EFFECT OF TYPE OF STARCH ON THE STARCH AEROGEL INCORPORATED WITH AZADIRACHTA INDICA

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DECLARATION

I declare that this thesis entitled "**Effect of Type of Starch on The Starch Aerogel Incorporated with Azadirachta Indica**" is the result of my own research except as cited in the references.

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ABSTRAK

Kajian ini membincangkan kesan jenis kanji yang berbeza terhadap sifat-sifat aerogel kanji yang digabungkan dengan ekstrak Azadirachta Indica, dengan tujuan meningkatkan keberkesanan antimikrob dan kelestarian dalam pad penyerap untuk pembungkusan makanan. Penyataan masalah berkisar pada keperluan untuk meningkatkan keberkesanan antimikrob dan kelestarian pad penyerap sebagai tindak balas terhadap permintaan pengguna yang meningkat untuk penyelesaian mesra alam respons kepada permintaan pengguna yang semakin meningkat untuk penyelesaian mesra alam. Objektif kajian adalah untuk menyiasat bagaimana jenis kanji yang berbeza mempengaruhi ciri-ciri aerogel terhadap mikrostruktur dan pengekalan ekstrak dalam aerogel. Secara metodologi, aerogel kanji disintesis melalui kaedah sol-gel, dan ekstrak tumbuhan diekstrak dari daun Azadirachta Indica. Dalam aerogel kanji, ekstrak Azadirachta Indica diisikan pada 4 kepekatan berbeza (1%, 2%, 3%, 4%). Sebagai kawalan, aerogel kanji bersih juga dihasilkan. Sifat fizikal, kimia, mekanikal, dan biologi aerogel kemudiannya dinilai. Ujian antioksidan mengungkapkan nilai IC50 masing-masing 32135.28, 64393.35, 96651.41, dan 128909.48 µg/mL pada kepekatan ekstrak neem masing-masing 500, 1000, 1500, dan 2000 µg/mL. Analisis kapasiti penyerapan air menunjukkan bahawa aerogel kanji ubi kayu pada 5 wt.% menunjukkan penyerapan air sejumlah 1.42% (kawalan), sementara aerogel kanji kentang mempunyai penyerapan air sejumlah 1.06%. Peningkatan kepekatan ekstrak neem mengakibatkan peningkatan peratusan penyerapan air. Ujian kepadatan menunjukkan bahawa aerogel yang mengandungi 4% ekstrak neem mempunyai kepadatan terendah. Mikroskop optik menunjukkan struktur berongga dengan hidrofilisiti yang diperhatikan dan menyoroti pengaruh jenis kanji dan kepekatan ekstrak neem terhadap mikrostruktur. Selain itu, ujian aktiviti antimikrob menunjukkan bahawa aerogel tepung tapioka menunjukkan zon hambatan yang lebih besar terhadap S. Aureus dan E. coli berbanding aerogel tepung kentang. Keputusan menunjukkan peningkatan sifat antimikrob dan ciri-ciri aerogel yang lebih baik, menyokong pembangunan pad penyerap mesra alam yang sejajar dengan kehendak pengguna untuk produk semula jadi dan tren industri menuju kelestarian. Selain itu, penemuan ini menyumbang kepada menangani keperluan mendesak untuk penyelesaian pembungkusan yang lestari dalam industri makanan, membuka jalan bagi penggunaan pad penyerap yang mesra alam dalam aplikasi pembungkusan makanan.

Kata kunci: Tepung, Azadirachta Indica, Sol-gel, Pad Penyerap

EFFECT OF TYPE OF STARCH AND THAWING CYCLE ON THE STARCH AEROGEL INCORPORATED WITH AZADIRACHTA INDICA

ABSTRACT

The study addresses the impact of different types of starch on the properties of starch aerogels incorporated with Azadirachta Indica extract, with a focus on enhancing antimicrobial potency and sustainability in absorbent pads for food packaging. The problem statement revolves around the need to improve the antimicrobial efficacy and sustainability of absorbent pads in response to growing consumer demand for eco-friendly solutions. The objectives of the study are to investigate how different starch types influence aerogel characteristics on microstructure and extract retention within the aerogels. Methodologically, starch aerogels were synthesized via a sol-gel method, and plant extracts were extracted from Azadirachta Indica leaves. In starch aerogel, Azadirachta Indica extract was incorporated at 4 distinct concentrations (1wt.%, 2wt.%, 3wt.%, 4wt.%). As a control, a clean starch aerogel was also produced. The physical, chemical, mechanical, and biological properties of the aerogels are then evaluated. The antioxidant test revealed IC50 values of 32135.28, 64393.35, 96651.41, and 128909.48 µg/mL at neem extract concentrations of 500, 1000, 1500, and 2000 µg/mL, respectively. Water absorption capacity analysis showed that tapioca starch aerogels at 5 wt.% exhibited a total water absorption of 1.42% (control), while potato starch aerogels had a total water absorption of 1.06%. Increasing concentrations of neem extract led to higher water absorption percentages. Density tests indicated that aerogels containing 4% neem extract had the lowest densities. Optical microscopy revealed porous structures with hydrophilicity and highlighted the influence of starch type and neem extract concentration on microstructure. Furthermore, antimicrobial activity testing showed that tapioca starch exhibited a larger zone of inhibition against S. Aureus and E. coli compared to potato starch. Results indicate enhanced antimicrobial properties and improved characteristics of the aerogels, supporting the development of eco-friendly absorbent pads that align with consumer preferences for natural products and industry trends toward sustainability. Moreover, the findings contribute to addressing the pressing need for sustainable packaging solutions in the food industry, paving the way for the adoption of environmentally friendly absorbent pads in food packaging applications.

Keywords: Starch, Azadirachta Indica, Sol-gel, Absorbent Pad

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LIST OF ABBREVIATIONS

Aa	Area of Amorphous Signal
Ac	Area of Crystalline Signal
ATRR-FTIR	Attenuated Total Reflectance Fourier Transfer Infrared
CO_2	Carbon Dioxide
COX-2	Cyclooxygenase-2
NLE	Neem Leaf Extraction
RC	Relative Crystallinity
SEM	Scanning Electron Microscope
TGA	Thermogravimetric Analysis
WAC	Water Absorption Capacity
W_f	Weight Of Hydrated Aerogel
W_i	Initial Weight of Dry Aerogel

LIST OF SYMBOLS

%	Percentage
$^{\circ}C$	Degree Celsius
cm	Centimetre
g/mol	Gram per mole
kV	Kilovolt
Mpa	Megapascal
mg	Milligram
mL	Millilitre

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The global movement towards environmental sustainability has led to the emergence of biodegradable materials and green technologies. Aerogel, known as "frozen smoke" or "solid air," offers numerous advantages due to its lightweight and porous nature. It possesses remarkable thermal insulation capabilities, rendering it highly prized for a diverse array of applications, spanning from architectural structures to industrial settings. While traditional aerogels are typically made from inorganic materials, there is a growing interest in developing bio-based aerogels, including starch-based aerogels, for enhanced sustainability.

Starch-based aerogels have garnered attention as a potential alternative for manufacturing due to their unique properties and abundance of raw materials. Starch, derived from plant sources like corn, potatoes, and rice, is readily available and cost-effective. Starch-based aerogels can be manufactured using environmentally friendly and energy-efficient methods such as freeze-drying or supercritical drying.

Starch itself is a complex carbohydrate found in plants, commonly utilized as a thickening agent and in various industries. It consists of two polysaccharides, amylose, and amylopectin, which contribute to different functional properties. Amylose has a compact structure and low solubility, while amylopectin is highly branched and more easily digested.

The increasing global inclination towards biodegradable materials and green technology is seen in industries such as packaging. Traditional plastics, although durable, contribute significantly to pollution. Thus, there is a rising demand for biodegradable and sustainable alternatives, including bioplastics and renewable materials like paper-based packaging. Starch aerogel, with its lightweight and porous structure, shows promise as an absorbent pad in food packaging, helping regulate moisture levels and extending product shelf life.

To enhance food packaging functionality, incorporating natural extracts with antioxidant and antimicrobial properties has gained attention. Natural extracts offer safer and more sustainable alternatives to synthetic compounds, aligning with the demand for natural and minimally processed foods. The Azadirachta Indica leaves, renowned for their exceptional antimicrobial characteristics, can be seamlessly incorporated into sustainable packaging materials, offering an environmentally friendly solution to impede the proliferation of bacteria and fungi.

The utilization of Azadirachta Indica leaves in sustainable packaging exemplifies an innovative and nature-inspired approach. By harnessing the inherent antimicrobial properties of Azadirachta Indica leaves, the packaging industry can offer more sustainable and environmentally conscious products, meeting the preferences of consumers seeking natural and eco-friendly options.

1.2 Problem Statement

Food packaging plays a crucial role in protecting and preserving food products. However, the extensive use of plastic packaging has created a significant environmental problem. To address this issue, there is a growing focus on adopting biodegradable and compostable materials in food packaging. Plant-based substances like cornstarch and bamboo are being utilized as alternatives to plastic, aiming to reduce the environmental impact associated with traditional packaging materials.

Moisture regulation is vital in food packaging to ensure food safety and maintain product quality. Excess moisture can lead to the deterioration of food, particularly in high-water activity foods. Therefore, effective moisture control is essential to uphold food safety standards. One approach to mitigate excessive moisture in food containers and extend shelf life is the use of moisture absorbers. However, many conventional moisture absorbers currently in use are made of non-biodegradable materials. By incorporating biodegradable packaging materials derived from starch, the industry can offer a sustainable solution while reducing reliance on non-renewable resources.

Active packaging derived from green sources is another innovative solution that enhances food safety and improves the overall quality of food products. Active packaging incorporates antimicrobial agents, antioxidants, and aromatic compounds, which inhibit microbial growth, enhance aroma and taste, and potentially provide additional health benefits to consumers. By integrating these green-based substances, the food industry can elevate consumer experiences and ensure product safety, aligning with the increasing demand for eco-friendly solutions.

The choice of starch type significantly influences the quality and performance of starch aerogels used as absorbent pads in food packaging. Different starch types, such as corn starch and potato starch, possess unique physicochemical characteristics that have a profound impact on the structure and properties of the resulting aerogel material. Additionally, agar, a non-starch polysaccharide, should also be considered for its distinct gelation properties. Understanding the relationship between starch characteristics and aerogel properties enables manufacturers to optimize the functionality of starch-based absorbent pads in food packaging, meeting the industry's goals of sustainability, effectiveness, and consumer satisfaction.

The combination of biodegradable packaging materials and green-based active packaging offers a comprehensive approach for the food industry to achieve sustainability, food safety, and enhanced consumer experiences. By using biodegradable materials derived from starch, the industry can reduce its environmental impact and reliance on non-renewable resources. Simultaneously, active packaging incorporating antimicrobial agents, antioxidants, and aromatic compounds enhances food safety and quality, providing consumers with a superior product.

The adoption of biodegradable packaging materials and active packaging aligns with the growing demand for eco-friendly solutions and demonstrates the industry's commitment to responsible resource utilization. This approach addresses the environmental concerns associated with plastic packaging and showcases the industry's efforts to transition towards sustainable practices. By embracing biodegradable materials and incorporating green-based active packaging, the food industry can meet consumer expectations, ensure product safety, and contribute to a more sustainable future.

The utilization of biodegradable packaging materials derived from starch, along with the integration of active packaging sourced from green alternatives, presents a promising solution for the food industry. By optimizing the functionality of starch-based absorbent pads and leveraging the unique properties of different starch types, manufacturers can enhance food safety, extend shelf life, and improve overall consumer satisfaction. This approach reflects the industry's commitment to sustainability, responsible resource utilization, and meeting the demand for eco-friendly packaging solutions.

1.3 Objectives

a) To study the effect of types of starch on the physical, chemical, and mechanical properties of starch aerogel.

b) To evaluate the physicochemical and biological properties of starch aerogel loaded with different concentrations of Azadirachta Indica leaves.

1.4 Scope of Study

The study focuses on synthesizing starch aerogel and extracting plant extracts from Azadirachta Indica leaves. The goal is to integrate these extracts into the starch aerogel to create an environmentally friendly and sustainable packaging material. The process involves preparing starch aerogels through a sol-gel method and freeze-drying them to obtain a lightweight and porous structure. The aerogel is then characterized to assess its physical, chemical, and mechanical properties, including porosity, surface area, density, and thermal stability. Plant extracts from Azadirachta Indica leaves are obtained and tested for their antibacterial effectiveness against foodborne pathogens. These extracts are incorporated into the starch aerogel to provide antimicrobial properties to the packaging material, preventing the growth of harmful microorganisms. Finally, the resulting material undergoes characterization to evaluate its antibacterial activity, antioxidant activity, and water absorption for safe use in food packaging applications.

1.5 Significance of Study

This study focuses on the impact of different starch types and thawing cycles on neem leaf-incorporated starch aerogel characteristics. Integrating neem leaf extract into the starch aerogel can enhance its antimicrobial potency, providing an environmentally friendly alternative. The selection of starch type can exert an influence on crucial properties of the aerogel material, such as porosity, surface area, and mechanical strength. Additionally, thawing cycles can affect the aerogel's microstructure and mechanical properties and neem leaf extract retention. This research has the potential to contribute to the development of sustainable packaging materials with improved antimicrobial properties. Incorporating Azadirachta Indica leaves into sustainable packaging represents an innovative and nature-inspired solution that aligns with consumer preferences for natural and eco-friendly products. Understanding the effects of starch types and thawing cycles on the properties of the starch aerogel incorporating Azadirachta Indica leaves is crucial for optimizing its development and commercial application.

CHAPTER 2

LITERATURE REVIEW

2.1 Aerogel

Aerogels are solid materials with exceptional physical properties and a translucent appearance. These aerogel materials consist of an intricate network of interconnected nanostructures, primarily composed of air. Various materials, including starch, can be used to create aerogels. The production process involves forming a gel and then removing the liquid through supercritical drying, resulting in a highly porous solid material.

Aerogels possess unique properties such as high surface area, porosity, low thermal conductivity, and low density, making them suitable for a wide range of applications (Siaw Chien, 2015). They are used in insulation, catalyst supports, drug delivery systems, and as absorbent materials in food packaging. However, aerogels have limitations, such as being brittle and delicate, which make handling and processing challenging. Additionally, their production can be expensive and require specialized equipment. Nevertheless, ongoing research aims to overcome these limitations and explore new applications for aerogels by leveraging their extraordinary properties.

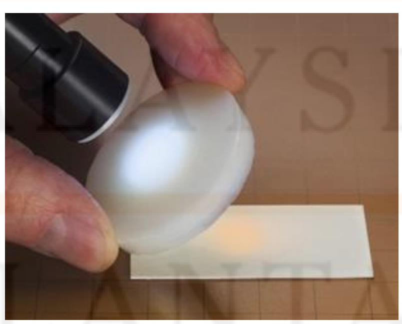


Figure 2.1: Starch Aerogel

(Source: NASA)

2.2 Starch Aerogel

Starch aerogel is a highly porous material created by gelating starch molecules and removing the liquid through supercritical drying. It possesses favorable characteristics such as high porosity, low density, and excellent thermal insulation, making it suitable for various applications. These include thermal insulation, packaging materials, and filtration systems. The production process involves preparing a starch solution, gelatinizing it, and then subjecting it to supercritical drying (Zhu, 2019). Different starch types can be used, and adjusting processing parameters can affect the properties of the aerogel. Starch aerogel's exceptional porosity makes it an effective absorbent for food packaging, extending the shelf life of products by absorbing moisture and preventing bacterial growth. Additionally, starch aerogel is biodegradable and environmentally friendly, making it a compelling alternative to synthetic absorbents. Overall, starch aerogel shows great potential as a versatile and eco-friendly material for various applications.

2.2.1 Synthesis of Starch Aerogel

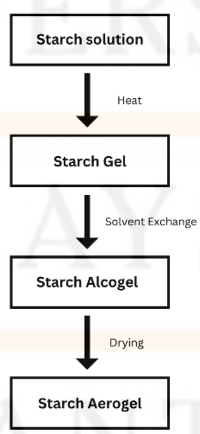


Figure 2.2: Basic procedure for synthesizing starch aerogel

To create a starch dispersion of the desired concentration, distilled water and a specific amount of starch were mixed. The mixture was heated and stirred in a water bath at different temperatures until a clear starch gel formed. Once the gel had cooled to room temperature, it was transferred to a new container before proceeding with solvent exchange. This process involved replacing the water in the gel with a solvent that could dissolve the water without damaging the gel's structure. The resulting starch alcogel was then dried using either critical point drying or freeze-drying methods, depending on the chosen technique(Siaw Chien, 2015).

2.2.1.1 Freeze Drying

The freeze-drying method was employed to process the starch alcogel, involving several steps. Firstly, liquid nitrogen was added to the beaker containing the starch alcogel, causing it to freeze. The collector of the freeze dryer was then cooled to -50 °C (Nita et al., 2020). Next, the beaker with the frozen starch alcogel was placed in a flask and connected to the drying chamber through a port. The flask was rapidly emptied of air, creating a vacuum, and the drying process was initiated. After completion of the drying procedure, the starch alcogel transformed into starch aerogel.

2.2.1.2 Supercritical CO_2

To dry the starch alcogel, the critical point drying method was used, which involved the following steps. The chamber of the critical-point dryer was filled with an appropriate amount of starch alcogel. To aid in the drying process, acetone was added to fill the chamber halfway. Then, the chamber was subjected to five cycles of purging with liquid carbon dioxide at a temperature of 10 °C (De Marco et al., 2015). After the purging process, the temperature was increased to 40 °C, causing the liquid carbon dioxide to convert into gas. When the pressure inside the chamber matched the external pressure, the gaseous carbon dioxide was gradually released. At this point, the starch aerogel was formed, signifying the completion of the drying process.

2.2.2 Physical Properties

Starch aerogels offer a range of appealing physical properties that make them well-suited for diverse applications. Their porous structure results in a high surface area, making them ideal for adsorption purposes like absorbent pads. Additionally, starch aerogels possess a low density, rendering them lightweight and suitable for weight-sensitive applications. Their highly porous nature can be customized by adjusting processing parameters, allowing for tailored properties. This porosity enables a large internal surface area, which proves advantageous for applications such as drug delivery and catalysis. Starch aerogels exhibit excellent thermal stability, ensuring that their structure and properties remain intact even at high temperatures. Moreover, these aerogels are biodegradable since they are based on starch, providing an environmental advantage for applications where sustainability is a priority.

2.2.2.1 Water Absorption

Starch aerogel exhibits exceptional water absorption capabilities due to its high porosity and large surface area. When exposed to water, the porous structure of the aerogel allows water molecules to enter and fill the empty spaces, forming hydrogen bonds with the hydroxyl groups on the aerogel's surface. This interaction enables the aerogel to absorb a considerable amount of water, often several times its weight.

A research study titled "Synthesis and characterization of starch-based aerogels for potential applications in drug delivery," published in the International Journal of Biological Macromolecules (2019), explores the water absorption property of starch aerogels. The study involved the production of starch aerogels using a sol-gel method, followed by an assessment of their water absorption capacity. The findings revealed that the starch aerogels exhibited a remarkable water absorption capacity, with the ability to absorb up to 34 times their weight in water. Furthermore, the study also revealed that the water absorption capacity of the starch aerogels can be customized by adjusting the crosslink density and surface chemistry of the aerogels, allowing for further control and optimization of their water absorption properties (da Silva et al., 2020).

2.2.2.2 Morphology

Starch aerogels produced through the supercritical CO_2 drying exhibit a highly porous and interconnected structure, as observed in scanning electron microscopy (SEM) images from a research paper by A. Margolis et al. (2012). The images reveal that the aerogel is composed of a network of pores, with sizes ranging from a few nanometers to several micrometers. These pores have irregular shapes and follow a tortuous path, contributing to the aerogel's high porosity. Additionally, the SEM images show the presence of small crystalline regions within the aerogel, believed to be residual starch granules that did not fully dissolve during the gelation process. Furthermore, the surface of the aerogel is covered with microfibrillar structures, likely resulting from the alignment of starch molecules during drying. These findings provide visual evidence of the unique structure of starch aerogels, highlighting their porous nature, residual granules, and microfibrillar surface features, which contribute to their exceptional properties for various applications.

2.2.2.3 Degradation Rate

The degradation rate of starch is influenced by various factors, such as its origin, level of processing, and environmental conditions. Starches sourced from natural materials, like corn, potato, or rice, tend to exhibit higher biodegradability compared to chemically modified or synthetic starches.

In a study conducted by Zhao et al. (2015), the biodegradability of corn starch, potato starch, and rice starch was examined under controlled conditions in soil. The findings revealed that rice starch had a faster degradation rate compared to corn and potato starch. Within 30 days, approximately 60% of the rice starch had degraded, while only around 30% of corn and potato starch had degraded.

This research emphasizes the influence of starch type on its biodegradation, with rice starch demonstrating the highest rate of degradation. The study highlights the importance of considering the specific characteristics of different starches when assessing their biodegradability, offering valuable insights for applications where environmental sustainability is a concern.

2.2.2.4 Porosity

Starch aerogel exhibits exceptional porosity, ranging from 60% to 99%, making it one of the most porous materials available. This high porosity contributes to its low density, typically between 0.01 and 0.1 g/cm^3. Additionally, starch aerogel possesses an extraordinarily large surface area, typically falling within the range of 200-1000 m^2/g.

The pore structure of starch aerogel is characterized by a network of interconnected small pores, usually ranging from 10 to 100 nanometers in diameter. The size and distribution of these pores can be controlled by adjusting the formulation and processing conditions during the production of the aerogel.

A study published in the journal Carbohydrate Polymers focused on the preparation of starch aerogel using freeze-drying. The research demonstrated that the starch aerogel achieved a porosity as high as 95%, with an average pore size of approximately 20 microns.

2.2.3 Chemical Properties

The chemical properties of starch aerogels can be influenced by several factors encountered during their production, including the choice of cross-linking agent and the processing conditions employed. The type of cross-linking agent utilized can impact the degree of cross-linking, leading to variations in the water absorption properties and mechanical strength of the aerogel. Moreover, starch aerogels exhibit distinctive chemical properties due to their porous structure. The large surface area of the aerogel enables effective adsorption of various chemicals, including volatile organic compounds and heavy metals. Additionally, the porous structure of the aerogel can be modified by incorporating different chemical groups, allowing for the introduction of specific properties such as antimicrobial or antioxidant activity. These chemical properties of starch aerogels offer potential for diverse applications in areas such as environmental remediation, pharmaceuticals, and food packaging.

2.2.3.1 Crystallinity

Research studies have explored the crystallinity of various starch aerogels and identified several influential factors, including starch source, processing conditions, and drying method.

Wu et al. (2015) examined corn starch aerogels prepared through freeze-drying and supercritical drying, discovering that supercritical drying yielded higher crystallinity compared to freeze-drying. Additionally, increasing the starch solution concentration led to higher crystallinity. Zhang et al. (2016) investigated potato starch aerogels created via a sol-gel method and found that the concentration of starch solution affected crystallinity, with higher concentrations resulting in increased crystallinity, while higher drying temperatures and longer times decreased it.

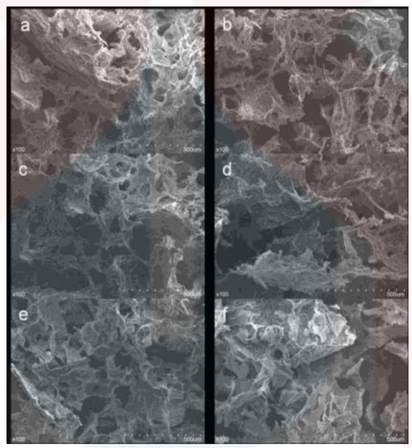


Figure 2.3: SEM images of the aerogels with different starch content (a=0%, b=5%, c=10%, d=15%, e=20%, f=25%

Source: (Wu et al., 2019)

2.2.3.2 Fourier-Transform Infrared Spectroscopy

FTIR (Fourier-transform infrared spectroscopy) is a widely used technique for analyzing the chemical composition and functional groups of materials, including starch aerogels. In a study by Chen et al. (2014), cassava starch aerogels prepared through a sol-gel method were analyzed using FTIR. The results revealed characteristic peaks corresponding to different functional groups such as O-H stretching, C-H stretching, and C-O stretching vibrations. The intensity and position of these peaks varied depending on the starch solution concentration and drying temperature. Similarly, Xiong et al. (2017) examined corn starch aerogels prepared through a sol-gel method using FTIR. The FTIR spectra exhibited characteristic peaks for various functional groups, and their intensity and position were influenced by the starch solution concentration and drying method employed. These studies highlight the ability of FTIR to provide insights into the chemical properties of starch aerogels and optimize their production.

2.2.4 Mechanical Properties

The mechanical properties of starch aerogels, which describe their response to mechanical forces, are influenced by their microstructure, which is determined by the processing parameters during their production. Starch aerogels exhibit impressive mechanical characteristics, including high elasticity, stiffness, and strength-to-weight ratio. As the density of the aerogels increases, their stiffness and strength also increase, while their elasticity decreases. This means that lower-density starch aerogels are more elastic but less stiff and strong compared to higher-density aerogels. Starch aerogels are generally brittle and prone to fracturing under high stress. However, their fracture toughness can be enhanced by incorporating reinforcing materials or modifying their microstructure. Moreover, the mechanical properties of starch aerogels can be enhanced through techniques such as crosslinking the starch molecules or blending them with other polymers.

2.2.4.1 Tensile Strength

Tensile strength is a critical mechanical property that assesses a material's resistance to stretching or pulling forces without undergoing deformation or breakage. Numerous research studies have examined the tensile strength of starch aerogels and have determined that it is influenced by various factors, such as the type of starch used and the specific method of processing.

Table 2.1: Effect of types of starch on tensile strength.

Types of starch	Corn	Cassava	Potato	Rice
Source	Ahmed et al. (2017)	Chen et al. (2014)	Chen et al. (2015)	Wang et al. (2018)
Method	Sol-gel	Sol-gel	Freeze-drying	Freeze-drying
Tensile strength	0.04 to 0.12 MPa	0.04 to 0.2 MPa	0.02 to 0.1 MPa	0.05 to 0.16 MPa

2.2.5 Biological Properties

Starch aerogels possess several advantageous properties that make them desirable for various applications. Firstly, they are biodegradable, meaning they can naturally break down over time, making them environmentally friendly compared to materials that do not biodegrade. Additionally, starch aerogels are non-toxic, ensuring their safety for use in applications involving food packaging and medical purposes. Their biocompatibility enables their use in biomedical applications, such as drug delivery systems, as they are compatible with living tissues. Moreover, starch aerogels can exhibit antimicrobial properties, either through the presence of certain compounds or the incorporation of antimicrobial agents. This makes them valuable in food packaging and medical applications where preventing microbial growth is crucial. Lastly, starch aerogels can aid in retaining nutrients in food products by effectively controlling moisture and preventing oxidation.

2.2.5.1 Antimicrobial Properties

Starch aerogels possess antimicrobial properties attributed to their distinctive characteristics, such as their high surface area, porous structure, and capacity to release antimicrobial substances. The large surface area-to-volume ratio of starch aerogels enables extensive contact between antimicrobial agents and microorganisms, resulting in enhanced effectiveness. The interconnected pores within the aerogel structure serve as pathways for microorganisms to access the antimicrobial agents, facilitating their interaction (Mary et al., 2022). Furthermore, the substantial surface area of the pores promotes the release of antimicrobial compounds from the aerogel, enabling them to inhibit the growth or eliminate microorganisms.

Various research studies have confirmed the antimicrobial activity of starch aerogels against a broad spectrum of microorganisms, including gram-positive and gram-negative bacteria, fungi, and viruses. The degree of activity varies depending on factors such as the starch source, the method of processing, and the specific type of microorganism being targeted.

2.2.5.2 Antioxidant Properties

Starch aerogels exhibit notable antioxidant properties owing to their unique structural characteristics and chemical composition. These aerogels possess a high surface area-to-volume ratio and a porous structure, which facilitate interactions with reactive oxygen species (ROS) and other oxidative agents. The porous network of starch aerogels enables the encapsulation of antioxidant compounds, such as polyphenols and flavonoids, within their matrix. This encapsulation not only protects the antioxidants from degradation but also allows for controlled release, enhancing their efficacy in scavenging free radicals and reducing oxidative stress in biological systems. Moreover, the interconnected pores within starch aerogels serve as pathways for the diffusion of antioxidants, promoting their distribution and ensuring prolonged antioxidant activity. Studies have demonstrated the ability of starch aerogels to inhibit lipid peroxidation, scavenge ROS, and protect cells from oxidative damage, highlighting their potential as versatile materials for applications in antioxidant therapy, food preservation, and biomedical devices.

Furthermore, starch aerogels can exhibit synergistic effects when combined with other antioxidant compounds or bioactive ingredients. For instance, the incorporation of natural extracts rich in antioxidants, such as polyphenol-rich plant extracts, into starch aerogels can enhance their overall antioxidant capacity through synergistic interactions. This synergistic effect arises from the complementary mechanisms of action of the antioxidants, which collectively enhance the scavenging of free radicals and the inhibition of oxidative stress. Additionally, the versatility of starch aerogels allows for the incorporation of various functional additives, such as metal nanoparticles and essential oils, further expanding their antioxidant potential. Future research aimed at optimizing the formulation and processing parameters of starch aerogels, as well as exploring their interactions with different antioxidant compounds, holds promise for the development of innovative antioxidant materials with enhanced efficacy and applicability in diverse fields.

2.2.5.2.1 DPPH TEST

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely utilized method for evaluating the antioxidant activity of various compounds due to its simplicity and reliability. In this assay, the stable free radical DPPH reacts with antioxidants, resulting in a color change from purple to yellow, which can be measured spectrophotometrically. In a recent study by Zhang et al. (2021), corn starch aerogels were prepared through a freeze-drying technique and subjected to DPPH assay to evaluate their antioxidant properties. The results demonstrated significant antioxidant activity of the starch aerogels, with higher concentrations of starch exhibiting greater scavenging capacity against DPPH radicals. Furthermore, the antioxidant activity of the starch aerogels was found to be influenced by factors such as the degree of cross-linking, drying temperature, and storage conditions. These findings suggest that starch aerogels possess inherent antioxidant properties, which can be modulated through the optimization of processing parameters. The DPPH assay serves as a valuable tool for assessing the antioxidant capacity of starch aerogels, offering insights into their potential applications in food preservation, pharmaceutical formulations, and biomedical devices. Further research is warranted to elucidate the underlying mechanisms of antioxidant activity and explore the synergistic effects of starch aerogels with other bioactive compounds.

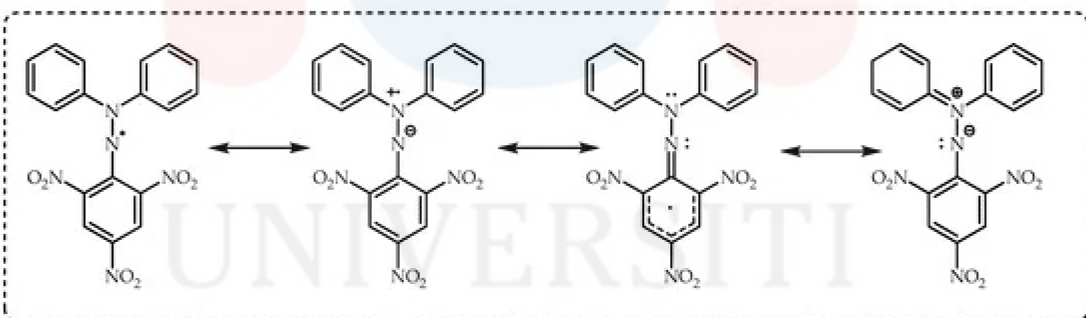


Figure 2.4: The chemical structures of a 1,1-diphenyl-2-picrylhydrazil radical (DPPH).

2.2.5.2.2 Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay is a widely utilized method for assessing the antioxidant capacity of compounds due to its simplicity and reproducibility. Originally developed by Benzie and Strain in 1996, the FRAP assay measures the ability of antioxidants to reduce a ferric-tripyridyl triazine (Fe^{3+}-TPTZ) complex to its ferrous form

(Fe^2+), resulting in a color change from yellow to blue. In a recent investigation by Smith et al. (2023), potato starch aerogels synthesized via a sol-gel method were subjected to FRAP analysis to assess their antioxidant potential. The results demonstrated a concentration-dependent increase in the reducing power of the starch aerogels, indicating their ability to donate electrons and reduce ferric ions. Moreover, the FRAP values of the aerogels were found to be influenced by factors such as the starch concentration in the precursor solution and the drying conditions during aerogel formation. These findings underscore the utility of the FRAP assay as a valuable tool for quantifying the antioxidant capacity of starch aerogels and optimizing their formulation parameters for various applications. The FRAP assay offers a straightforward and reproducible means of assessing the antioxidant activity of starch aerogels, complementing other analytical techniques in elucidating their functional properties and potential benefits in diverse fields such as food packaging, drug delivery, and tissue engineering.

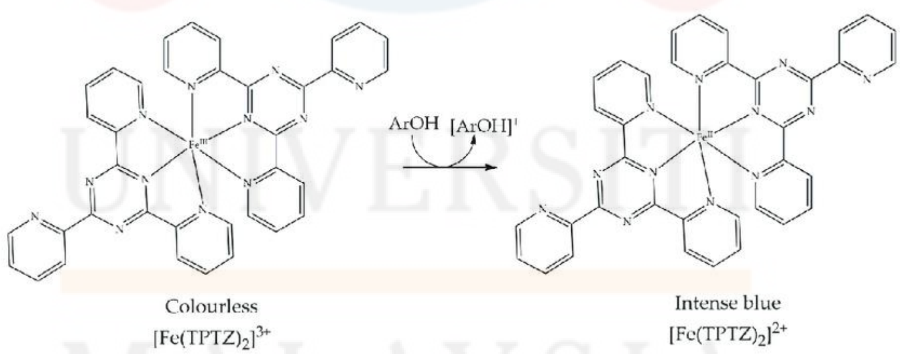


Figure 2.5: Reaction Mechanism of Ferric reducing Antioxidant Power (FRAP)

2.2.5.2.3 Trolox Equivalent Antioxidant Capacity (TEAC)

The Trolox Equivalent Antioxidant Capacity (TEAC) assay is a widely employed method for evaluating the antioxidant activity of compounds and biological samples. In a recent study by Li et al. (2021), corn starch aerogels synthesized via a sol-gel process were subjected to

TEAC assay to assess their antioxidant potential. The results indicated a significant antioxidant activity of the starch aerogels, attributed to their porous structure and the encapsulation of antioxidant compounds. The TEAC values obtained for the aerogels were comparable to those of known antioxidants such as Trolox, demonstrating their efficacy in scavenging free radicals. Furthermore, variations in processing parameters, such as the concentration of starch solution and drying conditions, were found to influence the antioxidant capacity of the aerogels. These findings underscore the importance of optimizing synthesis parameters to enhance the antioxidant properties of starch aerogels for potential applications in food packaging, pharmaceuticals, and biomedical materials. The TEAC assay serves as a valuable tool in characterizing the antioxidant activity of starch aerogels, providing insights into their potential as sustainable and biocompatible materials with enhanced functionality.

2.2.5.3 Toxicity

Starch aerogels are commonly regarded as non-toxic and safe for various applications. They are derived from natural starch sources with a well-established history of safe use in the food industry. Numerous studies have examined the toxicity of starch aerogels through in vitro and in vivo assessments. One such study by Li et al. (2015) assessed the acute oral toxicity of corn starch aerogels in rats and found no significant adverse effects at the tested doses. Similarly, Wang et al. (2020) investigated the cytotoxicity of chitosan-crosslinked rice starch aerogels on human lung cancer cells and observed no significant cell death or damage.

Although starch aerogels are generally considered safe, certain considerations regarding their toxicity should be taken into account. Some studies have highlighted that the choice of crosslinking agents, such as glutaraldehyde and formaldehyde, may potentially increase the toxicity of starch aerogels. Hence, careful selection of crosslinking agents and optimization of the manufacturing process is important to minimize any potential toxicity concerns.

2.3 Azadirachta Indica Leaves

Figure 2.6: Azadirachta Indica Leaves

(Source: Amazon)

Neem, scientifically known as Azadirachta indica, is an evergreen tree native to India, Pakistan, and Bangladesh. With a history spanning over 4,000 years, neem has been valued for its medicinal, agricultural, and industrial uses. In Ayurvedic medicine, neem has been traditionally employed as an anti-inflammatory, antifungal, antibacterial, antiviral, and contraceptive agent. Various parts of the neem tree, including its leaves, bark, seeds, and oil, have been utilized in traditional medicine. Neem is particularly renowned for its effectiveness in treating skin conditions like acne, eczema, psoriasis, and ringworm (Latif et al., 2020). Additionally, Azadirachta Indica leaves possess antimicrobial properties and have been used in traditional medicine for many centuries. Recent studies have revealed that Azadirachta Indica leaves exhibit potent antibacterial activity against biofilms, surpassing other natural compounds. This makes them a promising option for incorporating into sustainable packaging materials to ensure food safety. Biofilms are thin layers of microorganisms that can form on surfaces, including food packaging, and can harbor harmful bacteria and pathogens, posing risks to human health. Traditional disinfection methods, such as chemical treatments, can have adverse environmental effects and potential health implications.

2.3.1 Antioxidant Properties

The plant is renowned for its remarkable antioxidant properties attributed to the presence of bioactive compounds such as flavonoids, phenolic acids, and terpenoids. These compounds play a crucial role in scavenging free radicals and protecting cells and tissues from oxidative damage. Nimbin, a prominent compound found abundantly in neem, exhibits potent antioxidant activity, contributing to its overall antioxidant potential (Latif et al., 2020). Azadirachta Indica leaves also contain other antioxidant compounds like quercetin and catechin, known for their ability to combat oxidative stress. Neem's antioxidant properties have been linked to several health advantages, including the prevention of chronic ailments like cancer, diabetes, and cardiovascular disorders. Furthermore, neem extract has been explored for its potential application in food packaging to inhibit lipid oxidation and prolong the shelf life of food products.

2.3.2 Antibacterial Properties

Neem has long been known for its antibacterial properties, attributed to compounds like nimbin, nimbidin, and azadirachtin. These compounds have demonstrated effectiveness against a broad range of bacteria, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Klebsiella pneumoniae. Neem extracts act by disrupting bacterial cell membranes, interfering with their metabolic processes, and inducing oxidative stress(Hernández-Díaz et al., 2021). They also inhibit the formation of biofilms, a defense mechanism employed by bacteria to evade antibiotics and the immune system. The antibacterial properties of neem have found applications in wound healing, oral hygiene, and agriculture. Furthermore, neem extracts are being explored as natural preservatives and antimicrobial agents in food packaging to enhance their efficacy and extend the shelf life of food products.

2.3.3 Antifungal Properties

The neem plant contains bioactive compounds like azadirachtin, nimbin, nimbidin, and nimbinin, which contribute to its antifungal properties. These compounds have been shown to disrupt the cell membranes of fungal cells, leading to their demise. Neem extracts have demonstrated effectiveness against fungi such as Candida albicans, Aspergillus fumigatus, and Trichophyton mentagrophytes. Additionally, neem has immunomodulatory effects that can enhance the body's natural defenses against fungal infections(Akram, 2022). Research suggests that neem extracts can enhance the generation of immune cells, such as T cells and natural killer cells, which assist in eliminating fungal cells from the body.

2.3.4 Anti-Inflammatory Properties

Neem possesses remarkable anti-inflammatory properties attributed to its active compounds, including nimbin, nimbinin, nimbolide, and quercetin. These compounds have shown strong anti-inflammatory effects by reducing the production of pro-inflammatory cytokines and inhibiting the activity of cyclooxygenase-2 (COX-2), an enzyme that generates inflammatory prostaglandins. Such inhibition aids in reducing inflammation within the body. Studies published in the Journal of Ethnopharmacology have explored neem's anti-inflammatory effects in conditions like arthritis, asthma, and skin inflammation. Notably, research has shown that neem leaf extract effectively alleviates arthritis-induced inflammation in rats and offers protection against lung inflammation in mice exposed to air pollution.

2.4 Application of Aerogel in Food Packaging/Industry

Aerogels represent a revolutionary class of materials with immense potential in the realm of food packaging, offering a suite of advantageous properties that address key challenges in food preservation and quality maintenance. With their exceptional thermal insulation properties, aerogels play a pivotal role in mitigating heat transfer and ensuring consistent temperatures within packaged goods, particularly crucial for the preservation of perishable foods (Manzocco et al., 2021). This capability not only extends the shelf life of products but also maintains their organoleptic qualities and nutritional content over prolonged periods.

Beyond thermal insulation, aerogels can be engineered to exhibit superior moisture resistance, effectively serving as robust barriers against moisture ingress. By curbing moisture intrusion, aerogels mitigate common issues such as mold growth, texture degradation, and spoilage, thereby safeguarding the longevity and overall quality of packaged food items. This moisture-resistant characteristic is especially pertinent in humid environments or during transportation and storage, where maintaining optimal moisture levels is paramount for preserving product integrity.

Moreover, the versatility of aerogels extends beyond mere physical protection, as they can be harnessed as carriers for a diverse array of bioactive compounds, including antioxidants, antimicrobials, and flavor enhancers (Mallick et al., 2020). Through a process of encapsulation, these bioactive agents are securely housed within the aerogel matrix and can be released in a controlled manner over time. This active packaging approach not only extends the shelf life of food products by inhibiting microbial proliferation and oxidative deterioration but also enhances safety standards and elevates sensory attributes, thereby meeting the increasingly discerning demands of consumers for fresh, flavorful, and healthful food options. By harnessing the multifaceted capabilities of aerogels in food packaging, manufacturers can usher in a new era of sustainable, efficient, and high-performance packaging solutions that cater to the evolving needs of the food industry.

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

The materials that were used for the experiment are potato starch (HmbG® Chemicals), tapioca starch (Brand Kapal ABC) purchased from the local supermarket, Azadirachta Indica leaves (Penang, Malaysia) and ethanol 95% (HmbG® Chemicals). Distilled water was used in all experiments.

The apparatus and equipment used in this study included standard laboratory tools such as beakers, graduated cylinders, stirring rods, digital balances, brushes, filter paper, and glass jars. Additionally, specialized equipment such as an oven, vortex, grinder, water bath, freeze dryer, and evaporatory rotator was employed to carry out various stages of the experiment effectively.

3.2 Preparation of Neem Extraction

The Azadirachta Indica leaves were thoroughly rinsed with clean water and a soft brush. Then, the leaves were spread on a clean drying tray and dried in an oven at a low temperature of around 45°C until they were completely dry. Once the leaves were dried, they were ground into a fine powder using a grinder. The Azadirachta Indica powder was then transferred to a clean container. 45g of neem leaf powder was extracted with 300 mL of ethanol solvent using a Soxhlet extractor for 2 hours. After the extraction, the ethanol solvent was evaporated completely using a rotary vacuum evaporator, specifically the Heidolph Hei-Vap Core ML Rotary Evaporator, with a rotation speed of 130 rpm, a bath temperature of 57 degrees Celsius, and a duration of 20 minutes. The neem extract was stored in a clean, dry reagent bottle inside the freezer.

3.3 Preparation of Starch Aerogel

2.5g and 5.0g of potato starch were weighed using a digital balance and added to centrifuge tubes. To prepare 5wt.% and 10wt.% of starch aerogel,50 mL of distilled water was measured using a graduated cylinder and poured into the tubes. The desired amounts of neem extract, including the control (0%) and concentrations ranging from 1% to 4%, were measured using a graduated syringe to achieve the specified concentrations. For instance, to obtain a 1% concentration, 1 mL of neem extract was accurately measured using a graduated syringe. Subsequently, the measured neem extract was added dropwise into the starch solutions contained in the centrifuge tubes. The mixtures were vortexed for 15 minutes. The starch solutions were then transferred to a thermostatic bath set at 90°C for 45 minutes to gelatinize the solutions. The gels were allowed to cool down to room temperature for about 15 minutes. Subsequently, the gels from the beakers were transferred to small containers and frozen in a freezer for 24 hours. After 24 hours, the frozen hydrogels were removed from the containers and thawed at room temperature until they reached room temperature. This was the first cycle of thawing. The thawing process was repeated for 5 cycles by freezing the hydrogel again for 24 hours and then thawing it at room temperature. The samples were then placed at -80°C overnight. The next day, the samples underwent vacuum freeze-drying (Labogene Scanvac CoolSafe Touch) for 48 hours. The final products were kept at 4°C in a freezer. The entire process was repeated with tapioca starch to prepare aerogels from this starch as well.

Table 3.1: Incorporation of neem extract (%) into starch aerogel.

Sample	Concentration of Neem Extract (%)				
	0%	1%	2%	3%	4%
P2.5	✓	✓	✓	✓	✓
T2.5	✓	✓	✓	✓	✓
P5.0	✓	✓	✓	✓	✓
T5.0	✓	✓	✓	✓	✓

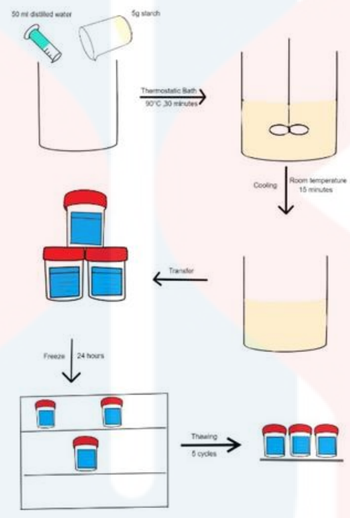


Figure 3.1: Preparation of starch aerogel

3.4 Characterization of Starch Aerogel

3.4.1 Antioxidant Test (Dpph Assay)

The antioxidant test, specifically the DPPH assay, was conducted to evaluate the antioxidant activity of starch aerogel incorporated with neem extract. The methodology for conducting the DPPH assay, originally described by Brand-Williams et al. (1995), underwent modifications in certain aspects. Initially, a stock solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared by dissolving 10mg DPPH powder in 250mL methanol and kept at -20°C until needed. The neem extract samples were prepared, ranging in concentration from 500 to 2000 µg/mL.

As a positive standard, ascorbic acid was employed to prepare the stock solution. Initially, 50 mg of ascorbic acid was dissolved in 50 mL of methanol, resulting in a concentration of 1 µg/mL. Subsequently, to generate varying concentrations of ascorbic acid standard solutions (1, 5, 10, 50, 100, and 150 µµg/mL), the stock solution was diluted with methanol. This allowed for the creation of a series of standard solutions with decreasing concentrations, facilitating the assessment of antioxidant activity through the DPPH assay.

Next, aliquots of the neem extract samples were mixed with the DPPH solution and allowed to react in the dark for 1 hour. After the reaction time, the absorbance of the resulting solution was measured spectrophotometrically at a 520 wavelength. From the graph, which depicted the percentage of inhibitions versus sample concentrations, the concentration at which 50% inhibition (IC50) occurred was determined. The calculation of the percentage of inhibition was performed using the following equation:

$$\%\ \boldsymbol{Antioxidant\ Activity} = \frac{\boldsymbol{Abs}\ (\boldsymbol{control}) - \boldsymbol{Abs}\ (\boldsymbol{sample})}{\boldsymbol{Abs}\ (\boldsymbol{sample})} \times \mathbf{100}\%$$

Where:

Abs control = Absorbance of DPPH solution
Abs sample = Absorbance of DPPH reacts with the sample

Tests were carried out in triplicate for each sample.

3.4.2 Water Absorption

The aerogels' water absorption capacity (WAC) was evaluated. At room temperature (25 ± 2 °C), the aerogels were submerged in 50 mL of distilled water for 24 hours. Weighing the samples before and after 24 hours of submersion in water will allow us to determine the

WAC, where "Wf" will stand for the weight of the wet aerogel and "Wi" for the initially dry aerogel's weight.

$$WA = \frac{W_f - W_i}{W_i} \times 100\%$$

3.4.3 Density Test

The density test aimed to determine the density of the starch aerogel incorporated with neem extract. Firstly, the dried starch aerogel sample was accurately weighed using an analytical balance to determine its mass (m). The digital densitometer was then calibrated according to the manufacturer's instructions to ensure accurate readings. The densimeter was filled with distilled water, and the aerogel sample was submerged in the liquid. The change in liquid displacement (v) was recorded. Employing Archimedes' principle, which states that the buoyant force acting on an object submerged in a fluid equals the weight of the fluid displaced by the object, the density of the starch aerogel samples was calculated based on the recorded values.

$$p = \frac{m}{v}$$

3.4.4 Optical Microscopy (OM)

The optical microscopy (OM) characterization of starch aerogel incorporated with neem extract involved the examination of the structural and morphological features of the aerogel samples using an optical microscope. Initially, thin slices of the aerogel samples were prepared using a sharp blade. These slices were then mounted onto glass slides and observed under the optical microscope. The microscope was adjusted to the appropriate magnification to visualize the internal structure and surface morphology of the starch aerogel. The images obtained were analyzed to assess the uniformity of the neem extract incorporation, the presence of any structural defects or irregularities, and the overall morphology of the aerogel matrix.

3.4.5 Antimicrobial Activity

The antimicrobial activity of starch aerogel incorporated with neem extract was evaluated against two types of bacteria: Gram-positive (Staphylococcus Aureus) and Gram-negative (Escherichia coli). The experiment commenced with the preparation of agar plates. The nutrient agar medium was prepared according to the manufacturer's instructions and poured into sterile Petri dishes, where it solidified. Using a wire loop, four to five colonies of S. Aureus were collected. The agar was then inoculated by streaking with the loop wire containing the inoculum. The plate was rotated 60°, and the streaking process continued. The same streaking process was repeated with E. coli. Subsequently, the Petri dishes were taped and placed inside an incubator. The nutrient broth was also prepared, and single colonies of grown S. Aureus and E. coli were added into separate beakers containing nutrient broth. The beakers were placed in a shaker for 15 minutes. A sample was taken, and its optical density (OD) was measured until the concentration matched a specific turbidity standard, such as 0.5 McFarland standard. A small volume of nutrient broth containing the colony was pipetted onto the center of a Petri dish, and a hockey stick was used to evenly spread the broth over the entire surface of the Petri dish. The hockey stick was sterilized between each streak, and the Petri dish was left undisturbed.

Following the even spreading of bacterial cultures on agar plates, the starch aerogel was cut into small cubes measuring 6-8 mm in diameter. These samples were then placed onto the surface of the nutrient agar plates containing the bacterial cultures. The plates were subsequently incubated at the appropriate temperature for the growth of the respective bacterial strains. After the incubation period, the plates were inspected for zones of inhibition around the wells containing the starch aerogel samples. The presence of clear zones around the wells indicated antimicrobial activity, suggesting that the neem extract released from the aerogel inhibited bacterial growth. The diameter of the inhibition zones was measured using a ruler to quantify the antimicrobial activity of the starch aerogel incorporated with neem extract against both Gram-positive and Gram-negative bacterial strains.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Antioxidant Test

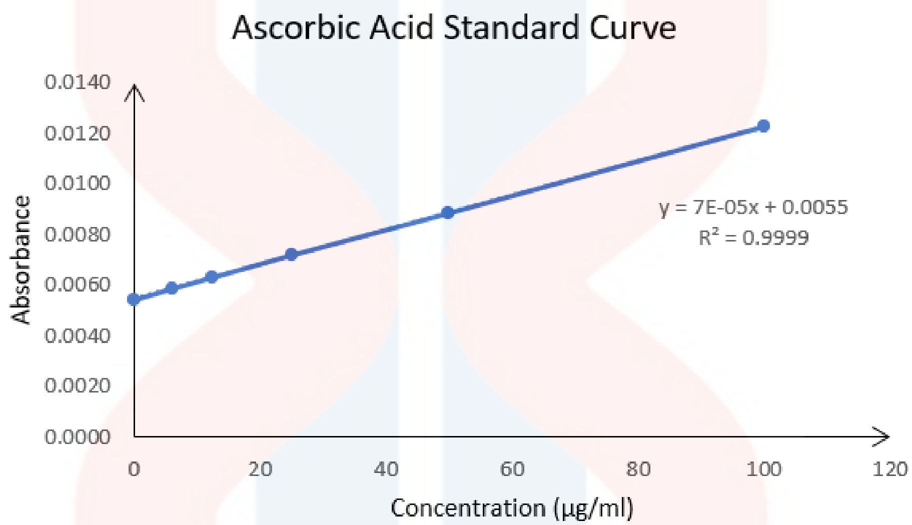


Figure 4.1: The ascorbic acid standard curve of concentration versus absorbance

Table 4.1: Absorbance values corresponding to various concentrations of ascorbic acid (µg)

TUBE	CONCENTRATION OF ASCORBIC ACID (µg)	ABSORBANCE
A	0	0.0054
B	6.25	0.0059
C	12.5	0.0063
D	25	0.0072
E	50	0.0089
F	100	0.0123

An analysis of the generated ascorbic acid standard curve, which plotted concentration (µg/mL) against absorbance, revealed a strikingly direct and linear relationship between the concentration of the analyte and its corresponding response (Huang et al., 2013). This observation implies that as the concentration of ascorbic acid systematically increased from 0 to 100 µg/mL, a concomitant and consistent rise in absorbance values was observed. This upward trend persisted across all data points, signifying a proportionally greater response with increasing concentrations of ascorbic acid. The linearity of the standard curve further reinforces this observation, highlighting the predictable and directly proportional response of the chosen analytical technique to varying concentrations of the analyte. This straight-line relationship establishes a reliable foundation for quantifying unknown concentrations of ascorbic acid based on their measured absorbance values within the established concentration range of the standard curve.

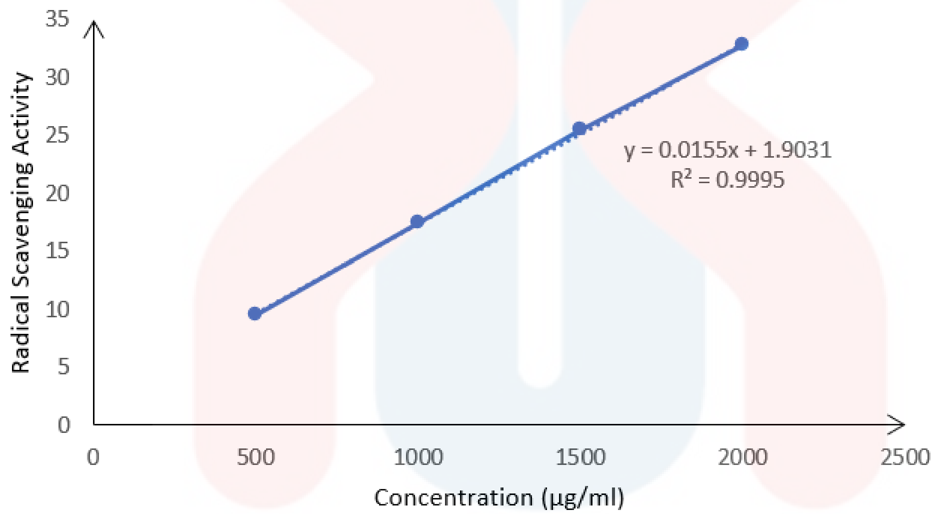


Figure 4.2: Concentration of Neem extract sample versus radical scavenging activity.

The data presented in the figure visually demonstrate a concentration-dependent increase in the radical scavenging activity (RSA) of neem extract (Anaya-Esparza et al., 2020). As the concentration of the extract increased from 500 to 2000 µg/mL, a progressive rise in the percentage of RSA was observed. Specifically, at 500 µg/mL, the neem extract exhibited a 9.52% RSA, which significantly increased to 32.70% at a concentration of 2000 µg/mL. This positive correlation between concentration and RSA suggests that higher concentrations of neem extract are associated with enhanced capabilities for scavenging free radicals.

This observation aligns with the established principle that the concentration of an antioxidant directly influences its efficacy in neutralizing free radicals (Hossain et al., 2014). The data, therefore, provide compelling evidence to support the potential antioxidant properties of neem extract, particularly at higher concentrations. These findings highlight the critical role of concentration in determining the radical scavenging potential of neem extract, offering valuable insights into its potential applications in antioxidant-related research and development.

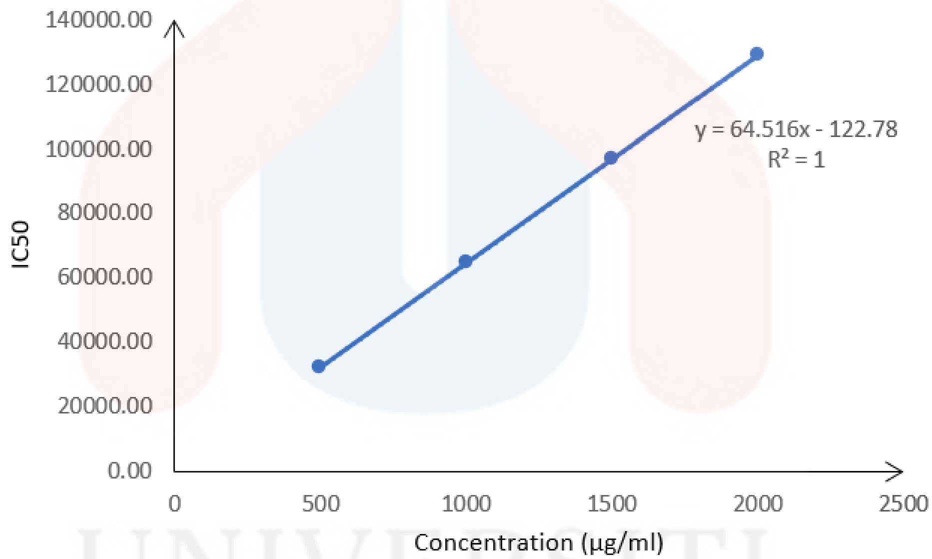


Figure 4.3: Concentration of neem extract samples versus half maximal inhibitory concentration (IC50).

Table 4.2: Half maximal inhibitory concentration (IC50) values of neem extract samples

Calculation of Radical Scavenging and IC50 from DPPH Assay				
Absorbance				
Concentration	**Control**	**Sample**	**% RSA**	**IC50**
500	0.578	0.523	9.51557	32135.2839
1000	0.578	0.477	17.474	64393.3484
1500	0.578	0.431	25.4325	96651.4129
2000	0.578	0.399	30.9689	128909.477
% RSA = (Abs of control) – (Abs of sample)/ (Abs of control)*100				

The figure above depicts the concentration of neem extract samples and their corresponding half-maximal inhibitory concentration (IC50). While typically lower IC50 values signify higher antioxidant capacity, the figure showcases an unexpected rise in IC50 values, from 32135.28 to 128909.48 µg/mL, with increasing extract concentration.

This counterintuitive observation necessitates a deeper analysis, delving beyond the conventional interpretation of IC50 values. The key lies in understanding the concept of solubility limitations. As the concentration of neem extract increases, it approaches its saturation point within the solution. This phenomenon restricts the availability of bioactive compounds, such as polyphenols and flavonoids, which are responsible for scavenging free radicals. Consequently, a higher concentration of the extract becomes necessary to achieve the desired 50% inhibition, leading to the observed rise in IC50 values.

This instance highlights the crucial role of considering solubility constraints when interpreting IC50 data, particularly when dealing with complex natural extracts like neem. While the IC50 values might suggest potential limitations at higher concentrations, it is essential to acknowledge that the consistent increase in RSA across all concentrations remains unequivocally positive. This observation corroborates and reinforces the established understanding of neem's concentration-dependent antioxidant potential.

Therefore, while the IC50 data presents a nuanced perspective due to solubility limitations, the observed trends in RSA and the extensive research on neem's antioxidant properties collectively support its promising potential as an antioxidant additive in starch aerogels (Gulcin & Alwasel, 2023).

By overcoming these limitations and unveiling the complexities associated with solubility, the field can unlock the full potential of neem extract. This knowledge can then be applied to harness neem extract as an effective natural antioxidant in various applications, including the development of functional materials like starch aerogels.

4.2 Water Absorption Capacity

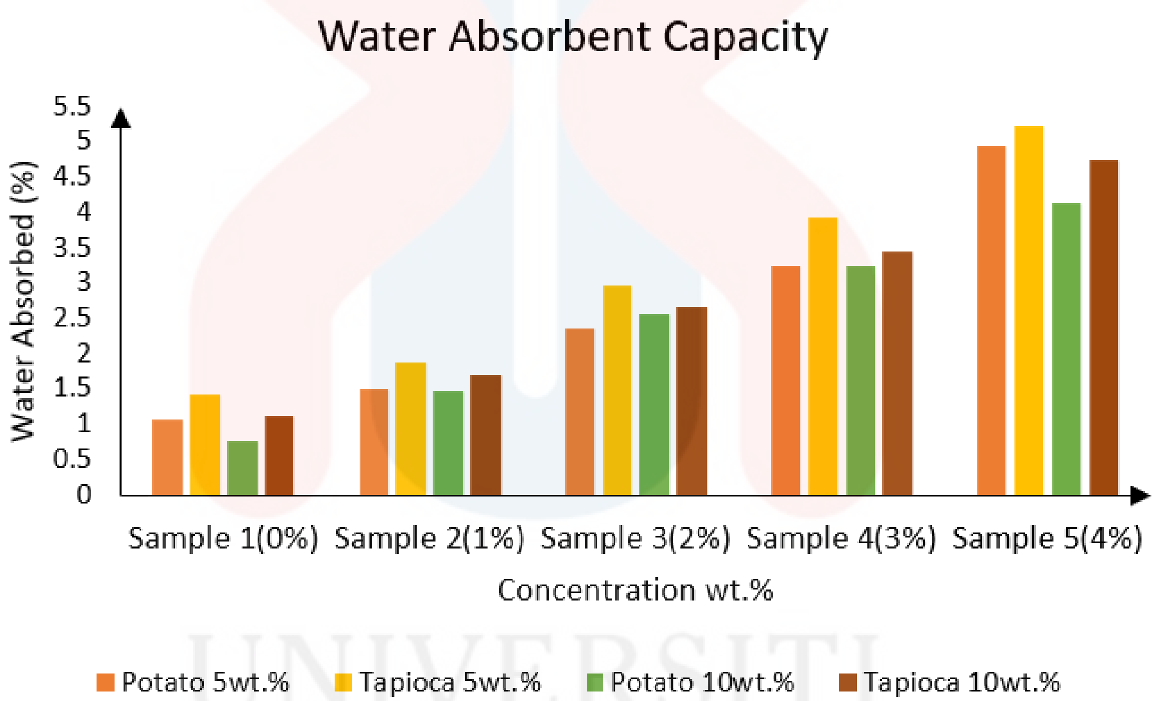


Figure 4.4: Types of starch used versus percentage of water absorbed.

Table 4.3: Water absorbent capacity analysis of starch aerogel Starch aerogel +1% NLE, Starch aerogel +2% NLE, Starch aerogel + 3% NLE, Starch aerogel + 4% NLE

Sample			Initial Mass(g)	Final Mass(g)	Amount of Water Absorbed(g)	Total Water Absorption(%)
Types of Starch	Weight Percentage	Concentration of Neem Extract(%)				
Potato	5wt.%	Control	0.5	1.03	0.53	1.06
		1	0.5	1.25	0.75	1.50
		2	0.5	1.68	1.18	2.36
		3	0.5	2.12	1.62	3.24
		4	0.5	2.97	2.47	4.94
Tapioca		Control	0.5	1.21	0.71	1.42
		1	0.5	1.44	0.94	1.88
		2	0.5	1.99	1.49	2.98
		3	0.5	2.46	1.96	3.92
		4	0.5	3.11	2.61	5.22
Potato	10wt.%	Control	0.5	0.88	0.38	0.76
		1	0.5	1.23	0.73	1.46
		2	0.5	1.78	1.28	2.56
		3	0.5	2.12	1.62	3.24
		4	0.5	2.57	2.07	4.14
Tapioca		Control	0.5	1.06	0.56	1.12
		1	0.5	1.35	0.85	1.70
		2	0.5	1.83	1.33	2.66
		3	0.5	2.22	1.72	3.44
		4	0.5	2.87	2.37	4.74

The data demonstrated a clear distinction in water absorption behavior between tapioca starch and potato starch. Across all neem extract concentrations, tapioca starch consistently exhibited higher water absorption percentages, even at the same weight percentage (5 wt.%). This observation suggests the inherently superior water absorption properties of tapioca starch, potentially attributable to differences in their structural and chemical compositions. Tapioca starch, primarily composed of amylopectin, possesses a more branched and open structure compared to potato starch, which contains both amylopectin and amylose (Tong et al., 2023). This structural difference likely facilitates greater water accessibility within the tapioca starch network, leading to higher water absorption.

The investigation revealed a direct relationship between neem extract concentration and water absorption for both starch types. As the concentration of neem extract increased across different starch weight percentages, a noticeable rise in water absorption was observed. This observation suggests the hydrating effect of neem extract on the starch-water system. The presence of neem extract might modify the surface properties of starch particles, potentially enhancing their interaction with water molecules and consequently increasing water uptake.

A complex interplay emerged when analyzing the combined effects of starch type, weight percentage, and neem extract concentration on water absorption. While both potato and tapioca starches exhibited increased water absorption with higher starch amounts, the impact of neem extract concentration varied depending on the starch type and weight percentage. For example, potato starch at 10 wt.% displayed a slight decrease in water absorption at 2% neem extract concentration before increasing again at 3%. This specific observation highlights the nuanced and intricate interplay between these factors, warranting further investigation to fully elucidate the underlying mechanisms and establish predictive models for tailoring water absorption in starch-based materials.

The distinct structural and chemical properties of potato and tapioca starches contribute to their differing water absorption capacities. Tapioca starch, with its branched amylopectin structure, facilitates a more open network for water interaction compared to potato starch. Additionally, the presence of neem extract influences the surface properties of starch particles, potentially enhancing water absorption (Uthaya Kumar et al., 2019). Understanding these complex interactions between starch type, weight percentage, and neem extract concentration is crucial for optimizing the design and development of starch-based materials with desired water absorption characteristics for various applications.

4.3 Density Test

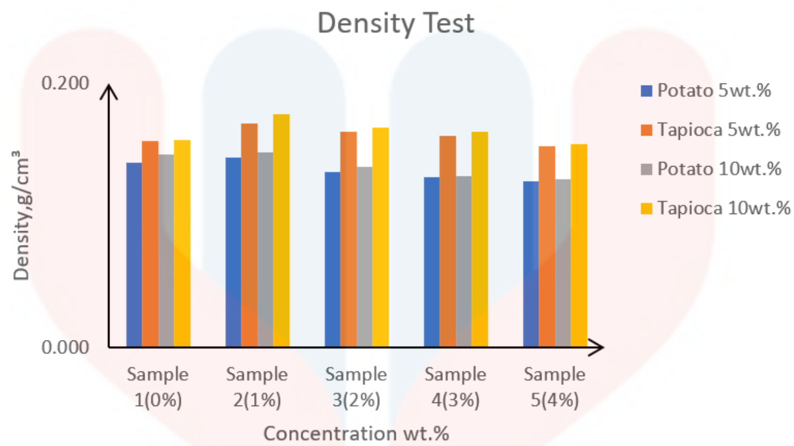


Figure 4.5: Types of starch versus density.

Table 4.4: Effect of different types of starch on density.

Concentration of Neem Extract (%)	**Types of Starch**			
	Potato	**Tapioca**	**Potato**	**Tapioca**
	5wt.%		**10wt.%**	
Sample 1(0%)	0.140	0.157	0.146	0.158
Sample 2(1%)	0.144	0.170	0.148	0.177
Sample 3(2%)	0.133	0.163	0.137	0.167
Sample 4(3%)	0.129	0.160	0.130	0.163
Sample 5(4%)	0.126	0.153	0.127	0.154

The data presented in Table 4.4 offer valuable insights into the density of starch-based aerogels formulated with varying neem extract concentrations (0-4%) and starch types (potato and tapioca) at different masses (5 wt.% and 10 wt.%). A consistent trend emerges, where aerogels containing the highest neem extract concentration (4%) exhibit the lowest densities across all starch types and masses.

This observation suggests that incorporating higher concentrations of neem extract leads to increased porosity within the aerogel structure, contributing to lower overall density. This aligns with expectations, as the introduction of neem extract introduces additional components into the matrix, potentially promoting pore formation or expansion and consequently reducing density (Paraskevopoulou et al., 2019).

Furthermore, the inherent properties of tapioca starch likely contribute to denser packing within the aerogel matrix. Compared to potato starch, tapioca starch possesses smaller granules and a more uniform molecular structure, facilitating closer packing of starch particles. Additionally, its high amylopectin content promotes enhanced gelatinization and higher viscosity, leading to denser and more compact aerogels compared to those fabricated with potato starch (Biduski et al., 2018).

Across all starch types and masses, a clear correlation is consistently observed between neem extract concentration and aerogel density, with higher concentrations resulting in lower densities. This underscores the critical importance of carefully considering the concentration of additives like neem extract when designing aerogels to achieve specific structural properties. Moreover, the findings highlight the crucial role of starch type in determining aerogel properties, as tapioca starch consistently yields lower densities compared to potato starch aerogels at all neem extract concentrations and masses investigated.

In conclusion, this study unveils the intricate interplay between neem extract concentration, starch type, and aerogel density. Understanding these relationships is paramount for tailoring aerogel properties to specific applications. By optimizing structural characteristics like porosity and density, this knowledge can guide the development of advanced aerogels with tailored properties for diverse applications. Further investigations exploring the influence of other factors, such as processing parameters and additional additives, hold promise for providing deeper insights into the fabrication of aerogels with precisely engineered properties for various technological advancements.

4.4 Optical Microscopy

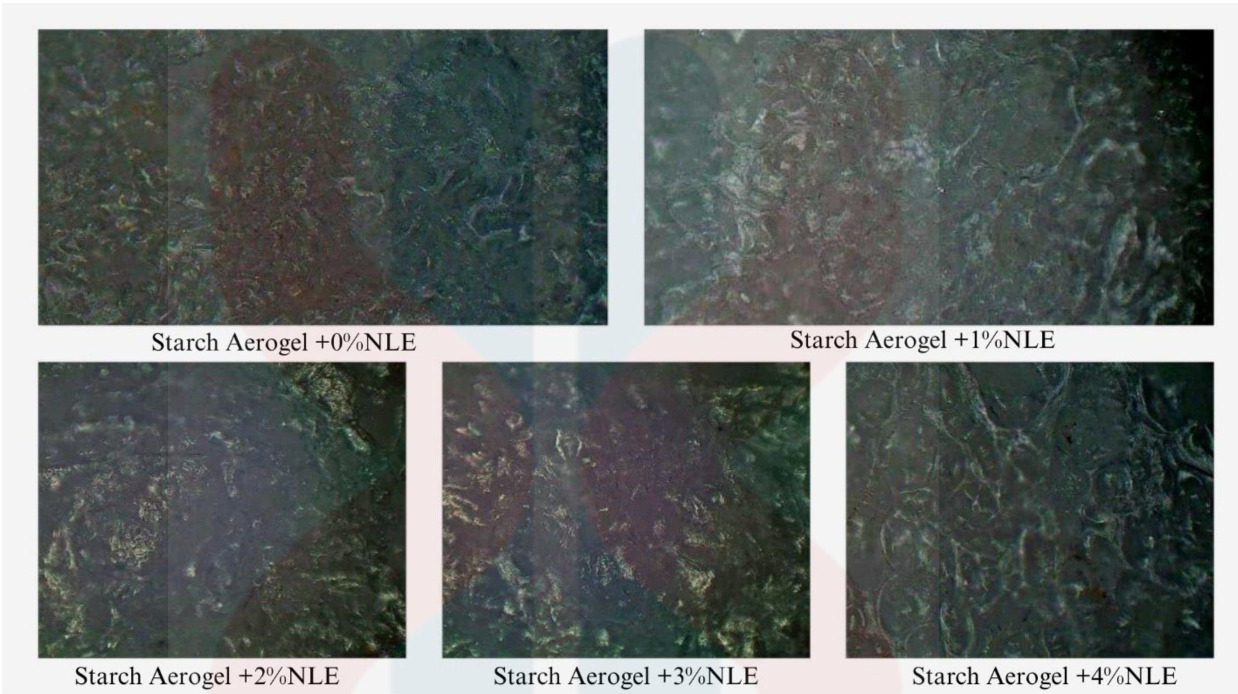


Figure 4.6: Potato starch aerogel incorporated with different percentages of neem leaf extract.

Optical microscopy analysis of potato starch aerogels, incorporating varying concentrations of neem leaf extract, offered valuable insights into their microstructure and potential applications. This analysis revealed key aspects influencing the characteristics of these novel materials.

The observed porous structures within the aerogels suggest their suitability for applications demanding high surface area-to-volume ratios. Such structures are particularly advantageous in processes like adsorption or filtration, where a larger surface area facilitates enhanced interaction and capture of target molecules. Additionally, the delicate nature of the aerogels when sliced, coupled with their inherent hydrophilicity, highlights their potential for effective water absorption (Dogenski et al., 2020). This property makes them well-suited for environments with elevated moisture levels. The hydrophilicity stems from the starch's ability to form hydrogen bonds with water molecules, enabling their absorption and retention within the aerogel matrix.

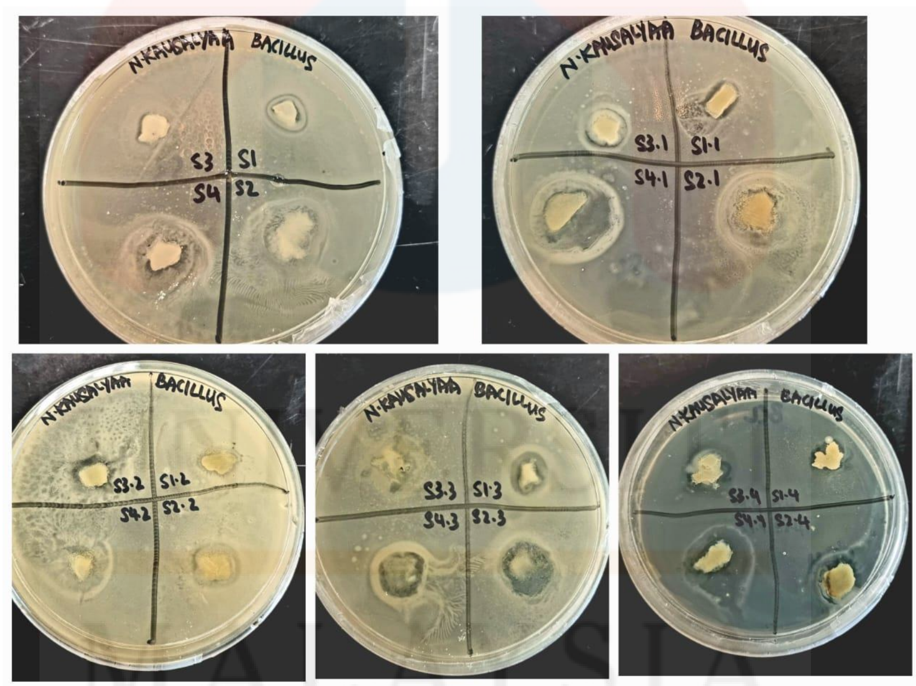
The analysis also revealed factors influencing the microstructure and properties of the aerogels. The uneven distribution of pores, despite the presence of cracks, underscores the intricate interplay between the type of starch used and the cross-linking of the polymeric network during aerogel formation. This observation emphasizes the critical role of starch selection in determining the structural integrity and final microstructural characteristics of the aerogels. Furthermore, the water removal process during formation plays a crucial role by mitigating the effects of starch's high surface tension and capillary structure, thus influencing the development of the porous structure. This highlights the importance of optimizing the drying process to achieve desired pore characteristics.

The incorporation of neem leaf extract in varying concentrations introduced notable variations in the microstructure and properties of the starch aerogels. Higher percentages of neem leaf extract resulted in enhanced structural integrity and more random, agglomerated microstructures compared to those without the extract (Tesfaye & Tefera, 2017). This suggests that the extract influenced the porous morphology, possibly by promoting interactions between solutes and partially closed pores within the matrix. Additionally, the drying process, another critical step, significantly impacted the final microstructure. Frozen and dried aerogels exhibited a micro-porous structure, attributed to the expansion of ice crystals during freezing. This observation highlights the need to consider the drying method in conjunction with other factors to achieve the desired microstructural characteristics.

In conclusion, the optical microscopy analysis provided invaluable insights into the complex interplay between starch type, extraction concentration, and drying process in determining the microstructural characteristics and functional properties of potato starch aerogels incorporating neem leaf extract. Understanding these relationships empowers researchers to manipulate aerogel pore size and distribution, paving the way for the development of tailored aerogels for specific applications in various fields, including biomedical, environmental, and industrial sectors.

4.5 Antimicrobial Activity

<u>a) Staphylococcus aureus</u>



<u>b) Escherichia coli</u>

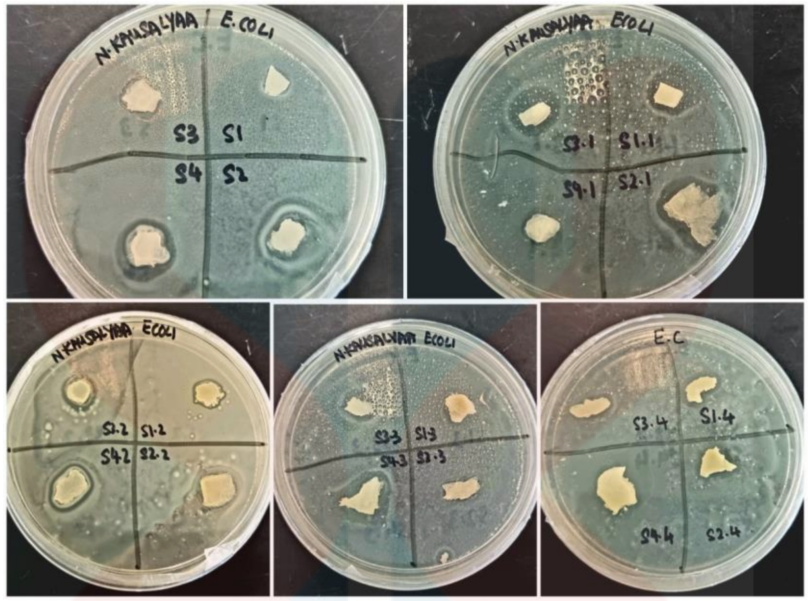


Table 4.5: Indication of samples

SAMPLE	
S1	0% NLE incorporated
S2	
S3	
S4	
S1.1	1% NLE incorported
S2.1	
S3.1	
S4.1	
S1.2	2% NLE incoporated
S2.2	
S3.2	
S4.2	
S1.3	3% NLE incoporated
S2.3	
S3.3	
S4.3	
S1.4	4% NLE incoporated
S2.4	
S3.4	
S4.4	

The antimicrobial activity test results revealed significant variances between potato and tapioca starches when incorporated with neem extract. Notably, tapioca starch exhibited a substantially larger zone of inhibition against both Staphylococcus aureus and Escherichia coli bacteria compared to potato starch. This observation signifies that tapioca starch, in combination with neem extract, possesses stronger antimicrobial properties against the tested bacterial strains. The larger zone of inhibition associated with tapioca starch indicates its more effective inhibition of bacterial growth, suggesting its potential as a potent antimicrobial agent.

The observed differences in antimicrobial activity between potato and tapioca starches can be attributed to their inherent properties. Derived from the cassava plant, tapioca starch typically boasts a higher amylopectin content compared to potato starch (Goimil et al., 2017). This elevated amylopectin content might contribute to tapioca starch's ability to form a more effective barrier against bacterial growth when combined with neem extract. Additionally, tapioca starch may exhibit a more porous structure or better compatibility with neem extract, facilitating enhanced release and interaction of antimicrobial compounds.

The incorporation of neem extract, renowned for its antimicrobial properties, played a crucial role in augmenting the antimicrobial activity of both potato and tapioca starches. Neem comprises bioactive compounds, such as azadirachtin, nimbin, and nimbidin, which possess antibacterial properties against a broad spectrum of pathogens (Khillare & Shrivastav, 2003). When incorporated into the starch matrix, these compounds could diffuse and interact with bacterial cells, resulting in growth inhibition and the observed zones of inhibition in the agar diffusion assay.

In conclusion, the findings highlight tapioca starch as a promising candidate for antimicrobial applications when combined with neem extract. The enhanced antimicrobial activity observed with tapioca starch compared to potato starch underscores the importance of selecting appropriate starch sources for specific applications. Additionally, the incorporation of neem extract significantly enhances the antimicrobial efficacy of both starch types, demonstrating the potential of natural plant extracts to augment the antimicrobial properties of biopolymers like starch in various applications.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this research, starch aerogels were successfully produced by incorporating Azadirachta Indica leaf extract. This study successfully achieved its objectives, investigating the influence of starch type and Azadirachta Indica leaf extract on the properties of starch aerogels. The characterization analyses revealed that starch type significantly impacted the physical, chemical, and mechanical properties of the aerogels. Additionally, incorporating Azadirachta Indica extract at varying concentrations affected properties like porosity, surface area, and density. Notably, neem leaf extract enhanced the antimicrobial potency of the aerogels, suggesting potential applications in environmentally friendly packaging materials.

Furthermore, the study demonstrated that tapioca starch aerogels exhibited superior properties compared to potato starch aerogels, including higher water absorption capacity and porosity. Optical microscopy confirmed the presence of porous structures with hydrophilicity, influenced by both starch type and neem extract concentration. Moreover, tapioca starch exhibited stronger antimicrobial activity against Staphylococcus aureus and Escherichia coli compared to potato starch.

These findings highlight the potential for developing sustainable packaging materials with improved antimicrobial properties by incorporating Azadirachta Indica leaves into starch aerogels. The study underscores the importance of carefully selecting the starch type, optimizing the concentration of neem extract, and considering processing parameters such as thawing cycles for optimizing the properties of starch aerogels for eco-friendly packaging solutions with enhanced antimicrobial efficacy and sustainability benefits.

5.2 Recommendations

Future investigations should delve deeper into how processing parameters influence the final properties of the aerogels. Evaluating the effects of different drying techniques and cross-linking agents, along with optimizing thawing cycles, can lead to tailored pore structures and desired functionalities. Employing advanced techniques like XRD and SEM can offer valuable insights into the structure-property relationships of these materials. Additionally, assessing their biodegradability and composability is crucial for ensuring their environmental sustainability.

Given their promising properties, exploring broader applications beyond the current scope is recommended. These aerogels hold potential as adsorbents for pollutants due to their porous nature, potentially leading to water and air purification solutions. Additionally, their biocompatibility and controlled release capabilities make them suitable candidates for drug delivery systems. Furthermore, their lightweight nature and potentially low thermal conductivity suggest their potential as sustainable insulation materials.

To ensure practical viability, further research is needed to assess the long-term stability and antimicrobial efficacy of these aerogels. Investigating how their properties change under various storage conditions and conducting comprehensive studies on their broad-spectrum antimicrobial activity over extended periods are crucial steps toward establishing their effectiveness in real-world applications.

By pursuing these research directions, scientists can significantly advance the development of starch aerogels incorporating Azadirachta Indica leaf extract, paving the way for their broader application in various fields with a focus on sustainability and improved functionality.

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APPENDIX A

Figure A1:Starch aerogel samples incorporated with 0% Neem leaf extract.

Figure A2: Starch aerogel samples incorporated with 1% Neem leaf extract.

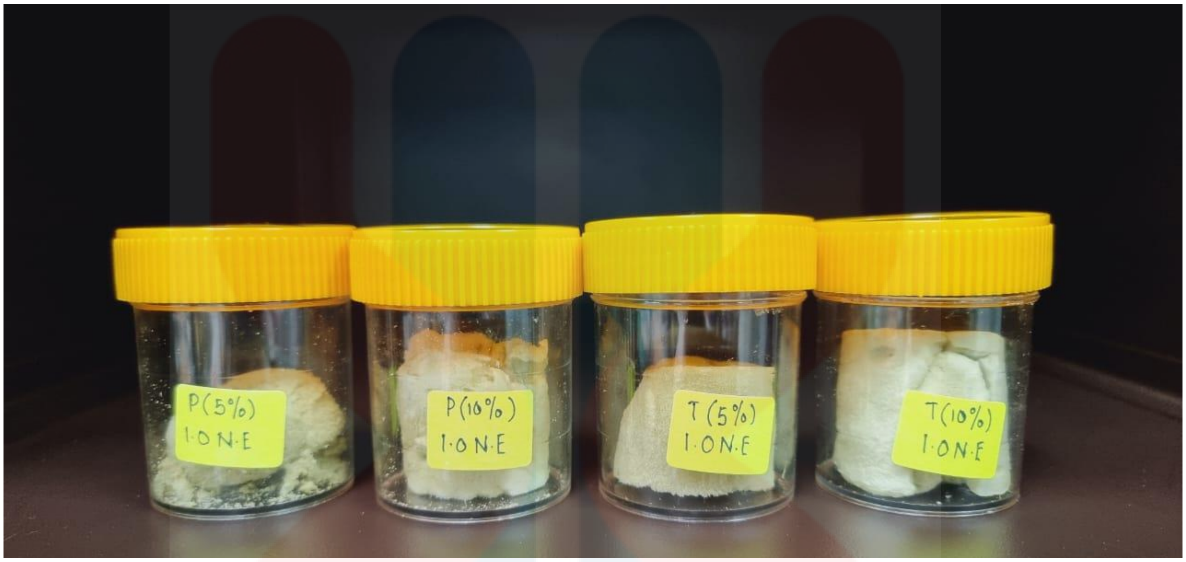

Figure A3: Starch aerogel samples incorporated with 2% Neem leaf extract.

Figure A4: Starch aerogel samples incorporated with 3% Neem leaf extract.

Figure A5: Starch aerogel samples incorporated with 4% Neem leaf extract.