a key role in detecting metal content and associated oxidation states in NCC-based composite materials [125], [129].

3.3 3.3. Fourier Transform Infrared Spectroscopy (FTIR)

A typical infrared (IR) spectrum consists of absorption bands corresponding to the vibrational frequencies of different atomic bonds within a material. Since each material has a unique atomic arrangement, no two molecules produce identical IR spectra. Consequently, IR spectroscopy serves as a distinctive chemical fingerprint for each sample [130].

Fourier Transform Infrared (FTIR) spectroscopy, commonly referred to as FTIR, provides several advantages over conventional IR spectroscopy, including an enhanced signal-to-noise ratio, improved resolution, and greater reproducibility. Numerous studies have reported FTIR data for cellulose and its derivatives, offering comprehensive peak assignments for IR spectra [131], [132], [133], [134]. ypically, cellulose nanocrystals (CNCs) exhibit two distinct absorption regions: one at higher wavenumbers (2700–3500 cm^{-1}) and another at lower wavenumbers (600–1800 cm^{-1}). The absorption bands at 3292 cm^{-1} and 3335 cm^{-1} are attributed to intermolecular and intramolecular hydrogen bonds, corresponding to –OH stretching vibrations [135] .

Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy is widely employed for characterizing chemical structures, analyzing structural modifications in functional groups, and comparing cellulose-based materials, including CNC systems. Due to its broad applicability, this technique is extensively utilized to examine structural transitions in cellulose nanomaterials, differentiate between crystalline and amorphous regions, and investigate functionalization processes [136].

3.4 Raman Spectroscopy

Raman spectroscopy is a widely utilized spectroscopic technique for the analysis and characterization of cellulose nanomaterials. This method has been extensively reported in the literature for providing critical insights into the structural properties of cellulose nanocrystals. Additionally, it plays a significant role in evaluating the accessibility of water to cellulose by distinguishing structural differences between crystalline and amorphous regions, thereby determining the extent to which water can penetrate cellulose fibers [137] Furthermore, Raman spectroscopy is effectively employed in the identification of cellulose II. Beyond these applications, this technique facilitates the quantification of cellulose nanomaterials, the structural analysis of cellulose nanocomposites, and the identification of sulfate esters and functional groups on the surfaces of cellulose nanocrystals[138].

3.5 X-ray Diffraction (XRD)

XRD is a diffraction technique used to analyze the atomic-level arrangement of crystalline structures. In the solid-state characterization of cellulose nanomaterials, XRD provides both quantitative and qualitative insights into the structural behavior of cellulose nanocrystals (CNCs). The XRD spectra of CNCs are recorded at room temperature (25 °C) using Cu–Kα radiation with a wavelength of 0.154 nm, within a diffraction angle range of 10–80°, and a scanning speed of 0.8° per minute. The diffraction peaks in XRD spectra appear at approximately 2θ = 15.3°, 16.4°, 22.5°, and 34.2°, corresponding to the crystallographic planes (110), (110), (200), and (040), respectively, which are characteristic of cellulose I [139].

The crystallinity index (CrI) of CNCs is determined from the obtained XRD spectra using both the peak height method and deconvolution approaches. Among researchers analyzing the CrI values of natural fibers and isolated CNCs, approximately 70–85% prefer the Segal peak height method over other techniques (such as deconvolution and amorphous subtraction) due to its simplicity. In this method, the crystallinity index of CNCs is calculated using the following equation (1) based on the peak height approach:

$$\boldsymbol{CrI}(\%) = \frac{\boldsymbol{I_{002} - I_{am}}}{\boldsymbol{I_{002}}} \text{x } 100 \qquad (1)$$

Here, I_{200} represents the maximum peak intensity of the (200) reflection, corresponding to the crystalline fraction of cellulose I, while I_{a} denotes the minimum peak intensity associated with the amorphous fraction. In the formula, the crystallinity index (CI) is initially calculated as the ratio of the crystalline peak intensity (I_{200} - I_{a}) to the total intensity (I_{200}). In the example XRD spectrum presented in Figure 1, the peak intensities and diffraction patterns are illustrated on the spectrum.

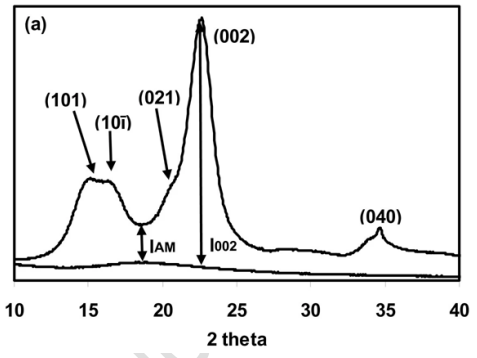

Figure 2. X-ray diffraction spectra of Avicel PH-101, illustrating (a) the peak height method, which is the most commonly used approach for CI calculation [140].

Despite its widespread use and acceptance, the Segal peak height method has certain limitations, including the underestimation of the amorphous contribution and the calculation of cellulose crystallinity based solely on the most intense crystalline peak, (002). However, the contributions of other cellulose crystalline peaks associated with different crystallographic planes, such as (110), (110), and (004), are disregarded in this method. This approach consequently leads to an evaluation of the cellulose crystalline structure from a single orientation [141].

For these reasons, the Segal peak height method has been reported to be useful for comparing relative differences between samples but unsuitable for accurately determining the absolute crystalline and amorphous content in a given sample [142].

In recent years, researchers have employed the peak deconvolution method to address the shortcomings of this approach and to calculate cellulose crystallinity (CrI) and the crystalline fraction (Crs) in cellulosic materials. Peak deconvolution involves modifying the observed pattern in the XRD profile to include an additional broad peak (or peaks) to account for both the apparent peaks and the amorphous content, as illustrated in the figure. In some cases, peaks are added at their expected positions using Gaussian, Voigt, or Lorentzian functions based on known unit cell dimensions. The peak parameters are then optimized using curve-fitting software, with adjustments made to ensure alignment with the experimental pattern.

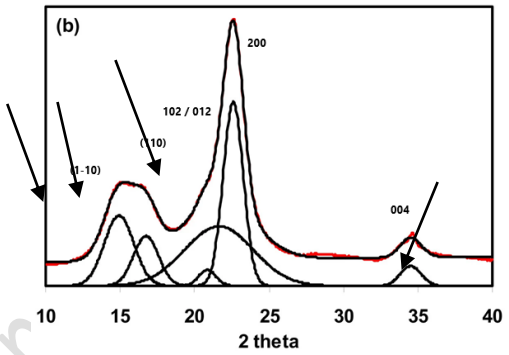

Figure 3. X-ray diffraction spectra of Avicel PH-101, demonstrating the most commonly used primary method for Carıcalculation, (b) Peak Deconvolution Method [143].

Typically, five crystalline peaks indexed as 1̄10, 110, 012/102, 200, and 004 are considered while using Gaussian and Voigt fitting functions. The 1̄10, 110, and 200 indexed peaks are clearly observable in most cellulose samples, while the 012/102 reflection often appears as a shoulder. However, in some samples (e.g., bleached eucalyptus), these peaks may overlap and cannot be clearly separated. Another diffraction peak, visible around 34.5°, typically appears as a broad peak indexed as 004, but it is actually a composite of several neighboring peaks.

The Voigt (commonly adapted-Voigt) function was used to fit the five peaks observed in the XRD diffractogram of cellulose samples. Figure 4B is a typical example of such a fit. However, when using the Lorentzian fitting function, four crystalline peaks indexed as 1̄10, 110, 200, and 004 are considered because the 012/102 peak, which often appears as a shoulder, cannot be clearly separated.

The crystallinity index (CrI) is calculated as the ratio of the area under all the crystalline peaks to the total area of the sample.

$$CI = \frac{A_{cr}}{A_{sample}} = \frac{\left(\int_{2\theta_1}^{2\theta_2} I_{cr} d2\theta\right)}{\left(\int_{2\theta_1}^{2\theta_2} I_{sample} d2\theta\right)} \qquad (2)$$

Here, CrI represents the crystallinity index; Acr is the area under all crystalline peaks; A_sample is the total area of the sample; I_cr is the integral sum of the intensities of the crystalline peaks; and I_sample refers to the total intensity across the entire diffraction space.

Amorphous contribution is typically modeled by adding a broad peak between 18° and 21° using general peak fitting software or XRD-specific software. Since more peaks are considered compared to the Segal method, it has been

suggested that the peak fitting method may be more reliable in comparing the crystallinity index of different samples. However, there are also criticisms that the peak area method does not sufficiently account for all factors contributing to peak broadening, such as crystal irregularity, crystallite size, and heterogeneous stress within the crystal[144] .

3.6 Dynamic Light Scattering (DLS)

The determination of dispersion quality is a critical factor in the characterization of cellulose nanocrystals (CNCs). Dispersion quality refers to the degree to which a material is homogeneously distributed within another medium (typically a liquid or polymer matrix). The homogeneous dispersion of nanoscale filler materials, such as cellulose nanomaterials, in bio-based polymer composites directly affects the mechanical and physical properties of the composite.

The hydrodynamic diameter of the cellulose nanomaterial suspension is recorded using Dynamic Light Scattering (DLS) techniques based on the Microtrac and Nanotrac wave methods. The diffusion coefficient of cellulose nanomaterials is measured using light scattering properties applied by the DLS device [145], [146].

The success of the DLS method depends on sample preparation, aggregation state, equipment used, and the protocol followed. However, it is not suitable for measuring the hydrodynamic radius of cellulose nanofibrils with high flexibility and aspect ratio. This method is primarily used to assess the aggregation state of CNCs in aqueous media based on changes in pH and ionic strength. Although DLS cannot determine the exact size of CNC particles, it is preferred for its ability to quickly and easily analyze dispersion quality. Alternative methods may be more precise but are generally more expensive, time-consuming, and complex, which is why DLS is often used as a preliminary analysis method [145].

3.7 Zeta Potential

Colloidal stability is a critical parameter in cellulose nanocrystal (CNC) suspensions, and the zeta potential is a fundamental measure for evaluating this stability. The zeta potential is related to the electrostatic repulsive force created by the charged chemical groups on the surface of the material. The electrophoretic mobility of the particles is measured in mm/s using electrophoretic mobility analyzers. The negative surface charge of CNCs originates from anionic functional groups such as sulfate and carboxyl groups present on their surface. If the zeta potential of the nanocellulosic material is between -15 mV and +15 mV, the electrostatic repulsive forces are insufficient, causing the particles to aggregate. In contrast, zeta potential values below -30 mV or above +30 mV stabilize the suspension. CNCs hydrolyzed with HCl exhibit low zeta potential values in measurements due to the lack of ionic charge on their surface, resulting in an unstable suspension structure[147], [148].

In summary, improper dispersion of CNCs leads to agglomeration, which can cause deterioration in the mechanical properties of bio-based polymer composite materials. Therefore, the various techniques and their precision used to determine the dispersion quality of the material play a critical role in defining the structural properties of the final product.

Morphological analysis is of great importance in determining the sizes of nanoparticles and investigating the structural properties of materials. This analysis also contributes to understanding the physical characteristics of the materials. In the field of nanocellulose, accurate determination of the nanometric sizes of CNCs plays a crucial role in quality control, gaining more insight into production processes and applications, comparing different products in the market, and facilitating their industrial use. Microscopic techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) are commonly used to detect the size and morphology of CNCs [149].

3.8 3.8 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) and Field Emission SEM (FE-SEM) are imaging techniques used to examine both the structural properties (shape and morphology) and the sizes of cellulose nanocrystals (CNCs) without requiring surface contact. In these methods, a 10 µl sample of a dilute material suspension at a 1–2 wt% concentration is first dropped onto a 10 x 10 mm glass slide and allowed to dry for approximately 3 hours under room temperature and ambient lighting. The fully dried CNCs are then coated with platinum to enhance surface conductivity. During SEM imaging, the device's operating voltage is maintained within the range of 10–20 kV [150].

In the literature, it has been noted that the interactions between particles during SEM or FE-SEM imaging of CNCs can complicate the morphological measurement of CNCs. Due to the tendency of nanomaterials to aggregate, the contrast of individual nanocrystals may not be sufficiently distinct [151]. o address this issue, Mattos *et al*. (2019) demonstrated that the negative contrast SEM method provides both fast, practical imaging and high contrast. However, direct comparison of results obtained from different microscopic techniques is not always possible. SEM, being faster and more practical than AFM, allows Mattos *et al*. (2019) to relate negative contrast SEM measurements (width/length) to AFM measurements (height/width), aiming to improve sample characterization with negative contrast SEM alone [152] Additionally, images obtained using FE-SEM cover a wider size range compared to those from TEM (Transmission Electron Microscopy) and AFM (Atomic Force Microscopy), allowing for the examination of the sample at different scales [150].

3.9 Transmission Electron Microscopy (TEM)

TEM is a technique that offers higher spatial resolution than scanning electron microscopy (SEM) and allows for detailed characterization of nanoparticles. This method is effectively used to determine the morphology, size, and aspect ratio of individual nanocrystals [153]. Although TEM images help satisfactorily determine the size of cellulose nanocrystals, the complex sample preparation processes and the expertise required for its use are among the biggest challenges of this technique (Kaushik *et al.,* 2014, 2015). However, TEM stands out by offering approximately five times the magnification contrast compared to SEM [150] However, the small size of CNCs, strong hydrogen bonding, and low electron density still make it difficult to visualize them at high resolution and magnification levels. To overcome these challenges, staining procedures and dispersion techniques have been developed to enhance contrast. In the literature, different dyes, dispersion agents, and support materials have been investigated to improve the visibility of individual CNCs and achieve high contrast using Transmission Electron Microscopy (TEM). Stinson and colleagues (2018) identified that the most successful method involved using CNCs at low concentration

along with the addition of bovine serum albumin as a dispersion agent and Nanovan® as a contrast-enhancing substance. In this method, the use of a silicon monoxide-coated Formvar TEM grid enabled sharper and more detailed imaging of CNCs [154].

Despite the high magnification and detailed analysis advantages provided by microscopic techniques, there are concerns regarding how representative and verifiable the data obtained is, due to the small sample size and limited field of view. Additionally, the time-consuming nature of sample preparation, observation, and image analysis can limit the practical application of these techniques. Therefore, the development of relevant characterization methods and the improvement of existing techniques to make them more efficient are significant aspects being studied in the literatüre [155] .

3.10 Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC)

The thermal properties of cellulose nanocrystals (CNCs) are determined using Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). DSC is commonly used for thermal transitions, glass transitions, and heat changes, while TGA primarily provides data on thermal degradation, decomposition, and weight loss. The combined use of these two methods provides a more comprehensive analysis of the thermal properties of CNCs.

Thermogravimetric analysis is performed to predict the rate of mass loss with temperature (°C), and this process is typically carried out in a nitrogen atmosphere (the reported flow rates in the literature usually range from 20 mL/min to 60 mL/min) [92]. In this analysis process, dry CNCs are placed in an aluminum pan within the analyzer. The temperature is increased at a rate of 20°C/min from room temperature to 600°C. The first observed degradation in the thermogram typically occurs around 100°C, due to the evaporation of moisture present in the CNCs, and the final degradation occurs in the temperature range of 250–350°C. This final degradation temperature can vary depending on the raw material source from which the CNCs are isolated and the process conditions. Isolated CNCs exhibit lower thermal stability compared to raw fibers. This data is attributed to the larger surface area of CNCs (due to their nanoscale size) and the presence of sulfate groups in the structure [156]. The literature specifically reports that CNCs produced by chemical methods such as TEMPO-mediated oxidation or acid hydrolysis have lower thermal stability compared to the lignocellulosic fibers from which they were produced [157].

reported that acid hydrolysis carried out with sulfuric acid negatively affects the thermal stability of cellulose nanocrystals (CNCs) due to the presence of sulfate groups on the surface. Sulfate groups reduce the activation energy for degradation, thereby decreasing thermal stability. Additionally, it has been reported that the carbon residue at 500 °C is high, and this is attributed to the dehydrating effect of the sulfate groups. However, mechanically extracted cellulose nanofibers (CNFs) exhibit higher decomposition temperatures and thermal stability compared to CNCs obtained by acid hydrolysis [92].

Thermal properties of materials are commonly investigated using Differential Scanning Calorimetry (DSC), which analyzes endothermic and exothermic reactions, phase transitions, and specific heat capacity [158]. DSC detects thermal transitions such as melting, crystallization, and glass transition by measuring changes in heat flow, while Thermogravimetric Analysis (TGA) determines thermal stability and decomposition temperatures by measuring mass loss [159].

Reviewing the literature, studies on cellulose nanocrystals (CNC) report two distinct endothermic peaks in DSC curves within the 30–350°C temperature range. The first peak typically occurs between 100 and 210°C and is associated with thermal events such as moisture loss, evaporation, and the decomposition of thermally unstable components. The second endothermic peak, observed in a narrower temperature range of 280–300°C, corresponds to the melting process associated with the decomposition of CNC crystallites [33], [88], [160].

The thermal degradation temperatures of CNCs can vary depending on factors such as the raw material used, production methods, and surface modifications. In cases where amorphous cellulose is present, the second endothermic peak may appear at lower temperatures, around 190°C. This is primarily due to the lower melting point of amorphous cellulose compared to the crystalline form of CNCs. Mandal and Chakrabarty, in their study on CNCs derived from sugarcane bagasse, reported similar endothermic events (moisture loss and melting point). Additionally, the melting temperature of the CNC produced was found to be consistent with the values reported by Huang *et al.* for CNC derived from corn stalks and comparable to the thermal properties of CNCs reported by Maiti *et al.* [36], [159], [162]. Upon reviewing similar studies in the literature, it can be concluded that CNCs possess excellent thermal insulating properties, offering a wide range of material options for material engineers working in fields where thermal performance is essential [156], [160], [161], [162], [163].

4 Conclusions

The depletion of natural resources, the effects of global warming, and increasing environmental pollution highlight the issues of reducing energy consumption, efficiently utilizing non-renewable resources, and promoting renewable alternatives. One of the key objectives of studies in this direction is to ensure sustainable transformation and enhance the efficiency and applicability of environmentally friendly technologies.

In this context, many researchers and the scientific community are focusing on the development of bio-based and ecological nanomaterials to minimize environmental harm. Nanocellulose, derived from lignocellulosic fibers and recyclable cellulose materials, emerges as a highly durable and sustainable material. Due to its large specific surface area, superior barrier properties, biocompatibility, non-toxicity, and biodegradability, it is preferred in various fields ranging from engineering to the packaging industry. In particular, it has high potential for use as a reinforcing component in sectors such as structural materials, printed electronics, paper production, and food packaging.

This review aims to provide a comprehensive evaluation of cellulose nanocrystals (CNCs) production processes, fundamental properties, and characterization methods. The main findings of the study can be summarized as follows:

Raw lignocellulosic fibers are not suitable for direct use as reinforcing materials due to the significant presence of hemicellulose, lignin, and wax-like compounds. Therefore, to obtain high yields, the biomass must first undergo chemical pretreatment. The conditions and effectiveness of this

purification process play a decisive role in determining the physical and chemical properties of the final product.

The production of cellulose nanocrystals (CNCs) through mineral acid hydrolysis involves drawbacks such as low thermal stability, equipment corrosion risks, high water consumption, and environmental sustainability concerns. To minimize these disadvantages, researchers are turning towards hybrid acid hydrolysis methods that include organic acid hydrolysis and controlled use of mineral acids. Literature reports that CNCs produced using organic acid hydrolysis possess high thermal stability, superior colloidal stability, structures that allow acid recovery, and systems that reduce equipment corrosion. Furthermore, enzymatic hydrolysis, considered an environmentally friendly alternative, is also under investigation. However, due to long processing times, low efficiency, and high production costs, its applicability at an industrial scale remains limited. Therefore, enzymatic hydrolysis is often combined with mechanical or chemical pretreatment processes.

Today, the increasing demand for environmentally friendly and sustainable materials is encouraging the development and detailed investigation of nanocellulose-based materials and their commercial applications. To enhance the functionality of these materials and better evaluate their industrial potential, their surface morphology, chemical composition, and physicochemical properties should be comprehensively examined. In this regard, this study addresses the characterization techniques that play a critical role in determining the structural properties of CNCs, aiming to provide a fundamental resource for researchers working in this field.

The integration of bio-based materials into sustainable applications remains at a limited level. A comprehensive and innovative approach should be adopted to develop environmentally sensitive and ecosystem-compatible nanocellulose-based materials. This review article aims to provide a comprehensive evaluation of cellulose nanocrystals' production and characterization techniques, establishing a solid informational foundation for sustainable growth in interdisciplinary applications based on material properties. Future research should explore industrial scale-up challenges of CNC production, including economic feasibility, regulatory frameworks, and long-term material performance in real-world conditions. In addition, application-specific modifications of CNCs, such as surface functionalization for biocomposites, should be further developed for integration into commercial sustainable materials platforms.

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6 Author contribution statements

Within the scope of this review article, the author contributed to the development of the idea, the design and structure of the manuscript, the literature review, and the writing and content revision.

7 Ethics committee approval and conflict of interest statement

There is no need to obtain permission from the ethics committee for the article prepared.

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