CHAPTER 1

INTRODUCTION

Cellulose is the world's greatest source of organic carbon, with plants producing about 180 billion tons of it annually (Sundarraj et al., 2018). As one of the most abundant biopolymers globally, cellulose is biocompatible, biodegradable, and non-toxic, having no negative impacts on the environment or human health (Sharma et al., 2024). Since its discovery in 1838, global cellulose production has surpassed 10^{12} tons (Pitkanen et al., 2014). This polysaccharide is an essential structural element of the major cell wall in oomycetes, several types of algae, and green plants (Dufresne, 2013). Depending on the source and extraction technique, native cellulose can have a crystallinity of 40% to 70% and contains both ordered (crystalline) and disordered (amorphous) regions. Greater reactivity with other molecular groups is shown in the amorphous regions, which are less dense than the crystalline ones (Heinze, 2016). In contrast to their amorphous counterparts, crystalline domains typically exhibit greater resistance to mechanical, chemical, and enzymatic treatments.

Nanocellulose, an engineered nanomaterial (ENM) with a diameter of less than 100 nm, can be produced by bacteria or derived from plants (Thomas et al., 2018). Because of its large specific surface area, remarkable mechanical strength, lightweight nature, non-toxicity, sustainability, and superior biodegradability, it has garnered a lot of interest (Patel, 2020). Although the potential of nanocellulose for food applications was initially investigated in 1983, the high costs and energy requirements of commercialization created difficulties. Since then, extensive research has been conducted on the chemical and physical characteristics of cellulose, supported by the establishment of numerous production facilities expected to significantly increase output to several tons per day.

1.1. Sustainable nanocellulose from food waste

Nanocellulose offers intriguing applications in the food industry, including its use as emulsion stabilizers (Sarkar & Dickinson, 2020), food additives (DeLoid et al., 2018), biosensors (Schyrr et al., 2014), environmentally friendly packaging materials (Vilarinho et al., 2018), 3D-printed hydrogels (Hausmann et al., 2018), and pharmaceutical drug carriers (Sheikhi et al., 2019). The processing of fruits, vegetables, and their by-products produces a substantial amount of waste, estimated at around 0.5×10^9 tons annually (Andrade et al., 2022). Since these discarded materials are abundant, cost-effective, and rich in cellulose, they present an excellent opportunity for producing renewable and sustainable nanocellulose.

1.2. Sources of cellulose and nanocellulose

Cellulose is a linear homopolysaccharide made up of repeating β-(1-4) linked D-glucose units. Different sources of cellulose can produce various forms and structures, with properties that may vary depending on factors such as the degree of polymerization, crystallinity, and the presence of other components like hemicellulose and lignin. The specific characteristics of cellulose are influenced by its maturity, natural source, origin, pretreatment, and processing techniques (Trache et al., 2017). For instance, banana rachis and pineapple peel, both by- products of cultivation and processing, serve as excellent sources of cellulose, underscoring the importance of utilizing these materials for human benefit.

Cellulose is found in various morphological forms, including fibers, microfibrils/nanofibrils, and micro/nanocrystalline cellulose. This diversity stems from the inherent variability among organic material sources and the particular circumstances of biosynthesis and processing that affect the size and shape of cellulose particles (Seddiqi et al., 2021). By employing chemical, mechanical, biological or physical techniques, cellulose fibres can be converted into nanocellulose, which is an aqueous suspension of nanoscale fibres with a high aspect ratio, huge specific surface area, and remarkable Young's modulus (Abitbol et al., 2016). Derived from biomass, nanocellulose has gained attention as a sustainable alternative across various industries because of its remarkable physical characteristics and renewable nature (Lu et al., 2021). Bacterial nanocellulose (BC), Cellulose nanofibrils (CNFs), and cellulose nanocrystals (CNCs) are the three types of nanocellulose, each successfully isolated from numerous sources, including soy hulls, pineapple, corncobs, cassava bagasse, sugarcane bagasse, hemp fibers, rice husks, bananas, bamboo, teak wood, and wheat straw fibers (Costa et al., 2013; Dai et al., 2013; Johar et al., 2012; Kaushik and Singh, 2011; Neto et al., 2013; Pasquini et al., 2010; Silverio et al., 2013).

1.3. Extraction of nanocellulose

One effective method for extracting cellulose nanofibrils is the chemo-mechanical technique, which removes non-cellulosic components from wood and non-wood fibers through alkali treatment. This process enhances the solubility of lignin and hemicellulose by cleaving ether linkages (Xiao et al., 2001). Alkali treatment causes lignin to depolymerize and hemicellulose to partially hydrolyze, producing sugars and phenolic resin molecules that are partially soluble in water (Fernandez et al., 1999). Successive bleaching treatments can further eliminate lignin and hemicellulose, yielding pure cellulose that is ideal for extracting nanocellulose. The

nanocellulose that is produced by hydrolyzing microcrystalline cellulose with acid usually comes in a variety of sizes (Mandal and Chakrabarty, 2011). Controlled acid hydrolysis of native cellulose fibers disrupts the fibers, enabling the dispersion of rod-shaped crystalline microfibrils.

1.4. Pickering emulsion

The concept of using colloidal particles to stabilize fluid-fluid interfaces, known as Pickering emulsions (PE), was established over a century ago (Robin et al., 2022). A defining characteristic of PE is their exceptional stability, which results from solid particles adsorb irreversibly at the interfaces (Chevalier & Bolzinger, 2013). These emulsions have gained increasing attention due to their numerous advantages, such as enhanced resistance to coalescence, adjustable permeability, and favourable elastic properties (Sharkawy et al., 2020). The stability of PE stems from a densely packed layer created through the particles' irreversible adsorption at the oil/water interface, which effectively limits coalescence and Ostwald ripening (Ortiz et al., 2020).

Nanocellulose can effectively stabilize PE, where droplet stability is improved by solid particles at the liquid/liquid interface and provide an electrostatic protective barrier (Saffarionpour, 2020). Nanocellulose is highly valued in PE as an eco-friendly stabilizer, utilizing its anisotropic fiber structure to effectively stabilize oil/water interfaces even at low concentrations (Khan et al., 2018). Common stabilizers for PEs include safe inorganic particles like silica, calcium carbonate, and hydroxyapatite, as well as food-grade organic components such as proteins, lipids, and carbohydrates (Yang et al., 2017). These solid particles offer advantages over traditional surfactant-stabilized emulsions by reducing toxicity concerns. Using nanocellulose as a stabilizer aligns with the growing demand for sustainable and environmentally friendly emulsifiers (Khan et al., 2018). However, research on the use of nanocrystalline cellulose extracted from banana rachis and pineapple peel for stabilizing PE, particularly in food systems, remains limited.

1.4.1. Pickering emulsions as a carrier of bioactive compounds

In recent years, there has been an increasing focus on designing emulsions that enable the controlled release of bioactive substances into the gastrointestinal system and bloodstream (Shah et al., 2016). Moreover, PEs can be tailored to encapsulate a variety of bioactive compounds, with research exploring the encapsulation of nanoparticles either at the liquid-liquid interface or within the oil phase (Wei et al., 2022). Notably, various sources of nanocellulose, including banana peels (Costa et al., 2018), ginkgo shells (Ni et al., 2020), lemon seeds (Zhang et al., 2020), pistachio shells (Kasiri & Fathi, 2018), and bamboo shoots (He et al., 2021), have been successfully utilized to stabilize PEs.

Carotenoids are a class of naturally occurring pigments that dissolve in lipids and are present in most fruits and vegetables, including kale, tomatoes, and carrots. They are prized for their taste, color, and health benefits (Park et al., 2018). Among these, β-carotene stands out for its bioactivity (Weber & Grune, 2012). Numerous studies have been conducted to improve β- carotene's stability and bioavailability using different delivery methods, such as nanoparticles, gels, and emulsions (Mao et al., 2018). However, the limited bioavailability and poor oxidative stability of β-carotene pose challenges for its incorporation into functional foods and pharmaceuticals. Encapsulating β-carotene within lipid-based delivery systems has become a widely adopted approach to tackle these challenges (Liu et al., 2018). Food emulsions have demonstrated efficacy as β-carotene delivery strategies, facilitating its integration into oil droplets and enhancing absorption when consumed with lipids (McClements, 2010). This method improves the bioavailability of β-carotene, promoting its uptake by epithelial cells and potentially contributing to better health outcomes (Liu et al., 2018; Wei et al., 2020).

1.4.2. Importance of lipid digestion

Obesity is a major health concern due to its links to increased risks of chronic diseases. A balanced diet and regular physical activity are essential for preventing obesity, highlighting the importance of developing food-based strategies that can be easily integrated into daily routines (McClements & Demetriades, 1998). Designing foods with lower fat content while preserving desirable textures can significantly aid in reducing fat intake. Nanocellulose-stabilized Pickering emulsions present innovative opportunities for creating low-fat products.

Mayonnaise, a popular condiment, is a stable emulsion of oil and water, traditionally stabilized using egg-derived components such as egg white and egg yolk (Akhtar & Masoodi, 2022). However, the high oil content and egg components in conventional mayonnaise can pose challenges for individuals with cardiovascular diseases and egg allergies. To address these issues, incorporating Pickering nanoemulsions (PNE) can stabilise mayonnaise-like emulsions by lowering the interfacial tension between the oil and polysaccharides, which significantly influence their physicochemical properties and stability, including rheology, texture, and viscosity (Hosseini et al., 2020).

The potential of banana rachis nanocellulose (BRNC) as a stabilizer in food systems remains largely underexplored. However, incorporating Pickering nanoemulsions (PNE) of sunflower oil infused with β-carotene and stabilized with BRNC into emulsion-based gels like mayonnaise could significantly enhance the solubility and stability of β-carotene. This strategy not only improves the nutritional profile of mayonnaise but also supports lipid digestion, which is crucial for maximizing nutrient absorption. Thus, integrating BRNC into mayonnaise offers a promising approach to enhance stability while accommodating diverse dietary needs and preferences, ultimately promoting healthier eating habits.

1.5. Role of hydrogel in food system

Three-dimensional, hydrophilic materials called hydrogels have the capacity to absorb and hold large volumes of water (Goncalves et al., 2019). Hydrogels were first presented by Wichterle and Lim in 1960, and since then, they have been widely used in many different sectors, including agriculture, food, cosmetics, biomedicine, tissue engineering, and drug delivery. Natural hydrogels, in particular, have gained popularity due to their biodegradability and biocompatibility. Cellulose-based hydrogels stand out among the wide variety of natural biopolymers due to their advantageous mechanical qualities, low cost, non-toxicity, hydrophilicity, and abundance of availability (Kabir et al., 2018).

In food systems, hydrogels play a vital role in enhancing texture, moisture retention, and overall product stability. They can be used to develop innovative functional foods that not only improve sensory attributes but also offer health benefits. Effective modification is made possible by the distinct surface properties of nanocellulose structures, which enable the creation of intelligent hydrogels that react to external stimuli. Hydrogels derived from banana rachis present exciting opportunities for creating functional foods, as they can incorporate bioactive compounds, improve nutrient delivery, and enhance the overall eating experience.

1.5.1. Nanocellulose and protein as hydrogel components

Hydrogels made from natural polymers, such as proteins and polysaccharides, have recently become popular in functional foods because of their low toxicity, cost-effectiveness, edibility, biodegradability and biocompatibility (Fathi et al., 2022). Research shows that functional substances can be efficiently encapsulated in hydrogels with stable network architectures, improving their bioavailability and physicochemical stability (Mao et al., 2020).

Nanocellulose is an excellent candidate for hydrogel formulation due to its mechanical strength, high surface area, and biocompatibility. When combined with proteins such as soy protein isolate (SPI), which has a protein content of around 90%, nanocellulose can significantly enhance the properties of hydrogels (He et al., 2024). Although SPI has limitations, such as poor viscosity and mechanical strength, it also possesses benefits including swelling, solubility, foaming and gelling (He et al., 2024). SPI may interact with hydrophilic and hydrophobic materials to modify their characteristics, and it can be processed into gels (Aguilar et al., 2011) and emulsions (Song et al., 2011; Wagner & Gueguen, 1999).

In food production, SPI is frequently utilised as a dietary supplement, taking advantage of its gelling and emulsifying characteristics (Totosaus et al., 2002). The incorporation of polysaccharides into SPI can significantly enhance emulsion stability through rheological modifications and interactions with protein molecules (Pan et al., 2015). This combination can mimic the taste and texture of fat while reducing energy content, making it suitable for various food products aimed at replicating or replacing fat (Corredig et al., 2011). However, it is essential to consider that fat removal may adversely affect the structure, texture, and flavour of the final product.

1.5.2. Hydrogel-based bioactive compound encapsulation

Due to consumer demand for healthier options, the food industry is placing more and more focus on developing products enriched with bioactive agents including minerals, vitamins, and nutraceuticals (Mun et al., 2015). Nevertheless, a lot of these active compounds are susceptible to changes in storage and processing conditions, resulting in low bioavailability and stability due to factors like light exposure, pH sensitivity, and poor solubility. Nanocellulose-based hydrogels offer an innovative solution for enhancing the bioavailability and stability of bioactive compounds. These hydrogels effectively encapsulate sensitive compounds, such as carotenoids, by utilizing edible oils and fats to facilitate their dispersion (Nagao et al., 2013). The ability of various structures to transport and preserve β-carotene has been studied. These structures include emulsions, nanoemulsions, emulsion-filled hydrogels, liposomes, solid lipid particles, and lipid-based nano systems (Mun et al., 2015). Emulsion-filled hydrogels not only incorporate β-carotene into the lipid phase but can also be engineered to release entrapped oils during the intestinal phase, thereby enhancing nutrient absorption and bioavailability (Zhang et al., 2015). Consequently, one promising method for enhancing the stability and bioavailability of bioactive chemicals is the application of nanocellulose-based hydrogels, effectively addressing key challenges in both the pharmaceutical and food industries.

1.6. Aerogels: Properties and Applications

Food-grade aerogels have attracted considerable interest from both the food business and scientific community because of their remarkable qualities, which include high specific surface area, nanoporous structure, ultra-low density, and superior mechanical and thermal insulation characteristics (Selvasekaran & Chidambaram, 2021). These aerogels are primarily derived from safe-for-consumption polysaccharides such as alginate, pectin, starch, cellulose, κ- carrageenan, and konjac glucomannan (Wang et al., 2018).

To produce food-grade aerogels, three crucial steps must be followed: first, the precursor must be swollen in water or a suitable solution to form a hydrogel; second, water must be substituted with an appropriate organic solvent, usually ethanol, to form an alcogel; and third, the aqueous phase must be eliminated by freeze drying or supercritical CO2 drying to produce the final aerogel. Recent studies have investigated the incorporation of bioactive compounds, such as fruit extracts, into aerogels to enhance their functionality in food packaging, specifically targeting the preservation of meats and meat products (Rincon et al., 2023).

The unique properties of aerogels make them excellent candidates for encapsulating and releasing bioactive compounds in food systems, enhancing shelf stability and preserving nutritional quality. Their effectiveness as matrices for various compounds has garnered significant interest, underscoring their potential applications in food preservation and packaging (Oliveira et al., 2019a). With ongoing research and innovation, food-grade aerogels offer promising opportunities to improve the safety and longevity of food products.

1.6.1. Nanocellulose-based aerogels

According to some experts, materials should only be categorised as aerogels if they have meso- and macropores, which are characterised by sizes up to a few hundred nanometres and porosity greater than 95% (Garcia-Gonzalez et al., 2011). Among the various precursors used for aerogel production, polysaccharides, including pectin, chitosan, cellulose, starch, alginate, and carrageenan stand out due to their abundant natural availability. Polysaccharide-based aerogels offer significant advantages as packaging foams, contributing to efforts to reduce plastic usage.

Nanocellulose, derived from cellulose fibers, exhibits exceptional matrix properties for releasing bioactive compounds and effectively absorbing water (Oliveira et al., 2019). Aerogels based on nanocellulose have distinctive characteristics that make them ideal for a wide range of applications, including food packaging, environmental remediation, and drug delivery systems, highlighting their potential to promote both health and sustainability.

1.6.2. Incorporation of bioactive compounds in aerogels

Growing consumer awareness of the health benefits linked to nutrient-dense foods has resulted in the creation of perfect food products that incorporate functional ingredients. Vitamins, oils, and bioactive compounds are frequently added to these products to increase the amount of nutrients within them (Selvasekaran & Chidambaram, 2021). However, these functional components are often sensitive to unwanted conditions including light, high temperatures, oxygen, and moisture, which can lead to their degradation. Moreover, many bioactive compounds possess undesirable tastes and odours, which can restrict their direct application in food products.

To address these challenges, it is essential to develop suitable delivery systems for active substances (Hosokawa et al., 2018). One promising approach involves incorporating functional components into food-grade aerogels, which can act as effective carriers that control the release of bioactive compounds at targeted sites, thereby enhancing their bioavailability (de Oliveira et al., 2020). To fully realize their potential in real food systems, further development of specialized food-grade aerogels is necessary.

Although cellulose can be isolated from a variety of sources, the use of butterfly pea flower extract in this context, particularly for creating hybrid aerogels, has not yet been investigated. Butterfly pea flower has garnered attention for its diverse applications in agriculture, food (as natural colorants and antioxidants), and modern medicine (Oguis et al., 2019). These flowers offer several beneficial properties, including functioning as pH indicators, inhibiting DNA damage in cancer cells, and providing anti-inflammatory effects (Khoo et al., 2017). Despite the existing research on the bioactive components and biological activities of butterfly pea flowers, their potential application in bioactive aerogels remains unexplored.

1.7. Research Gap

Research on isolating nanocellulose from banana rachis and pineapple peel waste remains limited, with no comprehensive comparative studies. Despite the abundance of these agro-wastes, their potential for producing nanocellulose is underexplored, creating a gap in understanding how source-specific properties might impact its functionality across different applications. Limited studies have examined the use of banana rachis nanocellulose to stabilize Pickering nanoemulsions, which are gaining interest in food and pharmaceutical industries for their capacity to transport and encapsulate bioactive substances like β-carotene. However, the mechanisms and efficiency of nanocellulose in enhancing these emulsions, particularly for bioactive delivery, remain under-researched. Similarly, there is little research on nanocellulose/protein-based hydrogels using banana rachis nanocellulose to improve gelling properties in food systems. Despite the fact that hydrogels are necessary for modifying texture and regulating ingredient release, little is known about how nanocellulose, when combined with proteins, can enhance gel strength, water retention, and mechanical characteristics, which leaves potential for innovation. Additionally, the development of food-grade aerogels using banana rachis nanocellulose is scarce. Aerogels, known for their lightweight and porous structure, offer exciting potential for biodegradable packaging that can carry bioactive compounds, improving food preservation and quality. However, the application of banana rachis nanocellulose in aerogels, particularly in terms of safety, stability, and real-world packaging performance, remains largely unexplored, offering significant opportunities for developing eco-friendly, functional packaging solutions.

1.8. The objectives of the present investigation

To transform agricultural waste from banana farming and pineapple processing into valuable food applications, this study focuses on synthesizing nanocellulose from by-products like banana peels and pineapple residues. This approach promotes sustainable agricultural practices. By incorporating nanocellulose into food systems, the study aims to create a more sustainable food production method that aligns with global efforts to reduce waste and enhance food security. Therefore, the objectives were established as -

1. To investigate the properties of nanocellulose extracted from banana rachis and pineapple peel using various treatment methods.

2. To assess the impact of nanocellulose-stabilized Pickering nanoemulsion on β-carotene bioaccessibility in food systems.

3. To develop a nanocellulose/protein-based hydrogel that enhances gelling characteristics and explore its application in food systems.

4. To evaluate aerogels derived from nanocellulose and incorporate bioactive compounds for use in food packaging applications.

The flowchart presents the overall plan of work covering all the objectives.