BALL MILLING ISOLATION AND CHARACTERIZATION OF NANOCRYSTALLINE CELLULOSE FROM MICROCRYSTALLINE CELLULOSE DERIVED FROM OIL PALM EMPTY FRUIT BUNCHES

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ABSTRACT

This study investigates the production and characterization of nanocrystalline cellulose (NCC) from microcrystalline cellulose (MCC) derived from oil palm empty fruit bunches (OPEFB) using high-energy ball milling. The objective was to explore the feasibility of utilizing NCC from OPEFB for dental applications. The isolation process was conducted at room temperature with a ball milling speed of 1500 rpm and a specific ball-to-powder weight ratio, employing stainless-steel balls of 7.0 mm, 5.0 mm, and 2.0 mm. Characterization techniques included Field Emission Scanning Electron Microscopy (FESEM), Particle Size Distribution (PSD), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD). FESEM analysis confirmed size reduction of cellulose particles. PSD results showed that MCC had an average particle size of 913.7 nm with uniform distribution, while dry NCC had a bimodal distribution averaging 123.1 nm. Wet NCC, in contrast, exhibited a narrower and more uniform size distribution, averaging 793.8 nm. FTIR spectroscopy indicated that the chemical structure of cellulose remained unaltered post-milling, and XRD analysis confirmed increased crystallinity, particularly for wet-milled NCC. The findings suggest that wet ball milling is more effective in producing high-quality NCC with consistent particle size and enhanced crystallinity, supporting its potential application in dental materials. This research highlights the sustainable use of agricultural byproducts for creating value-added products suitable for industrial and dental applications.

Keywords: Nanocrystalline cellulose, Microcrystalline cellulose, Oil palm empty fruit bunches, High-energy ball milling, Agricultural byproducts.

ABSTRAK

Kajian ini menyiasat penghasilan dan pencirian selulosa nanohabluran (NCC) daripada selulosa mikrohabluran (MCC) yang diperoleh daripada tandan kosong kelapa sawit (OPEFB) menggunakan pengisaran bebola tenaga tinggi. Objektifnya adalah untuk meneroka kemungkinan menggunakan NCC daripada OPEFB untuk aplikasi pergigian. Proses pengisaran dijalankan pada suhu bilik dengan kelajuan kisaran bebola 1500 rpm pada nisbah berat bebola kepada serbuk yang tetap, menggunakan bebola keluli tahan karat dengan diameter 7.0 mm, 5.0 mm, dan 2.0 mm. Teknik pencirian termasuk Mikroskopi Elektron Pengimbasan Pancaran Medan (FESEM), Taburan Saiz Zarah (PSD), Spektroskopi Inframerah Transformasi Fourier (FTIR), dan Belauan X-ray (XRD). Analisis FESEM mengesahkan pengurangan saiz zarah selulosa. Keputusan PSD menunjukkan bahawa MCC mempunyai saiz zarah purata 913.7 nm dengan taburan seragam, manakala NCC kering mempunyai taburan bimodal purata 123.1 nm. NCC basah, sebaliknya, mempamerkan taburan saiz yang lebih kecil dan lebih seragam, dengan purata 793.8 nm. Spektroskopi FTIR menunjukkan bahawa struktur kimia selulosa kekal tidak berubah selepas pengisaran, dan analisis XRD mengesahkan peningkatan kehabluran, terutamanya untuk NCC kisaran basah. Penemuan menunjukkan bahawa pengisaran bebola basah adalah lebih berkesan dalam menghasilkan NCC berkualiti tinggi dengan saiz zarah yang konsisten dan kehabluran yang ditingkatkan, menyokong potensi penggunaannya dalam bahan pergigian. Penyelidikan ini menyerlahkan penggunaan mampan sisa pertanian untuk mencipta produk nilai tambah yang sesuai untuk aplikasi industri dan pergigian.

Kata kunci: Selulosa nanohabluran, Selulosa mikrohabluran, Tandan kosong kelapa sawit, Pengisaran bebola tenaga tinggi, Sisa pertanian.

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LIST OF SYMBOLS AND ABBREVIATIONS

CI	:	Crystallinity Index
EFB	:	Empty fruit bunches
FESEM	:	Field-Emission Scanning Electron Microscopy
FTIR	:	Fourier Transform Infrared Spectroscopy
MCC	:	Microcrystalline Cellulose
NCC	:	Nanocrystalline cellulose
OPEFB	:	Oil palm empty fruit bunches
PSD	:	Particle Size Distribution
XRD	:	X-ray Diffraction
O-H	:	Hydroxyl group
C=O	:	Carbonyl group
nm	:	Nanometer unit
Å	:	Angstrom

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

Nanotechnology has affected all aspects of life, and among all the fields that have been impacted, dentistry and healthcare are on the top list. Nanotechnology has the capability of developing materials that have certain properties that can be basically planned for. Nanocrystalline cellulose (NCC) can be considered as a highly prospective material due to its biocompatibility (Trache et al., 2017), renewable origin of the source raw material (Habibi et al., 2010), and high potential for applications (Lam et al., 2012). Thus, when considering materials that are more beneficial for health in a field where the concept of sustainable development is gradually becoming one of the main goals, NCC can be viewed as a promising opportunity, not only as a material that can be used in the processes of regeneration but also in a much wider range of applications, thus becoming a connection between medicine and materials as inspiring as the opportunity to develop a material that can control the delivery of medication.

Furthermore, based on the further analysis of the cellulose derivatives, it is possible to conclude that microcrystalline cellulose (MCC) is an essential component. MCC is mainly derived from raw materials like oil palm empty fruit bunches (OPEFB) and other biomass materials, thus making it a potential feedstock for NCC production. The use of empty fruit bunches (EFBs), which are also obtained from palm oil production, indicates that there is a move towards the use of environmentally friendly methods and affordable means of obtaining cellulose. Sustainability and cost-effectiveness are key considerations for researchers who are seeking to harness the full potential of cellulose and initiate the process of making sustainable materials.

Overall, the combination of the use of nanotechnology with sustainable manufacturing highlights the advancement of material science, especially in relation to NCC and its predecessor, MCC. Dentistry and other professions will be able to experience new innovations in biocompatibility, renewable resources, and cost effectiveness as long as the newest technology can be used in a manner that is friendly to the environment.

1.1 Background and Rationale

Cellulose, which is a biopolymer that is widely distributed in the rigid components of plant cell walls, has gained much attention in the past few years due to its numerous applications in various fields that range from biomedical applications to the production of environmentally friendly packaging materials (Yang et al., 2012). One such type of cellulose that has attracted the attention of both researchers and industries is its nanocrystalline form called NCC which has some unique properties such as high surface area (Brinkmann et al., 2016), biodegradability (Trache et al., 2017), and increased material properties. NCC can conveniently come into contact with other molecules and particles at the nanoscale, which make it suitable for drug carriers and composite reinforcement due to them having high surface area characteristics. NCC is also biodegradable, which is very ideal since it will decompose and not pollute the environment, especially with the increased use of the material in many applications (Julkapli & Bagheri, 2017; Lam et al., 2012). At the same time, the ability of NCC to enhance the strength of the final product enables the creation of strong (Leung et al., 2013) and durable goods for many industries (Coccia et al., 2014; Yan et al., 2019) . Thus, NCC is a universal and important material for numerous applications and will continue to stimulate fundamental research and practical developments in various areas.

The availability of cellulose has lately proved to have a very versatile application ranging from the advanced research in the biomedical sector to the fairly new sector of environmentally friendly packaging materials (Lam et al., 2012). Of interest are the NCC that have a large surface area, biodegradability, and capability of improving mechanical properties of the materials (Medeiros et al., 2014; Shanmugarajah et al., 2015). These incredible characteristics not only provide a solid foundation for the preferred properties of NCC but also make it a suitable material for use in various industries, especially the dental material innovation sector.

Among the potential sources for NCC, OPEFB stands out due to their abundance as an agricultural byproduct. With the palm oil industry producing very large quantities of OPEFB, utilizing this material not only addresses byproducts management challenges but also provides a sustainable resource for extracting valuable nanocellulose. OPEFB is rich in cellulose, making it an ideal candidate for producing NCC through efficient and environmentally friendly methods, such as ball milling. This approach not only promotes the sustainable use of agricultural byproducts but also contributes to the circular economy.

When it comes to dental applications such as fillings and crowns, materials are of great significance because they form the basis of the dependability and effectiveness of numerous restorative and prosthetic treatments. Although these conventional materials have been seen to work efficiently, the following shortcomings have been noticed which are the compatibility issues, non-satisfactory mechanical properties, and environmental impacts. This is where the unique appeal of NCC becomes apparent in offering potential improvements in biocompatibility, mechanical strength, and sustainability. NCC, derived from natural sources such as OPEFBs, can be incorporated into materials like polymethyl methacrylate (PMMA), a widely used material in denture bases and prosthodontics, to enhance their properties for dental applications. Due to its high biocompatibility and satisfactory mechanical properties, it can be considered a potential material for the dental applications that require the substitution of conventional materials. Therefore, taking into account the opportunities of NCC, dentists can increase the efficiency and longevity of dental interventions and offer patients effective and safe treatments following modern approaches to oral health care. Thus, it is possible to consider the identification of potential uses of NCC in the dental field as a significant contribution to the development of new practices and their optimization in terms of patients' comfort and the preservation of the environment.

The main problem that arises while trying to differentiate between NCC and MCC is that the separation entails the breakdown of cellulose's rigid crystalline network. This procedure requires one to disrupt the crystalline structures of cellulose while at the same time maintaining its properties. The ball milling method has been reported to be a suitable technique that has attracted the attention of researchers because of its effectiveness in isolating NCC (Naghdi et al., 2017; Zhang et al., 2015). This is due to the fact that mechanical forces applied in a ball mill will enable the researchers to control the size of MCC at the nanoscale level with respect to the dimensions of cellulose crystallites. This method not only results in the desired nanoscale size but also provides good control over the different parameters involved in the process (Zhang et al., 2015). This means that the researchers can manipulate the production of NCC in a way that will suit the intended use in different fields.

The major implication of this novel work can be attributed to its potential to provide a means of achieving the production of NCC suitable for dental use in a sustainable manner. This research aims at providing a more practical and efficient approach to the production of NCC suitable for dental applications by employing the widely available and renewable resource of OPEFBs and ball milling methods. Thus, the priceless information obtained by conducting detailed characterization and thorough evaluation at every stage of the research can make a significant contribution to the development of new dental biomaterials with high performance and environmentally friendly properties.

1.2 Problem Statement

The increasing demand for sustainable and biocompatible materials in various applications, particularly in the biomedical and dental fields, highlights the need for effective methods to produce high-quality NCC from renewable resources. MCC derived from OPEFB presents a potential source for NCC; however, the efficiency of the extraction process and the properties of the resultant NCC are critical factors that influence its applicability.

Despite the known benefits of NCC, there is limited research on the effectiveness of ball milling as a method for isolating NCC from MCC sourced from OPEFB. Furthermore, understanding the morphological, structural, and chemical properties of the isolated NCC is essential to evaluate its potential for various applications, especially in dentistry where material performance is crucial.

1.3 Objectives

1.3.1 Aims

The aim of this research is to explore the feasibility and effectiveness of using ball milling as a method for isolating NCC from MCC derived from OPEFBs and to characterize the resulting NCC for potential applications.

1.3.2 General Objective

The general objective of this research is to investigate the effectiveness of ball milling in isolating NCC from MCC obtained from OPEFB

1.3.3 Specific Objectives

i. To isolate NCC from MCC derived from OPEFBs using ball milling.
ii. To assess the morphological properties of the isolated NCC from OPEFBs
iii. To assess the structural properties of the isolated NCC from OPEFBs
iv. To analyze the chemical composition of the isolated NCC from OPEFBs

1.4 Scope and Limitations

The aim of this research is to carefully isolate and fully characterize the NCC derived from MCC derived from OPEFBs for its possible use in dentistry. The key technology employed in this project is a novel ball milling process for the effective extraction of NCC and subsequent characterization of the produced material for its application in dental composite materials. Hence, through a detailed analysis of the structural, morphological, and possible functional aspects of NCC, the study aims to provide valuable information on the possibility of using cellulose-based biomaterials in dental applications.

However, this study has some limitations and challenges regarding which the following considerations should be taken into account. First of all, the research focuses on the production of MCC from OPEFB only. The decision to use this agricultural byproduct is mainly based on its renewable nature and availability; however, this limits the applicability of the study results to other possible sources of cellulose. Also, the concentration on ball milling as the isolation technique may mean that other methods that could be more efficient or cheaper are not explored, and therefore, better methods may be missed out on.

Some difficulties are also observed in the characterization of NCC. Even though many improvements have been made in the analysis process, getting detailed information on NCC's characteristics involves the use of various spectroscopic, microscopic, and mechanical tests. In addition, the natural cellulose materials are known to be inherently heterogeneous due to factors such as geographical source and processing conditions used, which may result in variation in the data obtained and their interpretation.

However, one has to consider the following issues when trying to incorporate NCC into dental materials. Regardless of these encouraging characteristics, NCC has the potential of be used in dental formulations, and strict evaluation procedures have to be followed to confirm its effectiveness and safety. Other considerations include the ability of NCC-based products to harmonize with other dental materials, as well as the stability that such products can endure under oral cavities and biodegradability.

Despite these acknowledged limitations and challenges, the ultimate purpose of this research remains unchanged: to give valuable information on the potential and performance of NCC derived from OPEFB for possible use in dental applications. Thus, by outlining the scope of the study and discussing its limitations, this preliminary introduction establishes a basis for a detailed examination of the research questions and the subsequent results, thus offering a theoretical background for the further analysis of the importance and consequences of the proposed study.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction to Nanocrystalline Cellulose and its Importance in Dental Applications

NCC is quite special considering that it is a nanomaterial derived from cellulose which is a biopolymer that is readily available in natural sources such as plant materials such as pine wood and corncob (Ditzel et al., 2017), cotton linters , cattail, and red algae (Incani et al., 2013), kraft pulp (Aulitto et al., 2023; Xu et al., 2013), biomass and waste materials (Mishra et al., 2019). The exceptional characteristics of NCC, which encompass extraordinary strength (Lee et al., 2016; Saito et al., 2013), rigidity (Lee et al., 2016), biocompatibility (Trache et al., 2017), and eco-friendly renewability (Habibi et al., 2010), render it extremely sought-after across a wide array of fields. Notably, NCC has captured significant interest within the field of dentistry due to these exceptional properties. The inherent strength and stiffness of NCC make it an ideal candidate for applications that require durability and resilience. Moreover, its biocompatibility ensures that it can be used safely in contact with living tissues without causing adverse effects. Being sourced from a renewable and sustainable resource, NCC aligns perfectly with the growing demand for environmentally friendly materials (Zhao, 2013) in modern industries and offers a promising solution to current challenges in material science and technology.

NCC stands out for its structure consisting of tiny crystalline particles made from cellulose. These nanoparticles, typically ranging from a few to several hundred nanometers in size, are meticulously derived by breaking down cellulose fibers until they reach the nanoscale. The resultant NCC exhibits striking characteristics, including a high aspect ratio (Shrestha et al., 2018), an expansive surface area (Brinkmann et al., 2016), and a distinctive crystalline arrangement (Vasconcelos et al., 2017). These features collectively underpin its outstanding mechanical and optical properties, setting it apart from conventional materials. By virtue of its unique composition and properties, NCC finds versatile applications across various industries, from enhancing the strength of composites (Lee et al., 2016) to improving the transparency of films and coatings (Yang et al., 2012). The exceptional potential of NCC lies in its ability to revolutionize material science and open new avenues for sustainable and high-performance products.

The unique characteristics of NCC, including its exceptional biocompatibility, minimal toxicity levels, and remarkable capacity to establish robust interfacial connections, significantly contribute to its appeal as a promising material for various dental applications. Moreover, the versatility of NCC enables effortless functionalization and customization to fine-tune its attributes according to specific dental requirements, thereby broadening its scope for potential applications within the dental industry. This adaptability allows for a diverse range of tailored properties that cater to a multitude of dental needs, demonstrating NCC's versatility and potential as a versatile material in dental settings. By capitalizing on its inherent features and the ease with which it can be modified, NCC has paved the way for innovative solutions in dental care, offering a wide array of possibilities that can enhance treatments and procedures within the dental field. Ultimately, the multifaceted nature of NCC makes it a standout candidate that continues to push the boundaries of what is achievable in the realm of dental materials, underscoring its significance and promising outlook for the future of dental applications.

NCC is becoming more and more well-known in the dental field due to its remarkable qualities, which is capturing interest in a wide range of possible uses. Dental composites are an important discipline where NCC exhibits significant promise. By acting as a reinforcing filler, NCC plays an important role in improving dental materials such as glass ionomer cement (GIC) (Silva et al., 2016) utilized for restorative dental procedures and alginate (Huq et al., 2012). The integration of NCC into dental composites yields a multitude of benefits, primarily augmenting the mechanical attributes of the materials. Enhanced strength (Khan et al., 2012), sturdiness (Henriksson et al., 2008), and resistance to wear (Lin et al., 2018) are just a few advantages that arise from incorporating NCC, which ultimately will lead to the creation of durable restorations that exhibit superior performance. Furthermore, the use of NCC in dental composites opens up the possibility of developing innovative materials that can withstand the demanding conditions within the oral cavity while maintaining biocompatibility. As research in this area progresses, the potential for NCC to revolutionize the field of dentistry by improving the longevity and efficacy of dental restorations becomes increasingly apparent.

Furthermore, NCC-based materials have demonstrated promising versatility in various dental applications. For instance, they have proven effective in the development of advanced adhesive systems (Melo et al., 2013) for dental restorations, aiding in the longevity and durability of dental work. Additionally, NCC has been utilized as barrier membranes for guided tissue regeneration procedures (Peng et al., 2021), enhancing the success rates of regenerative treatments in periodontal therapy. Moreover, the adjustable properties and biocompatibility of NCC have paved the way for innovative drug delivery systems (Peng et al., 2021) within the field of dentistry, enabling targeted and sustained release of therapeutic agents for more efficient treatment outcomes. Furthermore, NCC-based materials serve as foundational components in tissue engineering scaffolds, fostering the growth and regeneration of oral tissues in reconstructive procedures. Through these varied applications, NCC addresses specific challenges in dental care, contributing significantly to the prevention and management of dental conditions such as caries, periodontal diseases, and the promotion of optimal oral tissue regeneration processes.

When comparing NCC to traditional materials commonly utilized in dentistry, it becomes evident that NCC stands out due to a multitude of advantages. Firstly, it is crucial to highlight that NCC is sourced from renewable materials, specifically cellulose-rich biomass like OPEFB, embodying a truly environmentally sustainable alternative. This unique origin not only underscores its eco-friendly nature but also signifies a step towards promoting sustainable practices within the field of dentistry. Additionally, NCC's exceptional biocompatibility and non-toxic properties not only make it well-suited for various clinical applications but also ensure its harmonious integration with oral tissues. By drastically minimizing the possibility of adverse reactions or inflammation compared to traditional materials, NCC serves as a promising avenue for achieving enhanced patient comfort and overall treatment efficacy. The inherent biocompatible nature of NCC not only broadens the scope of dental materials but also emphasizes the importance of innovation and sustainable practices within the dental industry.

Furthermore, due to its tiny size and expanded surface area, NCC presents remarkable opportunities for enhanced interactions with various dental materials, ultimately leading to notable improvements in mechanical characteristics and overall performance. This is particularly evident in its application within dental composites (Peres et al., 2019), where NCC effectively reinforces the material structure, significantly reducing the susceptibility to fracturing or abrasive wear. Beyond this, the utilization of NCC in material compositions could potentially introduce beneficial antimicrobial (Tavakolian et al., 2018) attributes, playing an important role in combating bacterial colonization and decay initiation within dental restorations. The unique properties of NCC not only contribute to the mechanical integrity of dental materials but also offer a promising pathway for developing advanced formulations that are highly resistant to microbial threats, thereby ensuring the longevity and effectiveness of dental treatments.

Therefore, the analysis of NCC in the dental field illustrates a high potential of assisting in the advancement of dental materials and treatments. NCC’s researchers and dentists are determined to generate new and innovative ideas through the identification of NCC’s core competencies and weaknesses to enhance patient satisfaction, oral care quality and sustainability of dental services. Thus, the transition to the use of NCC opens up possibilities for the creation of new solutions that aim at improving the patient’s outcome and shapes the dentistry as a profitable and eco-friendly sector. Thus, through the study of NCC, professionals are striving to introduce new technologies that may revolutionize oral health management and set new guidelines for dental materials and patient care. This move to embrace NCC in dentistry shows that the dental research and practice have shifted to a new level where new materials can be used to solve the current problems that patients present as they seek dental care while at the same time attaining the goals of dental health and sustainability respectively.

2.2 Use of Nanocrystalline Cellulose in Dentistry

The use of NCC has steadily gained interest within the domain of dentistry since this material may be suitable to a broad range of applications within the field (Bapat et al., 2019). This extraordinary material which bears attributes such as near purity (Liu et al., 2017), unbelievably large surface area (Grishkewich et al., 2017), harmonious biocompatibility (Tayeb et al., 2018), the mechanical strength that is second to none (Lee et al., 2016), and has become a favorite for improving and boosting the properties of sundry dental items and composites. It highlighted almost every aspect of using acrylic resin formulations (Du et al., 2018) right through to composite materials (Silva et al., 2016; Ventura-Cruz & Tecante, 2021), adhesives (Chaabouni & Boufi, 2017; Kaboorani et al., 2012) and coatings (Qing et al., 2016; Zhang et al., 2021) for its clients, NCC has proved to have come up with magnificent performance and longevity of dental solutions.

Presently, investigation is being made towards incorporating NCC into both acrylic monomers used in fabrication of dental prosthetic appliances and the composite material used for making the prostheses. This incorporation has provided exceptional improvements on such trends in mechanical property images of the developed composites such as tensile strength, flexural strength, and hardness that are crucial in ascertaining the sustainability and endurance of dental restorations. As such, it is hence more apparent that the field of dentistry is slowly embracing the possibilities of how NCC can help change the production of dental materials to develop improved and lasting dental products for everyone out there who needs them.

Also, NCC displays significant antibacterial properties (Fardioui et al., 2018), serving as a significant factor in frustrating the growth of various bacteria which are normally found in the mouth. This inherent property not only helps in reducing the risk of secondary caries, but also has a significant role in orofacial infections. It is also evident that NCC has a high level of antimicrobial activity (Sun et al., 2019), which is clearly reflected in its uses such as dental adhesives and coatings. In these contexts, the opportunities to fully harness NCC enhanced defense mechanisms against microbial infiltrations present the principal consideration since oral hygiene remains pivotal while preventing microbial colonization remains a vital necessity for maintaining oral health at the highest level. Therefore, besides fulfilling functional and mechanical properties, the incorporation of NCC in dental materials is an independent preventive defense mechanism against the microbial threat capable of damaging oral health integrity.

Evaluating the role of NCC in dental materials shows several benefits not only in terms of the properties of the material but also in terms of environmental impact (Kovacs et al., 2010). One more aspect that is important to mention is the possibility of using renewable materials like OPEFB for NCC, this aspect correlates with the existing trends in many industries for green production (Leung et al., 2013). This use of biobased materials can therefore be said to indicate favorable steps in the reduction of the carbon footprint as regards dental practices (Zhang et al., 2018).

Moreover, the biocompatibility of the materials generated from NCC-base also shows a significant aspect, which protects the life of the patients during dental treatments (Hosny et al., 2021). This generality of these materials to get along well with oral tissues puts them in good stead having low tendencies of the manifestation of these adverse reactions or irritation. Another measure of biocompatibility to consider is this biotechnical factor that is most important in its direct applications to intraoral tissues where the tissues are so sensitive and cannot withstand anything that elicits an adverse reaction. As a matter of fact, incorporating NCC appropriately contributes not only to boosting the technological factors of dental materials, but also to embracing a philosophy of responsible use of the resources while caring for the patients' welfare in the domain of dentistry.

While there are promising benefits of NCC in the field of dentistry, there are certain disadvantages that give rise to certain issues that need to be addressed in order fully to harness its potential in the field. However, there are different challenges that are associated with expanding the use of NCC in the field of dental applications and the first one is the problem of scaling for production and costs that are connected to it (Brinchi et al., 2013). One of the major challenges that have made it difficult for NCC to be included in routine dental care is the inability to produce the necessary amount for large-scale use at a reasonable cost (Islam et al., 2017). To counter this challenge, it is therefore necessary to direct more effort towards the identification and establishment of efficient and cheap extraction methods and processing technologies. By improving the organization and development of the cycle NCC, which increases the availability of this material in the industry, dentists and dental practitioners can use the properties of NCC to their advantage. Therefore, addressing the issues with the scalability and cost of NCC production not only contributes to making the compound more available but also helps to incorporate the material into dental operations and enhance the functionality of procedures that involve the use of composites; ultimately helping both patients and professionals.

However, there are some vital concerns to be looked into before NCC can be widely used for dental applications; these include the development of protocols for using NCC to reinforce dental materials (Huq et al., 2012) so that the properties of the resultant composite are optimized and compatible with existing dental materials and techniques, as well as more developmental studies needed to establish the longevity and biodegradability of NCC-reinforced dental materials. These evaluations are useful in defining the use of NCC in dental applications as well as the environmental consequences and safety factors linked with this application.

In addition, the behavior and interaction of NCC with the dental materials and in the oral environment can be better understood with more advanced knowledge to enhance the clarity of the performance and endurance of the material. This knowledge not only contributes to improving the quality of the NCC based dental products but can also contribute to development of proper solutions that will be able to fulfil the demand of the ever-evolving dental market. In addition, the development of specific procedures and recommendations for the production and use of NCC in dental materials will help to shorten the time required to material's experience the NCC acceptance and widely adopt it by the dentists. The purpose of the research is to create the bridges between the researchers, manufacturers, and dental practitioners to contribute to better integration of the NCC technologies into the dental practice hence, improving the patients' outcomes and general health of the oral cavities.

In conclusion, it is critical to conduct a thorough investigation of NCC-based dental materials in terms of their functionality, biocompatibility, and applicability for the further development of dental biomaterials. Therefore, based on more studies and investigation on the principles of standardization and interdisciplinary cooperation, it is possible for us to explore a progressive solution for the application of NCC in the current dental practice in terms of both sustainability and efficiency, so as to open the new chapter of development for the state-of-the-art dentistry.

As for future perspectives, more studies of dental materials are focusing on stabilization and enhancement of NCC on the basis surface treatment technologies and suitable functionalization. These approaches are directed towards controlling the properties of NCC to exhibit relevance in dental applications to the highest measure of accuracy. Additionally, the search for novel fabrication technologies, as well as the discovery of composite materials containing NCC and other types of nanofiller, appears to be another interesting line for enhancing the effectiveness and versatility of NCC-based dental materials. Through such research activities, the scientists and engineers are able to explore NCC in a more in-depth manner in an effort to expand the use of NCC in the field of dentistry to manufacture new generations of dental materials with even better attributes and performance in their functions. Due to the steady progression and synergistic work put into the studies of NCC for the present and future, these future approaches can create the new horizon of dental technology which will ultimately improve the additional quality in dental care.

Therefore, the application of NCC in the dental field has a huge prospect in enhancing the properties of many dental materials in terms of characteristics, durability and biocompatibility of dental materials in addition to dealing with the ecological concerns in the field of dentistry. This revolutionary technology is expected to bring a lot of changes and growth within the dental practice in the globe.

However, it can be stated that the range of opportunities is mostly significantly expanded and can be referred to as innovative with the help of NCC; however, it is possible to encounter certain problems concerning the development of NCC. These include factors such as: enhancing the production parameters in an effort to enhance the marketability of the processes, establishing the quality control measures that would enable the reproduction of the properties of materials that are reinforced with NCC, and evaluating the service life of dental products that have been reinforced with NCC. This is a critical step towards the improvement of NCC and the advancement of materials and technologies of the new generation in dentistry that will be useful in the growth of the field. Therefore, the above-mentioned challenges can be overcome and the dental community will be able to consider NCC as the long-awaited and long-deserved great chance for the material development in dentistry as the best chance to introduce new, better and more environmentally friendly materials into dental practice and into the treatment of patients.

2.3 Sources And Extraction Methods of Cellulose from Biomass, Specifically Oil Palm Empty Fruit Bunches

Consequently, OPEFB are today considered to be among the most promising sources of cellulose in the context of the sustainability industry due to the fact that they are available in large quantities, and, above all, they are renewable. Thus, the use of these biomass residues that could be obtained from palm oil production could be more viable than the conventional sources of cellulose like the wood pulp (Ali et al., 2020). This also reveals a good prospect and also a positive change orientation in the area of sustainability. Thus, based on the analysis of the process of cellulose extraction from OPEFB, it can be concluded that there are several ways to do so, and each of them has its advantages and disadvantages that should not be overlooked. Therefore, through the above mentioned strategies, it becomes apparent that both the scholars and the practitioners have the ability to maneuver through the many challenges that are aspects of extracting cellulose from these versatile palm wastes and at the same time create new possibilities in the sustainable material systems.

OPEFB is the parts of the oil palm fruit that are left after the oil has been extracted from the fruit and is therefore a type of agricultural residue that is abundant in the palm oil producing regions of the world. This is due to the fact that the bio-mass under consideration is known to contain a good amount of cellulose, hemicellulose and lignin (Aziz et al., 2002) which is used in the extraction of cellulose. Hence, applying OPEFB, the scientists and researchers contribute to the concept of waste valorization that makes the sustainable practices in the palm oil industry. This also goes a long way in reducing wastage and also suggests another way of reducing resource use and being environmentally conscious in the production of palm oil (Abu Bakar et al., 2011). Through the improvement of the application of OPEFB solutions, experts are coming up with solutions to the problems that affect byproducts management and, at the same time, promote the circular economy system in the agricultural sector, thus promoting sustainable agriculture and development in the agricultural sector (Rubinsin et al., 2020).

Malaysia, known as a well-endowed country in the agricultural sector, boasts a thriving oil palm industry that positions it as the second largest producer globally, following Indonesia. This industry contributes significantly to the world's palm oil production, with Malaysia alone accounting for more than 80% of the output (Anuar et al., 2019). The landscape of Malaysia is characterized by extensive stretches of oil palm plantations, totaling approximately 5.6 million hectares, with production of 4.77 tons per hectare across the country (*Malaysian Palm Oil Board (MPOB): Economic and Industry Development Division*). Consequently, Malaysia stands out as a preeminent exporter of various palm oil products worldwide (Saeyang & Nissapa, 2021), supported by its extensive plantation areas and a dense concentration of palm oil mills.

This is due to the large plantation areas and the increase in the number of palm oil mills which has made Malaysia the leading producer in the world. A substantial byproduct of the oil extraction process, large amounts of biomass production are derived from two key sources: on the large plantations and mills where they account for 90% of the total output (Kong et al., 2014). The palm oil industry is one of the most active industries because there is always the production of oil palm trunks and fronds from the plantations, and this will ensure that there is a continuous supply of biomass. At the same time, in the fresh fruit bunches' milling process, other biomass byproducts, including mesocarp fiber, kernel shell, and EFBs (Chiew et al., 2011), are generated as useful materials.

Specifically, Malaysia's annual OPEFB waste production is estimated to be at 22-23 million tons (Abdullah & Sulaiman, 2013). This high utilization of OPEFB as a non-wood fiber may be due to the low cost and favorable properties which makes it to be used in many industries. Therefore, OPEFB, which possess many positive characteristic features, and is readily available, can be deemed as a suitable feedstock for various industries, which can use it instead of expensive wood (Rafidah et al., 2017). Thus, Malaysia's agricultural growth and the increasing adoption of modern technologies make the application of OPEFB expected to contribute to the enhancement of sustainable industrial processes and the economy.

The management of OPEFB is usually through burning them in incinerators that are found in palm oil mills and the ash is used again in the plantation to manure the plants. However, the burning of OPEFB is not suitable nowadays due to the negative impact it has on the environment (Rahman et al., 2007). Therefore, due to current pressure on oil palm industry and the environment, it becomes crucial to search for the better methods of utilizing the lignocellulosic biomass waste. It is therefore necessary to discover other feasible means of utilizing OPEFB in a manner that is friendly to the environment and at the same time cost effective. Thus, along with the prevention of the issues connected with waste disposal, one can open new opportunities for the enhancement of waste utilization efficiency in the palm oil industry. Therefore, the strategic and comprehensive utilization of OPEFB is considered as valuable contribution in the enhancement of the environment management of the oil palm agricultural byproducts.

The two main process of isolating cellulose from OPEFB include acid hydrolysis (Azrina et al., 2017; Hastuti et al., 2018) and enzymatic hydrolysis (Gonzales et al., 2019). Acid hydrolysis can be defined as the process of using strong acids such as sulfuric acid (Zhang et al., 2012) or hydrochloric acid (Hastuti et al., 2018) to treat OPEFB in order to efficiently break down the lignocellulosic structure and get cellulose. This method is preferable due to its high cellulose output (Hastuti et al., 2018) and fast reaction rate (Rahman et al., 2007). Also, acid hydrolysis can be done under relatively low temperature (Sun et al., 2016), which can certainly minimize on energy used in the process (Pirani & Hashaikeh, 2013).

Nevertheless, the application of corrosive acids in acid hydrolysis (Yadav et al., 2017) has some environmental and safety issues (Santucci et al., 2018). It produces chemical waste that requires proper disposal hence, it raises the cost of the entire process (Lyu et al., 2023) as well as contributes to environmental pollution in the cellulose extraction from OPEFB. The consequences of using strong acids in the cellulose isolation process and the subsequent measures that should be taken to prevent the environmental impact are discussed. Also, there are possibilities of coming up with better and more efficient ways of extracting the mineral as well as the aspect of being friendly to the environment may also be considered in future. Thus, it can be concluded that although acid hydrolysis gives high cellulose values and fast reaction rates, the environmental impact of the extraction methods should not be overlooked in terms of both nature and human health.

Enzymatic hydrolysis is a biocatalytic process through which cellulase enzymes are employed to deteriorate cellulose to smaller (Chen et al., 2018) oligomers or monomers and has the following benefits. Further, one of the most important advantages is the mild conditions of the reaction combined with high selectivity and non-aggressive action towards the environment (Douka et al., 2018). In this case, enzymatic hydrolysis is carried out at ambient temperature and atmospheric pressure and thus there is potential of reducing the energy input and therefore reducing on the generation of hazardous waste (Saini et al., 2020). It should be noted that the cellulase enzymes applied in this process can be derived from renewable biological resources, thus adding to the biotransformation’s sustainability (Vasić et al., 2021). Nevertheless, the enzymatic hydrolysis is known to need longer time for the reactions (Wang et al., 2006) and slightly higher enzyme costs than the methods of acid hydrolysis (Arreola-Vargas et al., 2015). However, there are certain conditions that may influence the effectiveness of enzymatic hydrolysis including the requirement of substrate pretreatment (Mes-Hartree et al., 1988) and the requirement to maintain optimal enzyme activity levels throughout the process (Wu et al., 2020). However, it is important to pay attention to the fact that enzymatic hydrolysis, despite all the benefits and the use of environmentally friendly methods in the process, requires a certain consideration of factors and parameters when carried out in order to achieve the best results and consolidate its position as one of the most effective methods for cellulose degradation in the future of biotechnology.

On this basis, acid hydrolysis as well as enzymatic hydrolysis are regarded as viable methods for the production of cellulose from OPEFB. Among the reported methods that are said to give high cellulose yield and fast reaction rate is the acid hydrolysis. However, one has to look at the drawbacks of this system especially the environmental and safety aspect in relation to the use of acids and the issue of chemical waste disposal. However, it is impossible not to recall the results of the conducted studies, which confirmed the applicability of the acid hydrolysis method for the extraction of cellulose, as well as other benefits. But enzymatic hydrolysis has been found to be more eco-friendly since it does not require the use of strong acids and hence the environmental effects are reduced. This method is relatively slower than the acid hydrolysis but it is a better and safe way of getting cellulose. As a way of enhancing the efficiency while at the same time protecting the environment, more research is carried on and the comparison is made between the two methods used in extracting cellulose from OPEFB.

2.4 Ball Milling

Ball milling is currently a popular method of synthesizing NCC because it is very effective in breaking cellulose fibers down to the nanoscale (Piras et al., 2019). The process involves the application of high energetic ball milling to mechanically comminute cellulose material; this results into a reduction of the cellulose particle size by up to 75% (Avolio et al., 2012) and the production of NCC from MCC (Harini & Mohan, 2020). The process of ball milling which has the advantage of being able to process cellulose fibers in a systematic way has been recognized for its contribution in the production of NCC due to the fact that it offers a certain level of control over particle size that is essential in several industries (Hampsey et al., 2004). Hence, high-energy ball milling, which is a process that involves the use of advanced grinding equipment to apply force to cellulose material achieves a high level of control in the particle size reduction and at the same time makes it possible to control the creation of NCC thus enabling the generation of NCC with specific properties that are useful in a number of industries ranging from material sciences to biomedical engineering (Piras et al., 2019). This is because the properties of the NCC can be regulated through the controlled ball milling process which makes it suitable for the specific research and manufacturing needs that are of interest, thus increasing the application of this material in new technologies and green technologies.

Ball milling has been critical in many industries over the years as a versatile and effective means of accomplishing numerous operations (Piras et al., 2019) and NCC production among them. Ball milling process has been claimed to be useful in the production of NCC, and this is as early as the 1980s. During the early stage of its application in the area of NCC production, the main focus was on the control of the milling process with regard to the size of the crystalline particles and the crystallinity index (Avolio et al., 2012; Phanthong et al., 2016). This phase of experimentation and improvement can be considered as a stepping stone to the following works where the researchers enhanced the understanding on how the milling conditions influence the cellulose (Avolio et al., 2012). The advancement in the milling technology as well as the understanding of the characteristics of cellulose made it possible to improve the processes of NCC production systematically (Aulitto et al., 2023).

These improvements have not only widened the range of uses for NCC but have also highlighted ball milling's continued importance in materials research and industrial processes. Scientists and engineers have unlocked new possibilities for manipulating the properties of NCC and taking advantage of their unique traits for a wide range of novel applications across many industries by constantly pushing the boundaries of what is achievable.

2.4.1 Factor influencing ball milling

Several key factors play a significant role in shaping the efficiency and final results of the ball milling process in the production of NCC. Among these factors, ball size plays a significant role in shaping the milling process dynamics and the ultimate distribution of particle sizes (Zhang et al., 2015). This decision hinges on a delicate balance between various factors. For instance, when opting for smaller balls, one can expect to witness enhanced impact forces at play, leading to a more efficient breakage of particles. However, this efficiency might come at the cost of increased milling duration needed to attain the desired fineness of NCC. These potentially extended milling times may pose challenges in terms of process optimization and resource management. On the other hand, the utilization of larger balls presents a different set of considerations. While these larger balls facilitate a higher level of energy transfer per collision, thereby potentially accelerating the milling process, they also run the risk of yielding coarser NCC particles in the final product. This underscored trade-off necessitates a thorough evaluation of the specific milling requirements and objectives at hand, ensuring that the chosen ball size aligns seamlessly with the desired outcome and quality standards. In essence, the decision-making process concerning ball size in the context of milling operations involves a nuanced analysis of variables that intersect fluidly to shape the overall efficiency and outcome of the particle size reduction process.

Additionally, it is important to note that milling time emerges as an important parameter (Dai et al., 2018) that directly influences the procedural outcomes. This duration of mechanical treatment acts as a guiding force, determining the degree of particle size reduction and enhancements in crystallinity (Zhang et al., 2022). It represents a key point at which cellulose fibers convert into NCC particles. In essence, the longer the milling time, the more pronounced the refinement and size reduction of NCC particles tend to be. This progressive trend towards finer particles is driven by the prolonged mechanical forces applied during milling, which continuously break down the cellulose structure into its nano-sized constituents.

However, a precise balance must be found, since too extended milling durations can indicate potential hazards to the cellulose substance. Under excessive milling conditions, the risk of cellulose breakdown or undesired amorphization is significant (Avolio et al., 2012). The delicate line between producing excellent NCC properties and pushing the limits of degradation highlights the milling process's complexity. To successfully embrace this restricted line, one must be strict about time management and process control.

Therefore, when embarking on ball milling for NCC production, a thorough understanding of the interaction between milling time, particle size reduction, and crystallinity enhancement is important. This comprehension acts as a guiding compass, leading the process towards the desired NCC standards while avoiding the potential risk of over-milling. By carefully controlling the milling time and other relevant parameters, researchers and manufacturers can fully utilize the potential of ball milling to produce high-quality NCC with tailored properties for a wide range of applications in fields ranging from nanotechnology to sustainable materials.

The speed at which the milling process operates (Zhao & Shaw, 2017) is a critical factor that influences the overall efficiency and outcomes of ball milling. When the milling speed is increased, it enhances the level of mechanical forces and interactions occurring between the milling balls and cellulose particles. This intensified collision activity is essential for achieving a more refined reduction in particle size, which is instrumental in improving the overall effectiveness of the milling process. However, it is important to note that excessively high speeds can introduce challenges such as the generation of excess heat (Stolle et al., 2011). This excessive heat generation can be detrimental as it may initiate thermal degradation of the cellulose material, compromising the desired outcomes of the milling process. Furthermore, at extreme speeds, there is an increased risk of triggering unwanted reactions that can impact the quality and characteristics of the final milled product (Chen et al., 2010). Therefore, finding the optimal milling speed that balances efficiency, and the risk of thermal degradation is crucial to maximizing the benefits of ball milling for particle size reduction and ensuring the integrity of the final product. Ultimately, understanding the intricate relationship between milling speed and its impact on the milling process is key to achieving consistent and desirable results in the production of finely milled cellulose particles.

Another crucial aspect to consider in optimizing the milling process is the ball-to-cellulose ratio (Zhang et al., 2015), which represents the proportion of milling balls to the cellulose material within the milling jar. The significance of the ball-to-cellulose ratio lies in its role in facilitating the generation of adequate mechanical energy necessary for efficient particle size reduction. This optimal ratio not only ensures the successful application of mechanical forces to break down the cellulose particles but also plays an important role in preventing the milling jar from becoming overly congested with materials. Avoiding overcrowding in the milling jar is essential as it can interfere with the movement of the milling balls, hence restricting the milling process efficiency influencing the anticipated outcomes of particle size reduction. Therefore, maintaining the ideal ball-to-cellulose ratio is important in achieving the desired milling results effectively and consistently, optimizing the particle size reduction process in cellulose material milling operations. To keep the milling jars free of jams, it is important to carefully calibrate and monitor this aspect. A complete understanding and efficient control of the ball to cellulose ratio is necessary to maximize the productivity of the milling process and maintain the quality and consistency of the end products. Consequently, by carefully controlling and adjusting this ratio to meet operational requirements and to the material so that it is within the optimal parameters for the efficiency and effectiveness of the milling process, the performance and outcomes in applications such as processing cellulose material can be improved.

In addition to all that, the decision about dry or wet milling is also an important factor which determines the actual efficiency of the ball milling process for the preparation of NCC (Jung et al., 2015). Dry milling, as a process, concerns the milling of cellulose particles without the use of any liquid phase, which changes the flow patterns and all the effects taking place in the milling system. In this case, the term wet milling refers to the use of a liquid medium, including water or an organic solvent that enhance the milling process by reducing the particle sizes and discouraging formation of big lumps. Therefore, it important to highlight on the fact that every method of milling has its own benefits and disadvantages which must be taken into consideration in deciding the best method of milling in any given situation.

2.4.2 Dry and wet milling

Dry milling which does not involve use of a solvent has certain advantages which go along with wet milling. A great advantage is exclusion of the possible contamination of the obtained cellulose with solvents, as well as the ability to maintain the properties of the material by themselves. Meanwhile, wet milling has many benefits considering the possibility of regulation: with the use of liquid mediums, temperature regulation is possible and cellulose overheating is prevented. Furthermore, wet milling requires the use of water or other organic solvents since they assist in the process of dispersion of cellulose particles and enables a better control of particle size distribution compared to the dry processes (Kotake et al., 2011).

Moreover, the choice between dry and wet milling procedures impacts not only the process results but also factors such as energy demand (Williams et al., 2017), equipment, and overall effectiveness (Bu et al., 2020). Wet milling is often preferred because it can reduce energy consumption, thereby improving the productivity of the milling operation under favorable conditions (Hideno et al., 2009). Additionally, in the wet milling process, the medium is in liquid form, which helps minimize the frictional forces involved in milling (Kwon et al., 2014).

Consequently, the decision of whether to perform dry and/or wet milling methods influences considerably the properties of the final NCC product and points to the necessity of a critical appraisal of the benefits and drawbacks of each technique relevant to the overall process needs. Dry milling can be naturally preferred due to the absence of a solvent requirement and reduced degradation of the material, but wet milling has a clear advantage in its ability to provide better particle size distribution and improved efficiency. Therefore, the knowledge of each way of milling is crucial in order to attain high yield for the NCC production.

Hence, the various benefits and flexibility make the ball milling as a very viable approach in the fabrication of NCC from MCC from OPEFB. Thus, having given a general understanding of the ball milling process and its features but without delving much into the details of the ball milling technique, the need to get more detailed information and features about the ball milling technique, the history of the ball milling process and an analysis of the factors that may affect the efficiency of the ball milling process in the production of NCC becomes necessary as a new step towards finding better techniques, methods or improvements. This process of optimization also makes it possible to make further improvements on the manufacturing outcome and as such unveil other areas where NCC may be applicable in other industries but the field of dental manufacturing is the most appropriate one. Considering the situation in ball milling and analyzing the information in a way that a biology textbook would explain the structural and functional parts of a cell, one is able to grasp a certain fact that can be used as a lever to predict the future of NCC production. This paper presents the elaborated understanding of the relationships between various operating parameters and the environmental conditions that affect the efficiency of ball milling with the aim of enabling researchers and technologists to enhance the process and achieve reliable and consistent NCC yield and purity.

Also, the use of NCC for the enhancement of other fields is associated with numerous advantages and applications that may significantly improve the properties of dental materials and indicate new possibilities and approaches for the treatments. Hence it is important to acknowledge the comprehensive analyses and discussion of ball milling besides the historical background that is associated with it and the factors that determine the performance of NCC to ensure that the potential of this innovation is fully harnessed and its application is encouraged all through the industries, particularly in the dental industry where NCC's characteristics may be the key to new and improved solutions.

2.5 Characterization Techniques for Nanocrystalline Cellulose

Characterization techniques are indeed crucial when researching such a complex field as NCC and more specifically its incorporation into dental materials, where the knowledge of its characteristics and behaviors is critical. Numerous techniques are cautiously utilized for the comprehensive evaluation of the fine details regarding the structure, morphology, and chemical nature of NCC derived from the MCC extracted from OPEFB. This thorough analysis involves several typical characterization approaches, which provide a broad spectrum of perspectives on NCC. Such systematic approaches include but not restricted to, the Field-Emission Scanning Electron

Microscopy (FESEM) method, through which we have been able to capture images at microscale and the Particle Size Distribution (PSD) analysis which has helped in understanding the size distribution characteristics of NCC particles. Additionally, the essential X-ray Diffraction (XRD) technique reveals significant details about the crystal structure of NCC and its internal organization. Finally, the very comprehensive Fourier Transform Infrared Spectroscopy (FTIR) analysis provides a strong basis for understanding the chemical makeup of NCC and its further applications due to the complexity of its molecular structure. This article provides a detailed analysis of the dynamics and importance of these commonly applied characterization techniques to reveal the complexity of NCC and its potential for contributing to the field of dental materials (Valenti et al., 2024).

2.5.1 Field-Emission Scanning Electron Microscopy

FESEM was found to be another imaging technique that can be characterized as being complementary to other techniques in offering an overall picture of surface features of NCC. Since FESEM is a technique that may yield high image resolution (Zheng et al., 2017), the researchers benefit from the improved capabilities by being offered a view into the nanoscale world of cellulose crystals. Besides, use in observation, it also allows examination of such modes of the specimen as the dimensions, shapes and spatial distribution of cellulose crystals (Law et al., 2015), which cannot be easily investigated in any other way. Overall, FESEM does prove useful in providing successful differentiation of the sample in determining the impact of ball milling treatments on cellulose particles. They illustrate a map of how various forces that is being applied on the practice of milling influences the mechanical properties of the NCC products (Piras et al., 2019).

This can particularly be observed on how FESEM was able to establish that the NCC samples are homogenic and coherent even after several ball milling processes (Major et al., 2019). These FESEM produces highly resolved images enabling the scientists to start the analysis on a very detailed level to prove the homogeneity and, therefore, to reproduce the results of the experiments. Furthermore, in FESEM, among them are particle size and morphology and, to some extent in this regard, knowledgeable researchers may possibly make other decisions on how they would have it if they could having observed that the parameter in milling changed the property of the final NCC material. Researchers can also observe the efficiency and yields of the NCC isolation process established through FESEM by assessing the specimens and determining the qualities of the NCC products, which are either distinct or developed to possess certain characteristics to fit specific applications and properties.

Therefore, it can be stated that FESEM is the most efficient and rather effective tool in studying cellulose, particularly in the sphere of nanotechnology, which helps to investigate the nature of NCC and how it works. The potential to observe cellulose at the nanoscale, a material as complex as society that connects society, allows the scientific community to control decisions of fine mechanics that determine the potential for the development of science and technology of materials and biology. The knowledge can be further enhanced and improved through specific applications and through specifically designed NCC components where various structures and functions can be further understood and explored more deeply and at a more specific level as what the FESEM images above have demonstrated in the scientific research area of NCC.

2.5.2 Particle Size Distribution analysis

PSD analysis, a highly effective technique in nanotechnology applications, helps in characterizing the size distribution of NCC particles in the suspension solution (Runyan et al., 2020). With the use of techniques such as laser diffraction or dynamic light scattering, PSD analysis gives accurate information on the diameter of the cellulose crystals in addition to quantifying volumetric or numerical distribution of the particle within the suspension. This analytical approach proves very useful in determining the uniformity as well as the dispersion of NCC particles, which are two key factors that have a direct bearing on the mechanical strength and the flow characteristics of composites based on this material (Ma et al., 2021).

In the field of material science focusing on NCC material, it is crucial to identify the particle size distribution while creating new dental materials and products with tailored characteristics. By analyzing the relationship between particle size distribution and properties of dental materials, many researchers may design the dental composites, adhesives, or membranes for different clinical applications with high performance.

In addition, the data derived from PSD analysis is used as a starting point for developing NCC based materials with optimal structural strength and fine-tuning the rheological properties of materials. This knowledge is especially beneficial for researchers and engineers who are interested in using the properties of NCC for various applications such as regenerative medicine or biomedical implants.

In conclusion, the broad assessment of PSD and various PSD techniques is not only useful in imagination of the next generation of engineering materials, but also provides the possibilities for the development of new biomedical models to transform the current and future practice of healthcare. Thus, by exploring NCC particle distributions, the scientists can open opportunities for new advancements in dentistry and provide patients with better results and higher quality of dental care.

2.5.3 X-ray Diffraction analysis

In fact, XRD can be seen as an objectively fast and completely reproducible technique in research of multi-component powder crystals including NCC and precise identification of all the phases within this material with the help of high resolution (Garg & Rao, 2018). Similar to other fiber, diffraction patterns are obtained when samples of NCC expose to X-ray radiation, and this in return allow the scientists and researchers another chance to observe the patterns that are came about by the certain way of cellulose chains arrangement within the crystalline lattice (Ju et al., 2015). Though, the details obtained from such XRD analysis are not only sufficient to determine the quantitative extents of NCC such as crystallinity point, size of the crystal and its preferred orientation, but also adequate enough to give profound understanding about few of the fundamental principles determining mechanical strength, thermal stability and biocompatibility of NCC, which in fact makes XRD as one of the most essential and routine tool in the field of material science and nanotechnology.

In addition, XRD had the capability of not only identifying the format structure of NCC when the research was initiated in this study but it was also useful for the observation of change as influenced by ball milling process (Gayathri & Mukherjee, 2018). From the XRD analysis before and after the milling operation, researchers are able to identify various changes happening in the NCC which will then lead to further work being done to estimate the optimal conditions to be used for milling in an effort to enhance the crystallinity and purity of the material (Pourghahramani et al., 2008). This optimization is crucial in enriching two of the features of the NCC and that is the functionality and usability of the NCC in industries such as biomedical engineering and industries in composite material while at the same time creating an engineered material with the required properties. In other words, it is not sufficient, in this case, to read the mystery of the crystalline world of NCC and solve it simply through the comprehensive application of XRD analysis, but it also offers researchers the methods and knowledge they need to control and modify NCC for optimizing its performance and functions in different fields.

2.5.4 Fourier Transform Infrared Spectroscopy

FTIR spectroscopy, as one of the critical analytical methods, is widely employed for investigating the chemical characteristics and the complex arrangements of functional groups in NCC samples (Yuan et al., 2015). This non-destructive method takes advantage of the heating effect from infrared radiation to cause the cellulose molecules to vibrate in a specific pattern and produce different spectra that are incredibly informative about the basic chemical structure of materials (Berthomieu & Hienerwadel, 2009). Thus, by analyzing these FTIR spectra, scientists can determine whether cellulose, hemicellulose, lignin, or other contaminants are present in the NCC matrix and where they are located (El-Hendawy, 2006). This analysis also extends the objective of assessing the purity and elemental contents of the isolated NCC samples and affords a precise identification of crucial functional groups (Deygen & Kudryashova, 2016). For instance, hydroxyl and carbonyl functional groups are shown to have a more significant impact on the interaction between NCC and other materials in dental composites or adhesives applications. By using the FTIR analysis, researchers can have a further improved understanding on these intermolecular interactions, which represent whole picture of molecular factors relating to enhancing the performance and compatibility of NCC-based materials in a wide range of applications. Indeed, the systematic study based on the FTIR spectroscopy not only aids in understanding the chemical structure of NCC but also creates the solid knowledge base for the further development of NCC as a new material and its targeted application in various sectors (Dassanayake et al., 2023).

Hence, gaining a clear understanding of the characteristics of NCC is highly significant in order to fully harness its properties and improve its efficiency in different dental applications. The knowledge of surface morphology, size distribution, the crystalline structure, and chemical composition of NCC extracted from OPEFB by ball milling can be obtained from these techniques like FESEM, PSD analysis, XRD, and FTIR spectroscopy. Thus, by integrating these complex and advanced analytical techniques in the process of characterization, the scientists and researcher will be able to optimize the extraction methodology, tailor the properties of NCC-based dental materials, and promote the development of new and innovative oral health solutions. By carefully studying and improving these methods, the likelihood of creating new dental materials that possess superior properties and applicability is greatly increased. Such profound insights into NCC properties act as a basis for creating the new generations of dental materials and devices dedicated to therapies and oral care with enhanced performance, biocompatibility, and efficiency in dental practice. By incorporating the multiple benefits from all these characterization methods, the dental industry can move forward in to a future whereby biomaterials are integrated as part of patient care to address different dental needs and providing sustainable solutions.

CHAPTER 3: METHODOLOGY

3.1 Materials

MCC was supplied by Malaysian Palm Oil Board (MPOB), Kajang, Malaysia. Ethanol 95% and ethanol 99.8% were purchased from R&M Chemical (Semenyih, Selangor, Malaysia). Polyethylene glycol 4000 (PEG 4000, Merck Co.,Germany) was purchased from Sigma Aldrich (Subang Jaya, Selangor, Malaysia)

3.2 Preparation of Microcrystalline Cellulose from Oil Palm Empty Fruit Bunches

The MCC was prepared and supplied by the MPOB using the methodology described by Ismail et al. (2022). In brief, the waste OPEFB cellulose was hydrolysed using sulphuric acid. The mixture was subjected to autoclaving for one hour and ultrasonication at 50°C for three hours. After ultrasonication, the suspension was repeatedly washed with distilled water until it reached a near-neutral pH, indicating it was acid-free. The MCC was then separated by filtration, dried in an oven until it reached a constant weight, and ground into a fine powder. This resulted in the production of MCC.

3.3 Ball Milling Isolation Process

The MCC powder derived from OPEFB was further processed to synthesize NCC through the efficient Retsch Emax high-energy ball mill (Retsch, Haan, Germany) as shown in Figure 3-1. This enhanced process entailed the dry and wet milling of the MCC powder in two different 50 ml vials at room temperature.

Figure 3.1: Retsch Emax high-energy ball mill machine

High-quality hardened steel balls with a diameter of 7.0mm was used as starting media. Each vial was loaded with 34.6842 g of these stainless steel balls that were weighted using an analytical balance (Newclassic MS204S, Mettler-Toledo) as in Figure 3-2 and 3.4684g of MCC powder, to establish a ball-to-powder mass ratio (BPR) of 10:1 then placed in vial as in Figure 3-3.

Figure 3.2: Weighting stainless steel ball

Figure 3.3: MCC and stainless-steel ball in vial before milling process

It is necessary to note the details of the wet milling step that contributed to the total optimization of the process. To regulate the process and ensure that MCC powder was well dispersed, PEG4000, a vital process control agent, was prepared in a ratio of 5% of the MCC powder's weight to provide a suitable mixture. This unique mixture was diluted in 95% ethanol in a carefully structured MCC powder-to-liquid ratio of 1:2 to create a harmonious solution that was later introduced to the MCC powder, which greatly enhanced the milling efficiency in the second vial.

The final stage of this controlled experiment was to mill the MCC for a total of 2 hours at a constant speed of 1500 rpm. This detailed process assured that the particle size of the MCC powder was reduced adequately and that the powder was well mixed, producing a high-quality product with consistent and uniform characteristics that helped in the growth of material processing and nanostructuring methods.

The process of wet milling was followed by the recovery of the powders from the samples using a rotary evaporator (IKA RV-10 control, VWR, Madrid, Spain), as shown in Figure 3-4. The evaporator worked at a temperature of 40 ºC, a rotational speed of 35 rpm, and for a time of 60 minutes. The pressure of the evaporator's environment was maintained at 175 mbar, and the pressure within the evaporator was below atmospheric pressure. It was important to incorporate a rotary evaporator in the powder recovery stage to enhance the efficient separation of the required components after wet milling. These parameters, including the temperature and the speed, contributed to the evaporation and recovery processes of the liquid.

Figure 3.4: Rotary evaporator

The experimental procedure extended the investigation of the effects of different grinding media sizes on the final product size of MCC. The following milling sessions were conducted with a lot of precision using hardened steel balls with a diameter of 5.0 mm and 2.0mm as in Figure 3-5.

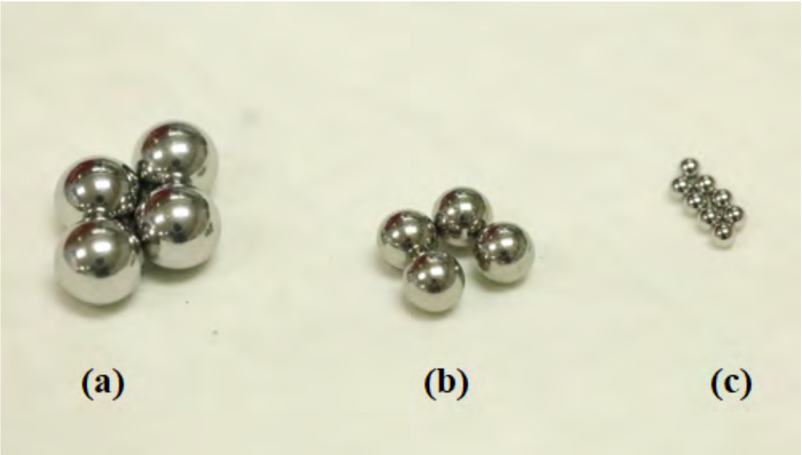

Figure 3.5: Stainless steel milling media size a) 7.0mm, b) 5.0mm, c) 2.0mm

3.4 Characterization Technique

3.4.1 Field-Emission Scanning Electron Microscopy

The structural characteristics of NCC were thoroughly analyzed and recorded by using a FESEM with a model number SU8010 (Hitachi, Ltd., Tokyo, Japan). The samples were placed on a thin layer of carbon tape on an aluminum stub, as shown in Figure 3-6 then the samples were sputter coated with platinum to make them conductive for the analysis.

Figure 3.6: Aluminum stub with carbon tape

These advanced tools offered significant information about the detailed features and characteristics of the NCC which helped in understanding the properties of the NCC at the nano level. When using the FESEM, the surface level analysis of the NCC was done to capture the topographical features and surface texture of the. Thus, the NCC's morphology was studied in detail with the help of these advanced imaging techniques, which helped increase the knowledge and understanding of this nanomaterial.

3.4.2 Particle Size Distribution

The volume-based particle size distribution was measured by laser diffraction (Mastersizer S 2000, Malvern Instruments Ltd., Worcestershire, UK) with the following parameters: The used lens was 300 RF, a small volume dispersion unit (1,000 rpm). The refractive index of dispersed particles is 1.596; and the refractive index for the dispersion medium is 1.0330. For all the measurements, ethanol at 95% was used as a disperser, and the obscuration varied from 11 to 16. The sample was sonicated in ultrasonic bath (WUC-A02H, Wise Clean,) for 10 minutes until solution appeared homogeneous prior to immediate PSD analysis.

3.4.3 X-ray Diffraction

The structure of the crystallinity of MCC and NCC was determined by powder XRD. The crystallinity of the powder samples was analyzed by wide XRD. A MiniFlex 600 (Rigaku, Japan) instrument has been used in the present work with λ = 1. 54, acceleration potential of 40 kV, current of 15 mA, and a copper target to measure the diffraction over the range of 2θ = 3°-80° at a scan rate of 3°/min. The XRD graphs were analysed and compared. The degree of crystallinity was characterized by crystallinity index (CI) by using the following Segal equation (1):

$$CI = (I200 - Iam)/ I200 \times 100\% \qquad (1)$$

Where: I200 = intensity of the crystalline peak corresponding to the crystalline plane 200 (2θ = 22.7°) and Iam = intensity of the amorphous peak between crystalline plane 200 and 110 (2θ = 18.8°)

3.4.4 Fourier Transform Infrared Spectroscopy

FTIR spectra of both MCC and NCC samples were directly recorded at the accessory of attenuated total reflectance with dimensions of 10 mm x 60 mm using a Nicolet 6700 FTIR spectrometer (Thermo Scientific, USA). The samples were placed in good contact with attenuated total reflectance (ATR) at a control temperature of 20 °C. At the vibrations' frequency range of 4000–650 cm−1, 32 scans of the FTIR spectra of the samples were accumulated with a resolution of 4 cm−1. Each of the obtained spectra was compared to its own spectrum. The spectra of all samples were rationed. Again, after each scan, a new background spectrum of the air at the reference level was obtained. These spectra were obtained as absorbance at each of these points, with three measurements for each data point. All spectra were treated and manipulated using the Omnic version 6.1 (Nicolet, Madison, WI, USA) software.

Procedure of this study can be summarized in Figure 3.7

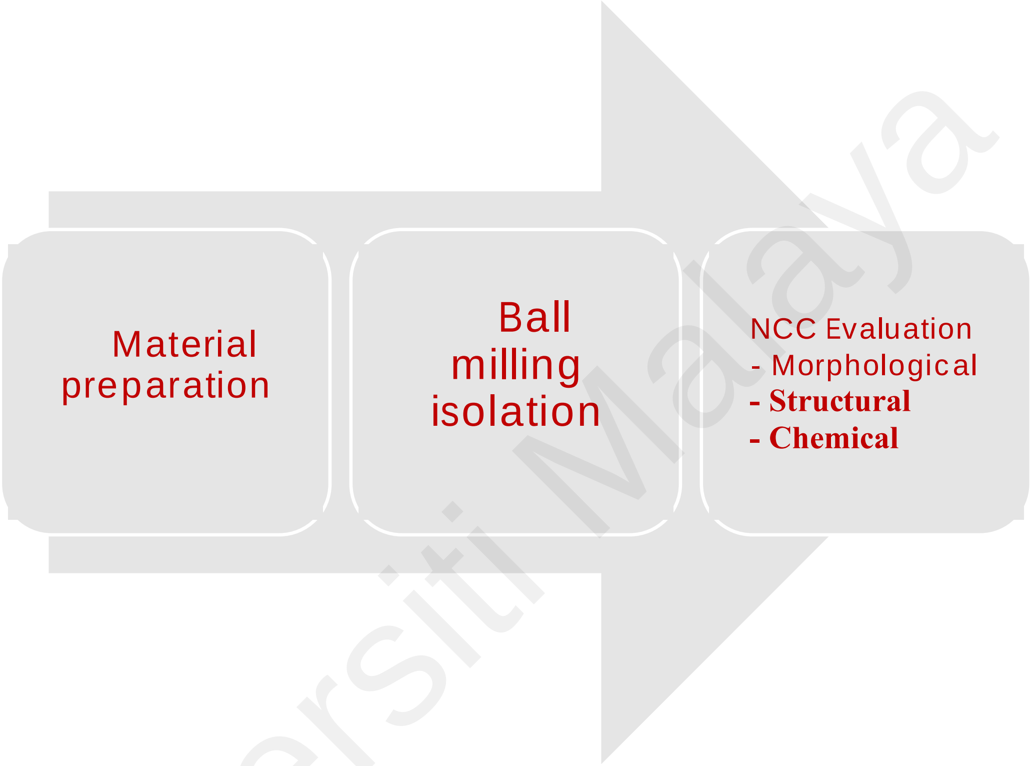

Figure 3.7: Summary of research procedure

CHAPTER 4: RESULT

4.1 Physical appearance of Nanocrystalline Cellulose after ball milling

Based on the provided image as in Figure 4-1, here is a description of the physical appearance of the powders:

Figure 4.1: Physical appearance of a) MCC, b) Dry NCC, c) Wet NCC

In the work conducted on the cellulose obtained from OPEFB, the physical characteristics of MCC and NCC were investigated in detail. This examination sheds light on the distinct characteristics brought about by different milling processes: also includes dry milling and wet milling using ethanol solvent.

MCC produced from OPEFB had a fair and white color. The powder was free flowing, non-lumpy, and had a uniform distribution, which is an indication of a uniform particle size distribution and a fine degree of process. This homogeneity was to make sure that the powder was well compressed and there was no formation of lumps. This consistency is vital in applications where the size of the particles must be consistent.

The effects of dry milling that resulted in the transformation of MCC to NCC were observed in the physical properties. The dry NCC was light grey and finer than the white and fine MCC. This irregular shape is quite common in dry milled powders because the high shear stress that is applied during the process may result in such a rough surface. The obtained powder was characterized by the presence of visible cracking and a brittle structure, which is typical for samples subjected to dry milling. The brittleness and the cracks observed on the particles indicate that the particles had gone through a lot of mechanical stress. In addition, the flowability of the dry NCC was poor, with some aggregation as seen in the loosely packed structure. This tendency has been often noticed in the dry milling processes where particles are not kept apart by any solvent medium.

On the other hand, NCC obtained by wet milling using ethanol solvent presented a different set of properties. It was noted that the color of the wet NCC was similar to that of the dry NCC sample, but the former was slightly darker. The powder that was milled using the wet-milling method had a denser and more uniform structure than the dry NCC. It is hypothesized that the prevention of particle agglomeration could be attributed to the use of ethanol solvent during the milling process thus making the product have a uniform texture. The wet NCC powder had a denser consistency and lower tendency to form agglomerates. This indicates that wet milling provided better results as compared to dry milling due to the production of finer and more uniformly sized particles.

In all the samples, there were certain trends that were noticeable with regard to colour and degree of compaction. All powders were off white in color. However, there was a slight difference in the shades due to the method of milling. The wet NCC was denser than the dry NCC which shows that wet milling is a more efficient method of breaking down the particles and avoiding overheating.

The above findings reveal the contrast in the physical characteristics of MCC and NCC that are prepared through dry and wet milling techniques. It is essential to comprehend these distinctions in order to enhance the milling process as well as the characteristics and potential of NCC in numerous industries. Through the proper choice of the milling process, the characteristics of cellulose can be controlled to address particular requirements for the improvement of the material’s properties in the final products.

4.2 Morphological properties

4.2.1 Microcrystalline Cellulose

The MCC has larger and less uniform in shape particles than the other types of cellulose. The morphology of the MCC particles can be explained by the heterogeneous nature of raw materials obtained from OPEFB, which leads to the formation of particles with a diverse and irregular shape. These particles do not have a specific shape, dense, has an irregular surface and is nearly spherical in shape (Haafiz et al., 2013) as in Figure 4-2.This feature in the MCC particles is quite unique and points to the variation in cellulose obtained from the OPEFB, which can be attributed to the variation in structure.

The average particle size of MCC is much larger compared to the milled one; the length and width are measured in micrometers in average 4μm -8μm. The aspect ratio is rather different, meaning that particles have different dimensions. This non-uniformity is typical of MCC before it is subjected to further milling procedures to achieve a more consistent particle size.

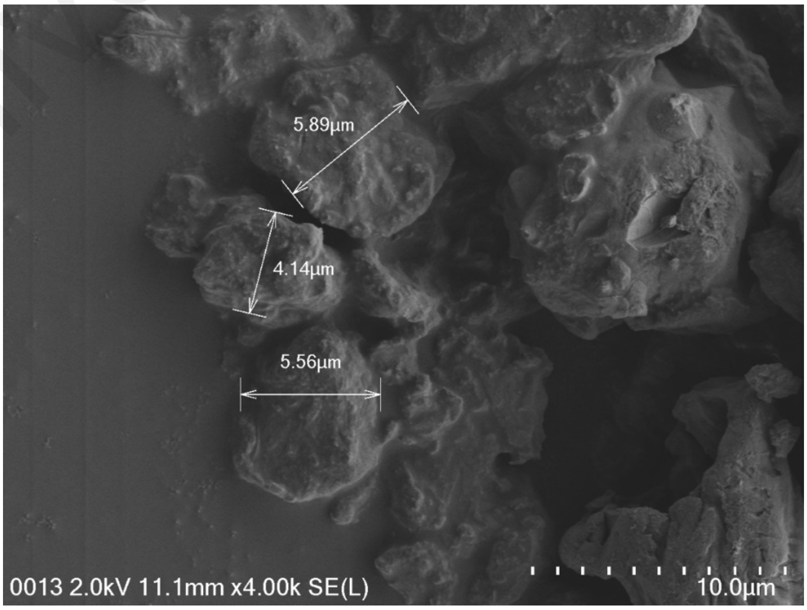

Figure 4.2: FESEM image of MCC

4.2.2 Dry Nanocrystalline Cellulose

The process of high-energy ball milling under dry conditions leads to a significant reduction in particle size, which can be clearly seen from the FESEM images as shown in Figure 4-3. These images clearly show that the particles are transforming into a smaller and more random shape than the primary state of the MCC. In this case, the dynamic milling process also assists in enhancing the surface roughness of the particles and at the same time enhances the structural defects in the material. Furthermore, the reduction in size is as a consequence of the controlled energy input that is as a result of milling which in turn leads to the development of certain surface properties that make the material more reliable. Also, the irregular shape of the particles shows that there is a change in the crystal structure of the particles, which points to the application of a lot of mechanical stress during milling. The milling treatment results in structural defects that help increase the area of contact between the material and other substances, thus increasing its reactivity and performance. In conclusion, the energy, the particle size reduction, the surface and structural changes all confirm that high energy ball milling is an efficient method that can be used to alter the properties of materials with high accuracy.

NCC after dry milling goes through a reduction in the average particle size and the size reduces to nanometer level for both length and width. This change is clear because the particles have sizes varying from 50 to 200 nm in length and width. It can be seen from the figures that these refined NCC particles have a higher aspect ratio when compared to the initial MCC particles which indicates the elongated shape of the particles after the milling process. This change in particle size and shape is a direct result of milling process that determines the NCC particles at the nanoscale level. It is seen that the aspect ratio of the milled NCC particles has improved; the structure of the particles has become longer and thinner; this is in accordance with the goal of the milling process to refine the particles to the nanometer scale. Hence, dry milling is a very efficient process that alters the properties of the NCC particles and opens a whole new angle of size and aspect ratio for the manipulation of nanomaterials.

The FESEM images clearly show the formation of several different nanocrystals of different shapes and sizes. These images are very useful in describing the morphology of the nanocrystals, which is one of their important properties. Nevertheless, a significant problem occurs during the dry milling process, which is widely used for size reduction and particle dispersion. It is a drawback of this process that no dispersing medium is used, which sometimes results in the formation of agglomerates. In essence, due to the lack of a proper medium to ensure uniform dispersion, nanoparticles are prone to clumping and aggregation, which is quite unfavorable for the NCC.

This tendency of nanoparticles to agglomerate becomes a major problem for the uniformity and consistency of the NCC. The clusters can sometimes form regions which are not homogenously distributed within the material, which may affect the structural strength and, in some cases, change the performance of the material. Consequently, it is essential to manage agglomeration since researchers and manufacturers working on nanocrystalline materials such as NCC are likely to encounter the problem.

The effect of agglomeration does not only pertain to the physical aspect of the NCC but also to its performance feature. The uneven distribution due to agglomeration may result in the material having different properties in different areas, which will influence the material's sensitivity to external stimuli or its interface with other components of a composite material. These variations can be significant in all those applications where NCC is employed for instance in increasing the mechanical properties of composites or in increasing the barrier properties of packaging materials.

Thus, the problem of agglomeration during the dry milling process indicates the need of proper dispersion techniques to preserve the quality and performance of NCC for a variety of uses, as illustrated in the FESEM image analysis

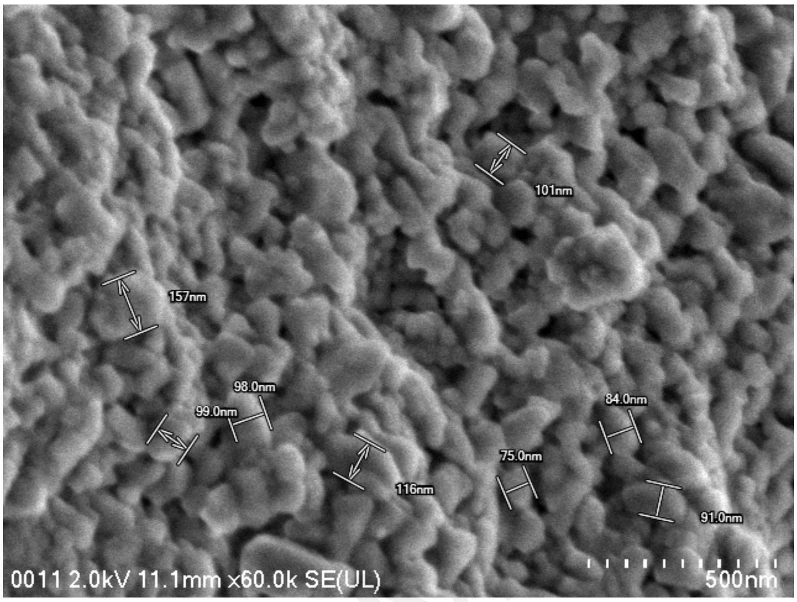

Figure 4.3: FESEM image of dry NCC

4.2.3 Wet Nanocrystalline Cellulose

When high-energy ball milling is carried out in the presence of ethanol, it is possible to notice a decrease in particle size as compared to the results of dry milling as shown in Figure 4-4. This decrease in particle size is due to the fact that ethanol, a solvent that is effectively used in the process, is present. Also, ethanol causes formation of smoother and more refined surface structures when milling which is beneficial for the quality of the final product. Ethanol is also vital in the milling process, and apart from that, it plays an important role in discouraging the formation of large lumps during the milling process. This prevention of particle clumping is very crucial in the formation of uniform and well-ordered nanocrystals thus enhancing the quality of the final nanomaterial. Finally, ethanol plays two roles in the high-energy ball milling process; it aids in the milling process and prevents particle aggregation, which directly determines the efficiency of the process and the final product’s quality in terms of uniformity and improved properties.

It is observed that after wet milling NCC with ethanol, the average particle size is greatly reduced compared to the one obtained under dry milling. The wet milling process leads to NCC particles with a relatively more uniform size distribution, the size of which is between 30 to 150 nm in length. It could be seen that the wet NCC samples have a higher aspect ratio than the dry NCC. This higher aspect ratio means that the nanocrystals have a more elongated shape which means that the overall structure is finer and there is better uniformity in the size of the particles. Thus, the wet milling method does not only help in obtaining smaller NCC particles but also provides better and smoother nanocrystal morphology. Thus, the wet milling technique effectively contributes to the improvement of the quality and properties of the resulting NCC through the reduction of particle size and the increase of aspect ratio, which may be beneficial in a wide range of applications with specific nanocrystalline structures.

Ethanol is used to avoid the tendency of the nanocrystals to agglomerate, which is a significant factor that influences the quality and characteristics of the fabricated nanocrystals when incorporated into the milling process. The above positive impact of ethanol is evident in the FESEM images, as illustrated by the prevention of the aggregation of the nanocrystals. Ethanol leads to the enhancement of the uniform distribution of the nanocrystals and decreases the tendency to form large agglomerates, thus improving the dispersion of the nanocrystals within the material.

Also, the surface of the nanocrystals in contact with ethanol is relatively smooth, suggesting that there are fewer defects that could be an indication of the improved quality of the crystals. Hence, ethanol does not allow the destruction of the nanocrystals' structure and ensures the even distribution of the nanocrystals during the milling process. This stabilizing feature, which is offered by ethanol, not only enhances the quality of the final product and assists in controlling the standardization of the manufacturing process but also enhances the production of nanocrystals that are of higher quality and have better performance.

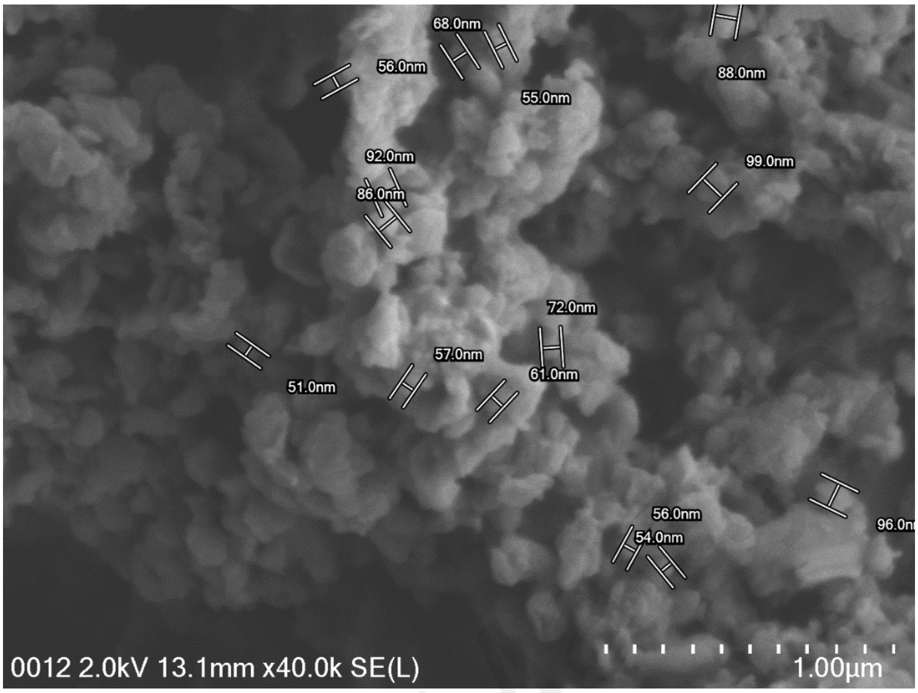

Figure 4.4: FESEM image of wet NCC

4.3 Particle size distribution

The PSD of MCC and its NCC derivatives after high-energy ball milling under dry and wet conditions gives valuable information regarding the efficiency of the size reduction and the degree of uniformity of the particles produced in the different milling processes.

From the PSD curve of MCC in Figure 4-5, it is observed that there is only one major peak at around 1000 nm with an average PSD of 913.7 nm. This shows that the particle sizes are closely squeezed around this value, thus meaning that MCC particles are of fairly similar sizes. However, the distribution is slightly broader compared to dry NCC meaning that there is some range in the size of the particles in the MCC sample but the range is not too wide as the particles are still relatively of similar size.

On the other hand, the PSD of the dry NCC as shown in Figure 4-6 is quite different and looks more like a combination of the two previous cases. The PSD curve for dry NCC displays two distinct peaks: two at one about 10 nm and the other at about 100 nm. These peaks have an average PSD of 123.1 nm which denotes that there is a bi-modal distribution of particles, that is particles of both small and large sizes. This bimodal nature suggests that when the drying milling process is employed, there is a production of a product with two main particle size distributions that are the small and large particles.

Wet milling process results in the production of NCC with different PSD characteristics as shown in Figure 4-7. When it comes to wet NCC, the PSD curve exhibits a single hump at around 1000 nm, as in MCC, but with rather a smaller average PSD of 793.8 nm. The spread is less than that of MCC suggesting that the particle size distribution is more even. This means that wet milling process is better at deboning and preventing the particles from sticking as compared to dry milling.

Therefore, the PSD analysis reveals that MCC has the largest particle size and the distribution is quite even. The particle sizes of dry NCC are the smallest among all the samples, however, the distribution is bimodal, suggesting that there are two types of sizes present. Therefore, wet NCC is observed to reduce particle sizes more uniformly than dry NCC producing a narrow size distribution. Based on the foregoing findings, it is clear that appropriate conditions of milling are crucial in getting the desired particle size distribution for the NCC that is produced from the MCC. Indeed, wet milling seems to provide better results in terms of reduction in size and uniformity of the particles when compared to dry milling, and this makes wet milling a better method for the production of high quality NCC.

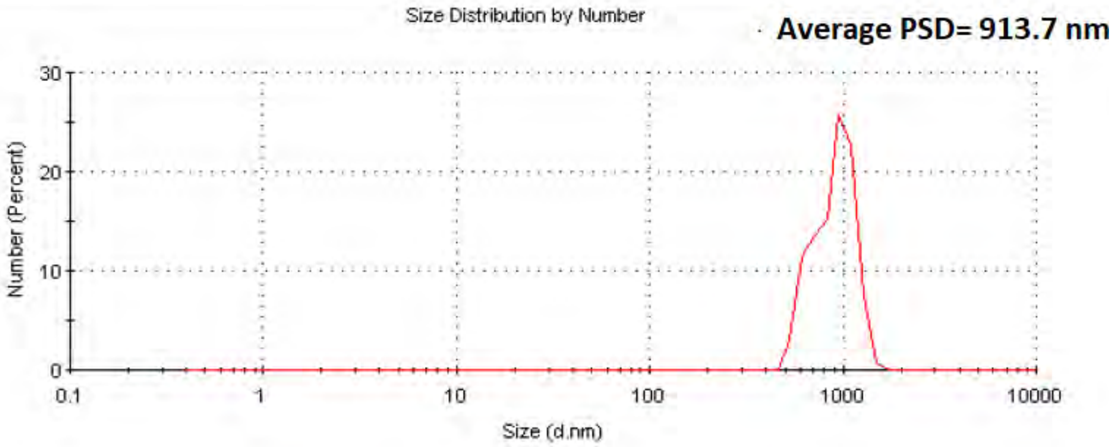

Figure 4.5: PSD result of MCC

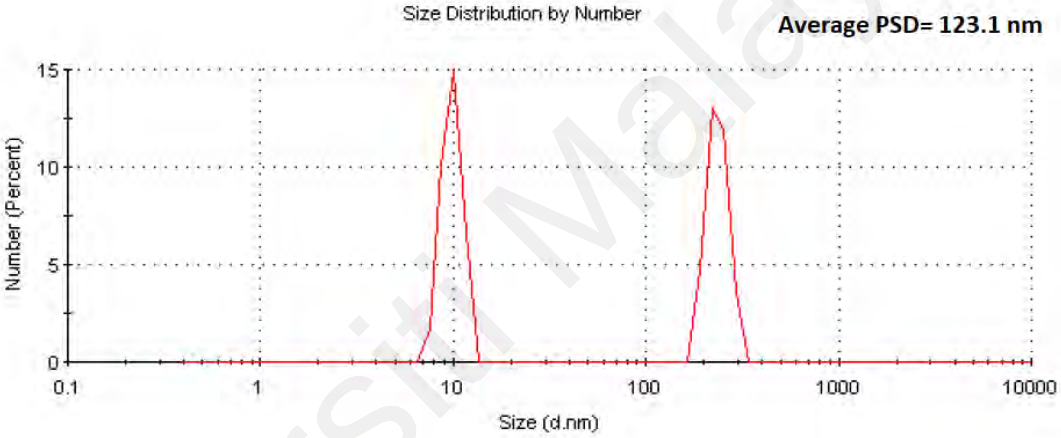

Figure 4.6: PSD result of Dry NCC

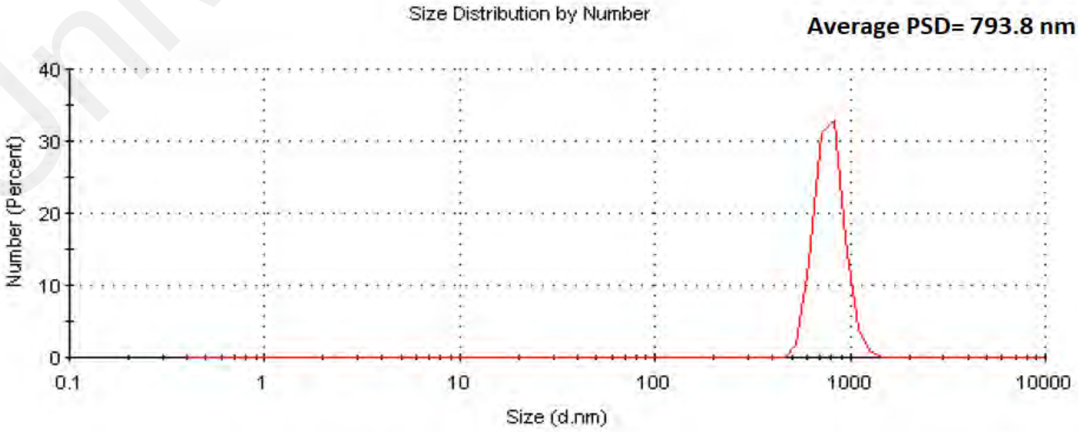

Figure 4.7: PSD result of Wet NCC

4.4 Structural properties

As seen from the XRD patterns of the nanocellulose samples in Figure 4-8, the crystalline nature of the samples is fairly well described. All three samples MCC, dry NCC and wet NCC have dominant peaks at the same position, which suggests that they have a common crystalline structure. However, the peak intensities differ, with MCC having the highest intensity compared to dry NCC and wet NCC. This difference in intensity shows that some of the samples might have been more crystalline than others.

The information on the peak width gives additional information on the crystallite sizes. The higher and narrower peaks of MCC sample depict that the crystals are larger in size, while the lower and broader peaks of the wet sample suggest that the milling process was wet. The dry sample has an intermediate peak width, which indicates that the crystallite size is between MCC and wet sample.

In all samples, there is a quite constant background signal with only mild changes in the signal intensity. It should be also mentioned that a slightly elevated background signal is observed for the dry sample as compared to the samples MCC and wet, which might be related to the differences in the level of amorphous phase or other structural characteristics.

In general, the MCC sample, as compared to the other two, shows sharper and more well-defined peaks, which is an indication of high crystallinity. On the other hand, the dry sample which had undergone dry milling process has the peak intensity reduced, thus implying that there is a reduction in the crystallinity of the sample. The wet sample, which was subjected to wet milling, has the smallest peak intensity and the largest full width at half maximum, meaning smaller crystallite size and higher reduction in crystallinity. The presence of sharp and narrow peaks at 14.8°, 16.4°, and 22.6° for all sample reveals that all the sample are predominantly in the cellulose I phase

These observations provide evidence of the effect of various milling method on the crystallinity of nanocellulose. Wet milling has the highest impact on the changes and significantly decreases both the crystallinity and the crystallite size. This detailed analysis helps the reader get a clear picture of how milling methods affect the fine structure of nanocellulose, its properties and how it can be used in different fields.

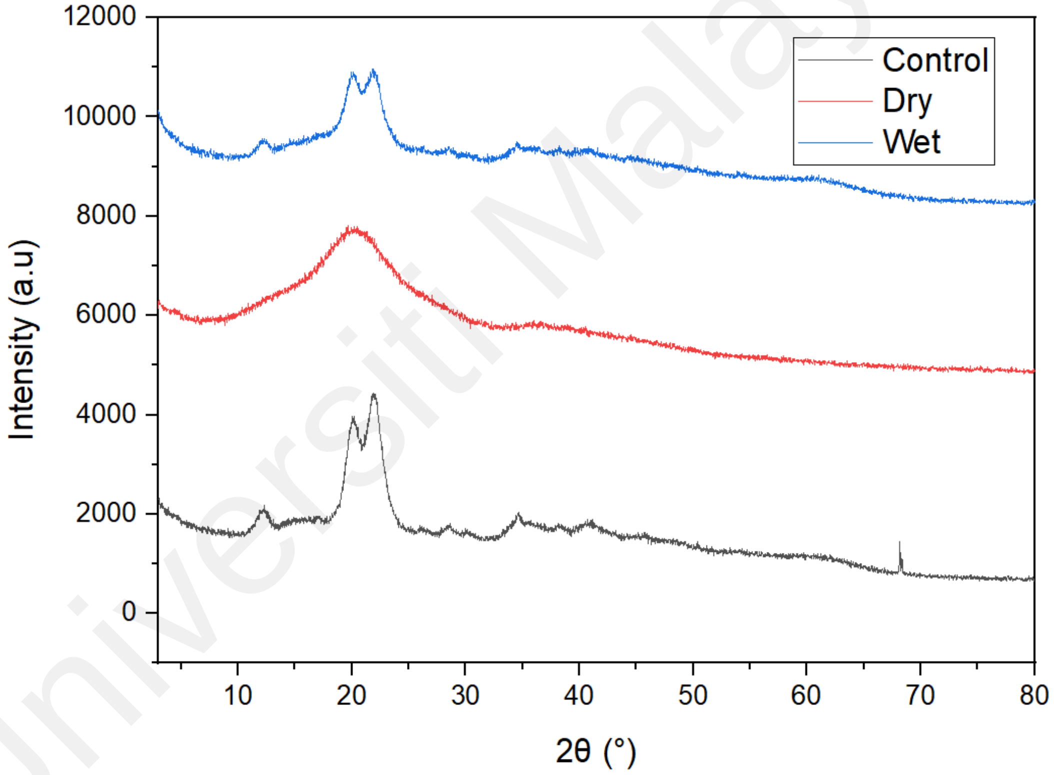

Figure 4.8: XRD pattern

From the measurement of the crystallite size in Table 4-1, it can be deduced that, dry milling has smaller crystallite size as compared to wet milling. Based on the analysis, the crystallite sizes of the dry NCC are 9.29 Å and 6.34 Å, while the wet NCC have larger crystallite sizes varying from 27.22 Å to 68.61 Å. It has been found that dry milling has significant advantage over wet milling; that is, the reduction in size of the cellulose crystallites is more effective in dry milling because there is no solvent in this process to help the formation of larger crystallites as opposed to wet milling.

Table 4.1: The crystallite dimensions of MCC, dry NCC and wet NCC

Sample	2Θ (º)	FWH (º)	Crystallite Size (Å)
MCC	12.1966	0.8091	103.13
	19.9835	1.3386	62.94
	21.8551	1.7559	48.13
	34.6645	2.1983	39.54
	41.1173	5.1923	17.07
	68.1635	0.0738	1357.36
Dry NCC	44.2444	9.6431	9.29
	56.8691	14.8822	6.34
Wet NCC	12.1166	2.0572	40.56
	20.032	1.2445	67.7
	21.9238	2.3782	35.54
	28.5413	1.2479	68.61
	34.5967	3.1921	27.22
	40.6627	6.5917	13.42
	45.0248	2.9334	30.62
	60.9794	4.1404	23.26

As a result of the above analysis, the FWHM values also confirm the crystallite size results. The FWHM values are extremely high for dry NCC, ranging from 9.6431° and 14. 8822°, it is noticeable that the wet NCC are higher, varying from 1.2445° to 3.1921°. The wider the FWHM, the smaller the crystallite size, as obtained from this research study, which means that the dry milling process leads to the creation of small crystallites.

This follows the perception that the forces produced by dry milling are mechanical in nature and produce larger particle sizes with ramped up structural randomness.

The characteristics of the crystalline structure also meet certain differences between the dry and wet milling processes in terms of 2-theta values. Comparing the two, the dry NCC shows higher 2-theta values of 44.2444° and 56°. In the dry NCC, no hemicellulose peak is noticed, while in the wet NCC, the hemicellulosis peaks are noticed at low 2-theta values varying from 12.1166° to 60.9794°. Such variations in 2-theta values may be suggestive of different crystal planes or phases dominant at the various milling regimens. A link between higher 2-theta values observed in dry milling may be due to more stressed and strained crystal structures than in wet milling, which shows a lower value.

Therefore, based on the data interpreted from crystallite size, FWHM, and 2-theta values, it can be identified that the milling conditions have a significant effect on structural properties of NCC. Dry milling results in more fragmented and distorted crystallites, which yield smaller crystallites, larger FWHM, and higher intensity of 2-theta peaks. However, wet milling produces larger crystallites with smaller FWHM, and smaller 2-theta peak angles indicating that this process does not so severely disrupt the cellulose structure. Consequently, these results depict the need to identify suitable milling conditions in order to possess desired structural characteristics in nanocellulose since it is related to its performance and use in different sectors.

Table 4.2: Crystallinity Index for MCC, dry NCC and wet NCC

Sample	I200 (2ϴ=22°)	Iam (2ϴ=18°)	Crystallinity Index (%)
MCC	4313	1851	57.08
Dry NCC	3284	3111	5.27
Wet NCC	3393	2053	39.49

Based on Table 4.2, MCC has a crystallinity index (CI) of 57.08%, which shows that it is a highly crystalline material. This is expected because MCC is one of the most crystalline products, and such characteristics are desirable for its wide range of applications in industries and pharmaceuticals. MCC has a high crystallinity index, which results in improved mechanical strength, thermal stability, and chemical stability of the material; thus, MCC is suitable for use in tablet formulations, as a filler in drug delivery systems and in other applications that require robust and stable cellulose.

On the other hand, dry NCC has a CI of 5.27%, which is a very low crystallinity index. This indicates that the dry milling process had a major effect on the cellulose crystallinity of the material and it became mostly amorphous. This might be attributed to the mechanical disruption of the crystalline regions during dry milling, since the degree of crystallinity is reduced by half. This could lead to a situation where dry NCC will have some other properties, such as flexibility and higher reactivity than other forms of cellulose and this could be of added advantage in certain applications where more active or flexible cellulose structures are needed.

Wet NCC has a CI of 39.49%, which shows that it has a moderate level of crystallinity compared to the other samples. In the context of comparing dry NCC with wet NCC, it can be noted that the latter leads to the maintenance of a more crystalline structure of cellulose. The utilization of ethanol as a solvent in the wet milling process might have contributed to the protection of the crystalline regions, hence a higher CI. This implies that wet NCC has an intermediate level of crystallinity and, therefore, possesses some of the positives of crystalline cellulose, including mechanical strength and stability, and some of the characteristics of amorphous cellulose, which are flexibility and reactivity. For these reasons, wet NCC is a suitable material for many uses requiring both strength and flexibility.

4.5 Chemical properties

FTIR analysis which is used to study the material structure was used to further investigate the chemical composition of the isolated NCC. In order to understand the changes that have taken place in the structure of the produced materials during the wet and dry milling processes, the FTIR spectra of the material obtained through both processes were compared to the spectra of the original MCC. Spectral analysis clearly showed that the basic chemical makeup of the NCC was largely intact, as demonstrated by the cellulose peaks at consistent frequencies.

When comparing the FTIR spectra as shown in Figure 4-9, interesting observations were revealed, namely, wet NCC had broader peaks in the range from 3400 to 3200 cm^{-1}. These sharp, broad peaks suggest that there may be a possible incorporation of more O-H stretching, which could be due to the effects of water molecules or new hydroxyl groups incorporated during the milling process. Moving to the range of 1800 -1500 cm^{-1}, probably corresponding to the C=O stretching from carbonyl groups, some more details pointed towards the chemical changes induced by wet milling.

When comparing the dry NCC with the other samples, there was a decrease in the intensity of the O-H stretching peaks. This decrease in the peak intensity indicated a decrease in the concentration of the hydroxyl group in the dry NCC in comparison with the wet NCC. These differences highlight the complex relationship between processing methods and the chemical properties of NCC, which may be useful for future research and applications in material science and nanotechnology.

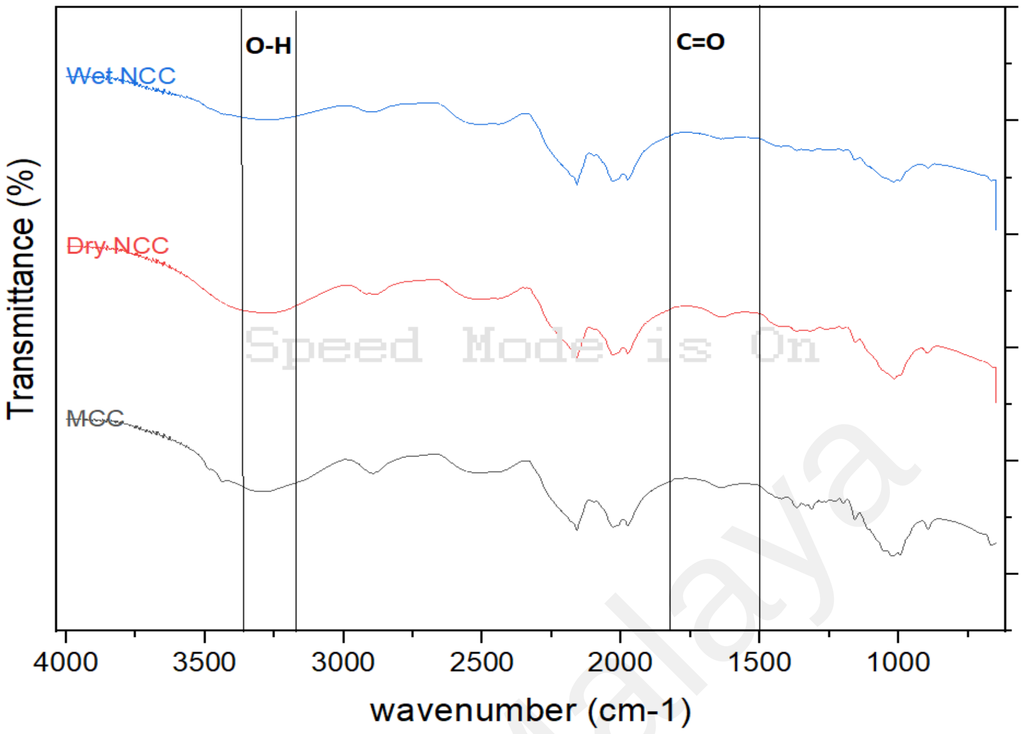

Figure 4.9: FTIR spectra of MCC, dry NCC and wet NCC

The findings of this research are summarized in Table 4-3

Table 4.3: Summary of finding

Characteristic	Aspect	MCC (Control)	Wet NCC	Dry NCC
Morphological	**Shape**	Irregular	Irregular	Irregular
	Particle Size	3-8 micrometer	30-150 nm	30-150 nm
	Particle Size Distribution	Not applicable (larger particles)	More uniform	Less uniform
Structural	**Crystalline Structure**	Original crystalline structure maintained	Better preserved, leading to higher crystallinity	Less preserved, lower crystallinity
	Mechanical Properties	Baseline	Potentially better	Potentially lower
Chemical Integrity	**FTIR Analysis**	Baseline chemical structure maintained	Unaffected by ball milling	Unaffected by ball milling

CHAPTER 5: DISCUSSION

5.1 Characterization of Nanocrystalline Cellulose properties

This research presents the results of the investigation on the production and characterization of NCC from OPEFB derived from MCC using high-energy ball milling and highlights the effect of milling conditions. Such differences can be seen in the physical, as well as morphological and structural characteristics of the NCC obtained. The following section presents a discussion of these findings in relation to the literature and expected results.

5.1.1 Physical characteristic

For the physical characteristics, all the powders developed were off white in color. This implies that regardless of the method of milling used, the cellulose base was the same. There was a variation in the shade parameter which was due to the different milling processes that were carried out. As for the density, wet NCC had a higher density compared to the dry NCC, this could be a result of the better packing of cellulose particles because of the wet milling process. The above finding is in accordance with previous studies that reveal that the type of solvent used during the wet milling process greatly affects the surface properties of the milled material to produce a smoother and a more uniform surface (Abraham et al., 2011). Wet NCC have a higher density because of the denser nanocellulose structure when ethanol is used as the milling medium (Khalil et al., 2014). The surface of the dry NCC was not as smooth and had visible cracks, as well as a tendency to clump together, possibly because there was no solvent used. On the other hand, there were wet NCC, and their surface was smoother and denser since the ethanol used in the process affected the surface tension and gave better and more compact particles.

5.1.2 Morphological properties

FESEM images of the samples were also used for the morphological analysis, where differences were well contrasted. MCC particles were larger, less uniform, and irregular in shape, which was due to the fact that the OPEFB raw materials used were rather heterogeneous. The size distribution was between 4 and 8 micrometers, and the shape was irregular with a non-uniform aspect ratio as obtained from raw MCC particles. High energy dry ball milling led to a decrease in particle size to smaller (50 to 200 nm) with a more random and rougher surface. The aspect ratio and roughness were higher as an indication of mechanical comminution and absence of dispersing medium, which often results in particle agglutination (del Carmen Jimenez de Haro et al., 2000). Ethanol based wet milling, on the other hand, was more effective at cutting particle size (30-150 nm in length) and preventing aggregation. The resulting particles had a better uniformity, a higher aspect ratio and a smoother surface because the ethanol medium hindered the particles from sticking together and provided a better distribution of the particles (Ben-Arfa et al., 2019). This is in agreement with the work of (Isobe et al., 2008) who have reported that wet milling in solvents produces better dispersion and fines with uniform size. Thus, distinctions in the morphology of nanocellulose obtained by dry and wet milling denote the role of solvent in obtaining the desired properties of nanocellulose.

5.1.3 Particle size distribution

From the particle size distribution analysis of the MCC, dry NCC and wet NCC prepared from OPEFB, the differences in size and distribution of particles can be clearly seen. The findings of this study can be compared with those of other studies to ascertain the results and the reasons for the disparities.

It has also been established that MCC usually has larger particle sizes because of its semi-crystalline nature. A study stated that MCC obtained from different biomass materials tends to have particle sizes less than 20 µm (Gaudreault et al., 2005). In the PSD curve of MCC in this work, the average PSD is 913.7 nm, which agrees with previous studies. The main peak at around 1000 nm indicates that there is a good dispersion of particle size, which is expected of MCC (Ioelovich & Leykin, 2008).

It has been reported that high energy ball milling in a dry mode can give NCC a broad particle size distribution (Jung et al., 2015). It has been observed that dry milling results in bimodal distribution as a result of partial agglomeration and dissimilar degrees of size reduction (Krause et al., 2011). This dry NCC in the present study has an average PSD of 123.1 nm with bi-modal distribution with modes at 10 nm and 100 nm. This bimodal nature shows that there are both small and large particles (Alamir et al., 2010) and dry milling produces particles of different sizes since milling is not usually complete and there is particle agglomeration (Jung et al., 2015).

Wet milling is commonly more efficient in producing a finer and well- sized particle (Juhnke et al., 2012), useful in preventing caking, and ensures uniform comminution (Carter et al., 1991), which leads to narrow PSD curves. The wet NCC in this study has an average particle size distribution of 793.8 nm with a single, relatively sharp peak at 1000 nm. This indicates that the particle size reduction is more uniform compared to dry NCC (Kotake et al., 2011) and stresses that wet milling is ideal for the production of uniformly sized NCC.

5.1.4 Structural properties

The peak intensity, peak width and crystallite size were also found to be quite different from the normal values as revealed by the XRD analysis. The MCC, however, had the highest peak intensity hence the highest crystalline character. The intensity of the peak of the dry NCC was intermediate between the two extremes, while the wet NCC samples had the lowest intensity because of the reduction in the degree of crystallinity. From peak width analysis, it was observed that MCC had higher and narrower peaks which was an indication of large crystallite size, while dry NCC had an intermediate peak width and crystallite size. The crystallite size of the wet NCC was the smallest and the peak was the broadest among all the samples. The higher background was observed in the dry sample, which pointed towards the increased amount of amorphous phase. Wet NCC had the greatest impact on the decrease in crystallinity and larger crystallite size (Ozcan et al., 2013), and wet milling affected the crystallinity of cellulose in a similar manner. Every sample exhibited a well-defined and narrow single peak at 14.8°, 16.4°, and 22.6°, characteristic of the cellulose I form, which proves that the crystal structure of cellulose is retained even after milling. These observed changes in crystallinity and crystallite size proved that wet milling causes more disruption of the crystalline structure than dry milling (Ozcan et al., 2013).

MCC is a material with a very high degree of crystallinity, thus its crystallinity index being 57.08 %. This high crystallinity makes MCC possess good mechanical strength and thermal stability thus making it suitable for use in many industries and in the production of pharmaceuticals. The property and production of NCC are, however, dependent on the milling conditions, as pointed out by the large difference in CI values between the dry and wet milling processes.

The degree of crystallinity of NCC is very much influenced by the milling conditions. The obtained CI of 5.27% in dry NCC means that the process significantly destroys the crystalline areas and leaves the cellulose mostly amorphous. This extensive mechanical disruption in dry milling could probably enhance the material’s flexibility and reactivity but weaken its structural soundness. On the other hand, wet NCC that uses a solvent such as ethanol seems to be less harsh on the crystalline regions, which gives a CI of 39.49%. This could mean that wet milling is more effective in maintaining the crystalline structure since the solvent has a way of reducing the mechanical strain that is inherent in the milling process.

The difference in properties of cellulose materials is determined by their crystallinity indices. In this case, MCC has a higher crystallinity, which means that mechanical strength and thermal stability are also high. These properties are very essential for applications that need materials that are strong and long-lasting. However, as seen from the CI values, dry NCC has lower crystallinity than wet NCC, and this is an advantage if there are applications that require more flexibility or chemical reactivity, such as in some composite materials or chemical treatments. Wet NCC has an intermediate level of crystallinity that might be useful for applications that require the material to be neither too rigid nor too reactive.

Therefore, between MCC, dry NCC, and wet NCC, the one to choose depends on the planned application as well as the required characteristics of the resulting material. MCC due to its high degree of crystallinity, is suitable for use in applications that call for high mechanical and thermal strength. Dry NCC has a low degree of crystallinity and a high degree of amorphous phases; hence, it may be suitable for applications where increased flexibility and reactivity are desirable. Wet NCC, which has an intermediate degree of crystallinity, can be used in applications where a material that is not too brittle but also not too ductile is required.

Therefore, it can be stated that the crystallinity index is a significant factor that determines the structure of cellulose materials. The distinctions in the CI between MCC, dry NCC, and wet NCC show how the milling parameters affect the material’s crystalline structure. These differences in turn influence the characteristics and applicability of the material in many fields. Hence, there is a need to pay keen attention to the milling process that will give the desired level of crystallinity when incorporating NCC in various industries and sciences.

5.1.5 Chemical properties

The chemical composition of the samples was analyzed using FTIR, which gave important information about the samples. The crystalline nature of cellulose did not change significantly in NCC, as the cellulose peaks were steady in all the samples. For wet NCC, a wider peak at 3400-3200 cm^{-1} indicated increased O-H stretching due to water molecule adsorption or the formation of a new hydroxyl group. Shifts in the range of 1800-1500 cm^{-1} pointed to C=O stretching from carbonyl groups that pointed to chemical modification as a result of wet milling. On the other hand, dry NCC had reduced intensity of O-H stretching peaks which suggested fewer hydroxyl groups than wet NCC. This is in accordance with the literature where the increase in hydroxyl groups and the broadening of the peaks in wet milling were documented by (Trache et al., 2017). The reduction of the O-H stretching intensity in dry milling agrees with the literature which reveals that dry milling does not significantly change the original hydroxyl content in the material (Habibi et al., 2010).

Collectively, based on the outcomes of this study, it could be concluded that high energy ball milling, especially wet milling in ethanol, is a more efficient method of producing uniformly distributed and high quality NCC from MCC obtained from OPEFB. The variations in the physical, morphological and structural characteristics when using dry and wet milling methods are significant. Ethanol wet milling is found to give better results in reducing particle size, avoiding aggregation, and producing denser and more uniform particles. This is in accordance with previous work that has stressed the advantages of using solvent assisted milling in the production of high quality nanocellulose (Phanthong et al., 2016). The wet NCC has smoother and more dense surfaces with improved flowability and low aggregation in accordance with the previous research that stated that solvents can improve particle dispersion and surface properties. The decrease in the degree of crystallinity and the size of the crystallites with wet milling is in agreement with the literature, thus highlighting the effects of the milling parameters on the nanostructure of cellulose. These findings are very valuable for the further development of NCC, the possibility of its improvement in the production process, and the improvement of its characteristics for various uses in material science and nanotechnology. The comparison with literature supports the expected results, indicating that the milling conditions significantly affect the characteristics of nanocellulose (Kano et al., 2019). Further research can be done on the basis of these findings in order to expand the possibilities of the usage of NCC in the various sectors of industry and technology.

5.2 Factors influencing the isolation process.

This research focuses on the synthesis of NCC from MCC obtained from OPEFB in relation to dental applications. The main goal is to enhance the ball milling process under definite conditions, which are dry milling and wet milling with ethanol as the solvent. The experimental setup involves maintaining a fixed room temperature, a rotational speed of 1500 rpm, a constant ball-to-powder ratio, and utilizing a sequence of stainless steel ball diameter: 7mm, 5mm and 2mm, respectively. This present work presents the factors affecting the ball milling process and their consequences for the isolation of NCC.

The process of ball milling is widely used in the reduction of particle size and the enhancement of the crystallinity of cellulose materials. Consequently, the use of steel balls of different sizes sequentially is proposed to increase the fragmentation and crystallization of MCC. The first stage using 7 mm balls is to ensure that there is enough force applied to the material to cause a comminution of larger cellulose fibers.

Consequently, the successive reduction to 5 mm and then to 2 mm balls is meant to enhance the particle size reduction and the surface area of the NCC. The size reduction process depends on the ball-to-ball collision and the ball to powder collision and the frequency of these collisions, and the energy with which they occur depend on the size of the balls and the milling time.

It is therefore important to look at the two conditions of dry and wet milling with a view to determining which of the two is most appropriate for the isolation of NCC. When in dry milling, the solvent is not used, the temperature rises, and there is a possibility of cellulose breakdown. The application of ethanol in wet milling is to prevent thermal degradation by carrying away heat and to provide a fluid to help hydrolyse the cellulose chains. The solvent is therefore very important in that it arrests the material, prevents it from clumping, and facilitates the breakdown of the crystalline structure into a nanocrystalline one.

The ball to powder weight ratio should be kept constant to enhance uniformity of the milling process and reduce the wear of the milling medium. This is to ensure that the energy that is applied to the cellulose sample is constant, which enhances the reliability of the outcomes. Some variations in the ball-to-powder ratio will cause improper energy transfer; others will cause too much friction, which will influence the milling process and the quality of the isolated NCC.

The constant milling speed of 1500 rpm is chosen as an optimal level between the energy applied to the cellulose particles and mechanical stress. The speed of the balls may be raised to cause them to have more kinetic energy, which in turn may cause overheating and material failure. On the other hand, low speed may decrease the effectiveness of particle size reduction mechanisms. The milling time, which was constant in this work, is an important factor that controls the level of size reduction and crystalline index improvement. It is therefore very important to determine the right milling time in order to get the desired NCC properties while at the same time preserving the cellulose structure.

The isolated NCC samples are analyzed by FESEM, PSD, XRD, and FTIR. FESEM helps to visualize the shape and size of the nanocrystals, while XRD gives information on the crystallinity index and phase of the NCC. The FTIR spectroscopy provides information on the chemical bonding and functional groups that exist in the NCC; thus, proving that the nanocrystalline structures are well isolated. All these characterization techniques, therefore, support the efficiency of the ball milling process in the production of NCC from MCC derived from OPEFB, which has a potential application in dentistry.

5.3 Implications for Dentistry

NCC obtained from MCC that is sourced from OPEFB provides new possibilities in the dental materials field. Due to its properties like high mechanical strength, biocompatibility, biodegradability, and large surface area, NCC can be used for dental applications. This section discusses the possibilities and identifies the merit or otherwise of applying NCC in dentistry with a view to determining its relevance to dental materials.

One factor that greatly affects the durability of dental materials is the mechanical properties of the material in relation to the stress of the masticatory system. It has also been observed that NCC has great potential to improve the mechanical properties of composite materials. Thus, when added to dental composites, NCC can enhance the flexural strength, modulus, and fracture toughness of the material. This means that NCC-reinforced composites are suitable in areas that require high strength, such as dental fillings, crowns, and bridges.

Biocompatibility is one of the main parameters of any material used in dental applications, as the biological response may vary. The NCC prepared from OPEFB has good biocompatibility since it has been proven to have low cytotoxicity (Imlimthan et al., 2020) and good cell adhesion (Barud et al., 2015), as seen from in vitro analysis. This, therefore, makes NCC a safe option when it comes to its use in dental restorations and implants. Also, since it is biodegradable, any remaining substance will be easily metabolized by the body with no negative consequences in the future.

Aesthetics in dental restorations is very important with special emphasis on the anterior teeth. The fact that NCC can produce clear and smooth composites is useful for making dental materials that have the color and surface of natural teeth (Parker et al., 2018). This property is very useful for dental veneers and bonding agents, as they require the material to be as translucent as possible and have a natural look.

NCC can also be used to improve the antibacterial aspects of dental materials when added to them (Hamouda, Makharita, et al., 2023; Hamouda, Qarabai, et al., 2023; Wang et al., 2019). Due to the high surface area of NCC, antimicrobial agents can be incorporated and gradually released to prevent bacterial adhesion and biofilm formation on dental restorations. This is especially relevant for the further development of secondary caries and for caring about the oral cavity.

The process of making NCC from OPEFB is economical and does not pose harm to the environment, as it utilizes abundant agricultural byproducts and avoids harmful chemical treatments (Kumar et al., 2020; Qing et al., 2020). This makes it an economical alternative to other types of NCC derived from wood or cotton, which involve more expensive raw materials and energy-intensive processes, making OPEFB-derived NCC highly suitable for dental. Moreover, the process can be optimized to obtain NCC with certain characteristics depending on the conditions of ball milling and solvent usage. This customization thus makes it possible to develop dental materials with specific characteristics for the various uses in dentistry.

The NCC obtained from OPEFB through the described ball milling process has properties that are suitable for dental materials. Ethanol as a solvent in both dry and wet milling conditions allows the production of NCC with the lowest levels of impurities and more uniform quality. The constant room temperature, high rotational speed of the mill at 1500 rpm, and sequential use of the stainless steel balls of different sizes (7 mm, 5 mm, and 2 mm) produce NCC with the desired particle size distribution and surface characteristics.

Characterization analysis of the produced NCC is done using XRD, FTIR, and FESEM, which proved the crystallinity, chemical composition, and morphology of NCC, respectively. The high CI and good morphological characteristics of NCC show great potential for increasing the mechanical properties of dental composites. The FTIR analysis reveals the presence of cellulose's functional groups and points to biocompatibility and chemical stability.

Thus, the isolation and characterization of NCC from MCC obtained from OPEFB have shown the material's high potential for use in dental applications. The following properties of the produced NCC mechanical strength, biocompatibility, aesthetics, antibacterial properties, and the ability to customize the production process make it a suitable candidate for dental applications. More studies and advancements can be made to utilize NCC in dentistry and to enhance the characteristics of dental materials.

5.4 Limitation of the Study

Despite the fact that the method of obtaining NCC from MCC by high-energy ball milling has certain advantages, there are certain drawbacks to it. These problems should be solved to improve the process, especially for dental applications, because dentistry is one of the areas that can benefit from the process.

First of all, the problem of heat generation and thermal degradation is critical. In dry milling conditions, the high energy used in the process results in generation of heat. Since there is no efficient way of removing this heat, there is a danger of the cellulose being thermally broken down. This degradation can pose a threat to the structural stability and crystalline nature of the NCC, which in turn lowers its quality. However, wet milling with ethanol can somehow solve the problem of heat build-up to some extent and thus may not affect the quality of NCC.

Contamination is another factor that can also be named as a major problem. Stainless steel balls pose the problem of metal debris contamination. When such balls are used in the mill for a very long time, there is a tendency for the balls to wear out and chip off small iron particles into the NCC. This is especially problematic for dental applications, where the material's contamination and biocompatibility are critical. Eliminating the risks of such contaminants in the final product is crucial for preserving the safety and effectiveness of the product in the dental field.

Another issue is the material loss that occurs during the transfer and drying phases of the milling process. Significant amounts of cellulose can be lost while moving the material between steps or due to evaporation of ethanol during drying. This material loss reduces the overall yield, affecting the efficiency and increasing the cost of production. It is important to minimize such losses to make the process more sustainable and economically viable, especially when scaling up for larger production runs.

One more issue that is rather problematic is the possibility of obtaining a uniform particle size distribution. The ball milling process creates a variety of particle sizes, which results in variability in the NCC properties. Such phenomena may influence the repeatability of the results and, therefore, the effectiveness of NCC in dental applications. Since NCC has a wide particle size distribution, it has an uneven mechanical property, which can limit the process of achieving the best NCC for certain applications.

The removal and recovery of ethanol are even more problematic. However, ethanol is a good solvent in wet milling, and it is imperative to properly eliminate ethanol from the final product since the presence of ethanol may be toxic in dental applications. Thus, ethanol is a highly inflammable compound, and its usage requires careful handling. In the same way, its disposal poses a threat to the environment. These problems should be solved in order to preserve process safety and minimize the negative impact on the environment.

Scale-up challenges also present a major problem. To scale up the high energy ball milling process from lab scale to industrial scale, there are issues of maintaining a constant milling condition and having a uniform particle size. This process of scaling up may be expensive and technically challenging since more and larger pieces of equipment would be needed, as well as more energy. For practical implementation, it is important to know that the process is efficient and economical when applied on a larger scale.

Milling time and efficiency are very related since the efficiency of the milling process is a function of time. It is sometimes required to mill the sample for longer periods to get the desired nanocrystalline grain size, which is both tedious and energy consuming. This requirement has the overall effect of slowing down the process and therefore, increasing the cost of operations.

Last but not least, during high energy milling, the structure and functional properties of cellulose are not preserved. The mechanical stress that is applied can lead to altercations in the chemical composition and the crystallinity of cellulose that might ultimately influence the mechanical and functional characteristics of the material. All these properties must be maintained while at the same time trying to get the desired nanocrystalline structure, as this will determine the effectiveness of the material in the intended applications.

Therefore, high-energy ball milling is a viable method for producing NCC from MCC, however, there are some issues that need to be considered and solved in order to apply the process in dental fields. These are: controlling heat generation, contamination, achieving a uniform size of the particles, handling and retrieval of solvents, process scaling, enhancing milling efficiency, and preserving the structure of cellulose. Subsequent studies should aim at further optimizing the mill parameters, improving the extraction of the solvent, and establishing methods to reduce contamination; all of which are critical for the increased use of NCC for dental applications.

5.5 Future Research Directions

The following can be suggested for future research in the field of NCC isolation and characterization based on ball milling process efficiency and its applications: Another consideration is the condition of the milling, which needs to be improved. To gain a better understanding of the milling process when variations in ball size and shape are introduced, further research should be conducted with different ball sizes and shapes. For example, non-spherical balls or different ratios of ball sizes could lead to a higher efficiency of particle size reduction and the NCC yield. Likewise, it is possible to control the milling time to study the effects on NCC, including crystallinity and particle size distribution. Thus, the milling time could vary, influence the final characteristics of the NCC, and give a more elaborate picture of the process.

Another important factor that should be analyzed is the ethanol concentration that was used during milling. The concentration of ethanol could influence the milling parameters and the characteristics of the NCC obtained. Different ratios of solvents could affect the degree of cellulose hydrolysis and characteristics of the produced NCC like purity, which might mean that there is better condition for production of high quality NCC.

Aside from these modifications, it is also possible to investigate other solvents and conditions in order to expand the possibilities of the study. Looking at other solvents or a combination of solvents might produce different NCC purity and properties. Green solvents or ionic liquids for instance can be used to lessen the environmental effects and therefore present a better method of getting NCC. Also, changes in the temperature during milling may affect the structure and mechanical characteristics of the NCC. It is possible that varying the temperature may give information on the thermal stability of the material and possibly improve the milling process.

Improvements in characterization are also critical to coming up with a better understanding of NCC. The use of advanced microscopy techniques like, for instance, Transmission Electron Microscopy (TEM) or Atomic Force Microscopy (AFM) could be useful in the identification of morphology and size of NCC at the nanoscale. Using other techniques like Raman spectroscopy or Nuclear Magnetic Resonance (NMR) could help in getting more information on the structure of NCC and the bonding between the atoms.

This means that specific studies are very important in an attempt to take research outcomes and apply them to a certain application. Subsequent works in the field of dentistry should focus on the utilization of NCC in dental materials, be it in composites, adhesives, or as a reinforcement in dental restoratives. Other important research has to include the biocompatibility and safety assessment of NCC. Clinical and experimental investigations could evaluate the NCC-based materials' biocompatibility, genotoxicity, and other adverse reactions before implementing them in dentistry.

Last but not least; the factors that include sustainability and environmental effects should not be forgotten. To assess the NCC production process's environmental impact, energy use, waste output, and resource usage, a life cycle assessment (LCA) can be performed. This assessment will help in determining the aspects that need to be changed and thus, the sustainability of the production.

Therefore, future work should focus on the optimization of ball milling process and the use of different conditions and solvents to produce better NCCs and their applications. Hence, exploring the application of NCC in certain fields, increasing the production capacity, and evaluating the viability of the material will be significant to build on the current knowledge and translate the research in the context of dentistry and other fields.

CHAPTER 6: CONCLUSION

In this study, NCC was synthesized from MCC derived from OPEFB using high-energy ball milling. The optimization of both dry and wet milling processes was carried out with ethanol as the solvent. Key conditions, such as temperature, mill speed (1500 rpm), and ball-to-powder weight ratio, were maintained constant. The milling process employed three different ball sizes in succession: 7.0 mm, 5.0 mm, and 2.0 mm diameter stainless steel balls.

The study concludes that high-energy ball milling is an effective method for converting MCC into NCC. FESEM analysis confirmed that particle size was well controlled, and the nanocrystalline nature of the cellulose was successfully developed. FTIR analysis demonstrated that the functional groups of cellulose remained intact, with no significant changes in structure during milling. PSD analysis revealed that particle size decreased with extended milling, while XRD patterns confirmed the conversion of MCC to NCC by displaying characteristic NCC peaks.

The comparison between dry and wet milling revealed that ethanol-based wet milling significantly improved size reduction efficiency and the overall quality of the NCC produced. The progressive decrease in ball size further optimized the synthesis process.

The study successfully isolated and characterized NCC from OPEFB-based MCC. Morphologically, wet-milled NCC provided a larger and more uniform particle size distribution. Structurally, wet milling better preserved the crystallinity of the NCC. Chemically, the integrity of the cellulose structure was maintained in all samples, highlighting the potential of this method for producing high-quality nanocellulose without altering its inherent properties.

Overall, the findings suggest that high-energy ball milling, particularly with ethanol as a solvent, is an efficient method for producing high-quality NCC from OPEFB-based MCC. Wet milling offers enhanced results, making it more suitable for dental applications. This study also emphasizes the potential of using agricultural byproducts to produce nanomaterials, offering a sustainable and cost-effective approach for generating NCC with tailored properties for the dental field.

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