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Biodegradable Packaging Made from Proteins

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Abstract

Protein films have gained significant attention in the development of sustainable packaging materials due to their exceptional properties and versatility. These films offer superior gas barrier properties, specific mechanical characteristics, and enhanced intermolecular connection capabilities compared to other biopolymers. Researchers are exploring innovative methods to enhance the film-forming properties of proteins, improve their mechanical strength, and optimize their gas barrier performance. Various protein sources, such as gelatin, whey protein, soy protein, corn zein, wheat gluten, and casein, are being investigated for film fabrication. Techniques to modify protein films, including the incorporation of additives, crosslinking agents, and nanomaterials, are being explored to enhance their properties. The development of protein-based composite films, by blending proteins with other biopolymers or synthetic materials, is also being explored to achieve improved performance and functionality. Advancements in processing technologies, such as film casting, extrusion, and electrospinning, enable precise control over the thickness, morphology, and structural properties of protein films. These films not only offer enhanced barrier properties but also possess biodegradability and renewable characteristics, aligning with the increasing demand for eco-friendly packaging solutions. The preparation and improvement of protein films hold significant potential for revolutionizing the packaging industry and contributing to a greener and more environmentally friendly future. This review provides an overview of current research and advancements in the field, addressing various protein sources, film modification techniques, processing methods, challenges, and future prospects.

Keywords: Degradable, Food packaging, Nanotechnology, Physicochemical properties, Protein

Introduction

Film-fabricating biopolymers play a crucial role in the development of sustainable packaging materials. Among these biopolymers, proteins have gained significant attention due to their exceptional properties and versatility. Protein films offer superior gas barrier properties, specific mechanical characteristics, and enhanced intermolecular connection capabilities compared to polysaccharide-based and lipid-based films (Balaguer *et al.*, 2013).

The unique ability of proteins to form intricate networks and induce plasticity and elasticity makes them an ideal choice for packaging applications. These films can be used as a component in biopolymer-based packaging materials, providing enhanced protection and preservation for various products (Du *et al.*, 2018).

The increasing demand for sustainable packaging solutions has led to a growing interest in the preparation and improvement of protein films. Researchers and scientists are

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exploring innovative methods to enhance the film-forming properties of proteins, improve their mechanical strength, and optimize their gas barrier performance (Garavand *et al.*, 2020).

Efforts are being made to identify suitable protein sources that can be used for film fabrication, such as gelatin, whey protein, soy protein, corn zein, wheat gluten and casein. These proteins offer a wide range of functional properties, making them suitable for different applications in the packaging industry (Yu *et al.*, 2021).

Additionally, researchers are investigating various techniques to modify protein films, including the incorporation of additives, crosslinking agents, and nanomaterials. These modifications aim to enhance the film's mechanical properties, increase its resistance to moisture and oxygen permeability, and prolong the shelf life of packaged products (Farhan *et al.*, 2017).

Furthermore, the development of protein-based composite films, by blending proteins with other biopolymers or synthetic materials, is being explored. This approach allows for the combination of desirable properties from different materials, resulting in films with improved performance and functionality (Winotapun *et al.*, 2019).

In recent years, advancements in processing technologies, such as film casting, extrusion, and electrospinning, have enabled the production of protein films with precise control over their thickness, morphology, and structural properties. These techniques offer opportunities to tailor the film properties according to specific packaging requirements (*Jariyasakoolroj et al.*, 2020).

The preparation and improvement of protein films hold significant potential for the development of sustainable packaging materials. These films not only offer enhanced barrier properties but also possess biodegradability and renewable characteristics, aligning with the growing demand for eco-friendly packaging solutions (Wang *et al.*, 2017).

This paper aims to provide an overview of the current research and advancements in the preparation and improvement of protein films for packaging applications. The various protein sources, film modification techniques, and processing methods employed to enhance the performance of protein films were discussed. The challenges and future prospects in this field were also addressed.

Overall, the preparation and improvement of protein films have the potential to revolutionize the packaging industry, offering sustainable alternatives to conventional materials and contributing to a greener and more environmentally friendly future.

Different Methods of Packaging Film Preparation

There are several different preparation methods for packaging films, each with its own advantages and suitability for specific applications.

The Solution Casting Method

The solution casting method involves dissolving the film-forming material in a solvent to create a solution, which is then poured onto a flat surface or a mold and allowed to dry or solidify, resulting in a thin film.

In order to use this method some crucial factors should be taken into consideration. The solvent must effectively dissolve the material without degrading it and should have a high volatility to facilitate easy removal. The solid material (polymer, composite, etc.) is added to the solvent and stirred at a controlled temperature until a clear solution is obtained. The concentration of the material in the solution is adjusted according to the desired final thickness and properties of the cast film. The prepared solution is poured into molds or on casting surfaces (e.g., glass plates) to achieve the desired geometry. Sometimes, methods like spin coating or knife-over-edge can be used to spread the solution uniformly over the substrate. The solvent is allowed to evaporate gradually at room temperature or under controlled heating conditions. The rate of evaporation influences the uniformity and properties of the final product. Care must be taken to avoid defects such as bubbles or uneven thickness during this stage. After solvent evaporation, the cast film may require curing (chemical crosslinking, thermal treatment) to enhance its properties. Further treatments such as annealing or surface treatment can be performed to improve film performance.

These kinds of films can be applied for packaging, electronic devices, and medical applications, coating technologies for enhancing surface properties, electrical conductivity, or barrier properties, composite materials**,** and nanocomposites.

The Extrusion

The extruder is a machine that consists of a screw inside a heated barrel. The screw rotates and conveys the raw material forward while also applying heat and pressure to melting the materials. The temperature in the barrel is carefully controlled to reach the melting point of the chosen material. As the raw material melts, it becomes a viscous mass suitable for further processing. There are several ways to form the film after extrusion. Blown Film Extrusion: The melted material is extruded through a circular die, creating a tube. Air is then blown into this tube, causing it to expand and form a thin-walled film. The film is cooled and pulled upward through an adjustable frame. Cast Film Extrusion: The melted raw material is extruded through a flat die, producing a continuous sheet. This sheet is immediately cooled on a flat surface (cast roll) and then wound up into rolls. In both blown and cast film processes, the film is cooled rapidly after exiting the extruder to stabilize its structure and hold the desired thickness. Once cooled, the film is typically wound into large rolls. This roll format is convenient for storage and shipping, and allows for further process such as printing or laminating.

Compression Molding

Compression molding involves shaping and solidifying the film-forming material in a mold cavity using heat and pressure, commonly employed for thermoplastic materials.

Lamination

Lamination is a process where multiple layers of films are bonded together to create a composite film, achieved through adhesive lamination, heat lamination, or extrusion lamination. Lastly, co-extrusion allows for the simultaneous extrusion of multiple layers of different materials, resulting in a multi-layered film with combined properties and functionalities (Yu *et al.*, 2021).

These are just a few examples of the various preparation methods for packaging films. The choice of method depends on factors such as the desired properties of the film, the film-forming material, and the intended application of the packaging film.

Physical Properties of Protein Films

Film Solubility

To assess solubility, conditioned protein films are weighed and immersed in distilled water. After stirring, undissolved material is centrifuged, dried, and weighed. Solubility is calculated by subtracting the weight of the remaining dry substance from the initial film weight (Pengbo *et al.*, 2023).

Protein films exhibit solubility ranging from 16-72%. Comparatively, gelatin films display higher solubility than whey protein films. Moreover, fish gelatin films demonstrate superior solubility when compared to mammalian gelatin films. This disparity may be attributed to reduced intra-chain and inter-chain connections, as well as the presence of lysine, hydroxyl-lysine residues, and their aldehyde derivatives. Conversely, whey protein films exhibit lower solubility due to protein denaturation and the loss of their native three-dimensional structure. Consequently, sulfhydryl groups surface on the protein molecules, leading to covalent connections between them, thus decreasing solubility

(Hadidi *et al.*, 2022). Water solubility is a significant characteristic for packaging films as it prevents water penetration or leakage in the packaged product. However, in certain applications like encapsulating nutrients or utilizing films as food additives, water permeability and solubility are evaluated as desirable features.

The solubility of whey protein films in water is reduced when subjected to UV treatment. This outcome is attributed to the impact of radiation on whey protein denaturation, resulting in the exposure of sulfide groups and subsequent intermolecular reactions. Notably, high-dose ultraviolet radiation, approximately 12 J/cm2, significantly decreases film solubility. Furthermore, denaturation induced by UV rays leads to the formation of hydrophobic surfaces in protein molecules, primarily through covalent bonding between aromatic amino acids and disulfide bonds. During the denaturation process, hydrophobic regions of the proteins migrate to the surface, thereby increasing the hydrophobicity of the protein film (Galus *et al.*, 2016).

Film WVP

Various techniques exist to measure water vapor permeability (WVP) of protein films. Here are some commonly employed methods in recent studies.

Gravimetric method entails monitoring the weight gain of a film sample over a specific duration when subjected to a controlled humidity environment (Chaudhary *et al.*, 2020).

Electrochemical sensors, such as quartz crystal microbalances (QCM) or surface acoustic wave (SAW) sensors, offer a means to evaluate alterations in film mass resulting from water vapor absorption (Velaga *et al.*, 2018).

Permeation cells comprise of two chambers that are divided by the film sample. The film allows water vapor to permeate through it, and on the opposite side, techniques such as gas chromatography or infrared spectroscopy are employed to measure the concentration of the permeated vapor (Kao *et al.*, 2014).

Dynamic vapor sorption (DVS) is a technique that assesses the sorption and desorption characteristics of a film in response to controlled humidity levels (Velaga *et al.*, 2018).

Coulometric Karl Fischer titration is a technique that entails subjecting a film to a controlled water vapor gradient and quantifying the rate of water vapor transmission through the film using Karl Fischer titration (Kim *et al.*, 2020).

Mathematical modeling employs Fick's laws of diffusion to estimate water vapor permeability. By taking into account factors such as film thickness, diffusion coefficient, and environmental conditions, these models can calculate WVP. However, for accurate modeling, it is necessary to possess knowledge of film properties and validate the models against experimental data (Pengbo *et al.*, 2023).

The selection of a method relies on factors including desired accuracy, sensitivity, equipment availability, and the specific properties of the protein film under examination. It is crucial to evaluate the limitations and benefits of each method when choosing a suitable technique for measuring WVP in protein films.

In the majority of food packaging studies, the assessment of water vapor permeability in the film is conducted using the method outlined in ATSM 1993 (Alak *et al.*, 2019). This method involves affixing films of precise dimensions and weight onto vials filled with dry silica gel. Subsequently, these vials are weighed and positioned within a conditioned desiccator containing distilled water at a temperature of 20 degrees Celsius. The rate at which water vapor passes through the films can be determined using the subsequent formula.

$$\mathrm{WVP} = \frac{\Delta w \times L}{\Delta t \times A \times \Delta P} \qquad (1)$$

Where $\Delta w/\Delta t$ is the mass change of vials per time unit, L is the thickness of the films, A is the exposed surface of the film and ΔP is the water vapor pressure difference between outside and inside of the film.

The soy protein isolate film exhibited a lower WVP compared to gelatin films due to its higher surface hydrophobicity (dos Santos Paglione *et al*., 2019).

Additionally, an increased protein content in the surimi film could potentially result in a higher concentration of polar groups. These polar groups have the ability to absorb more moisture from the surrounding environment, thereby influencing the WVP of the films. It was also suggested that film thickness played a role in this phenomenon.

Nevertheless, certain protein films, particularly the WPC film, demonstrated a low WVP. This could potentially be attributed to the denaturation of whey protein, which is facilitated by the intra-molecular and inter-molecular crosslinking of amino acid residues. Additionally, the formation of disulfide crosslinks and hydrophobic bonds may contribute to the reduction in WVP values. Typically, the moisture migration through films is regulated by the film's network. Films possessing a denser structure have the ability to more efficiently impede the moisture migration compared to those with a less compact structure (Galus *et al*., 2016).

Light Transmission

Various methods can be employed to measure the light transmission of biodegradable packaging films. Some commonly utilized techniques include spectrophotometry and haze measurement. Spectrophotometry entails passing a light beam through the film and assessing the intensity of transmitted light at different wavelengths. This method offers insights into the film's transparency and enables quantitative analysis of light transmission (Hadidi *et al*., 2022). Haze measurement, on the other hand, quantifies the extent of light scattering as it traverses the film, thereby indicating the film's clarity. Instruments like haze meters or haze-gloss meters are employed to measure haze values (Garavand *et al*., 2020).

Transparency can be visually evaluated by comparing the film to a standard transparent material. Although this method is subjective and may not yield precise quantitative data, it is commonly employed for quick evaluations (Yu *et al*., 2021).

Ultraviolet-visible (UV-Vis) spectroscopy measures light absorption and transmission in the UV and visible range. This technique offers insights into the film's capability to block or transmit specific light wavelengths (Velaga *et al*., 2018).

Fourier Transform Infrared (FTIR) spectroscopy gauges the absorption of infrared light by the film. It aids in analyzing the film's chemical composition and identifying specific functional groups that could impact light transmission (Nisar *et al*., 2018).

Colorimetry involves measuring the film's color using colorimeters or spectrophotometers. This method provides information about the film's color properties, which can indirectly influence light transmission (Nor Adilah *et al*., 2021).

It is worth noting that different methods may be more suitable for specific types of packaging films or research objectives. The choice of measurement technique depends on factors such as the desired level of precision, sensitivity, cost, and availability of equipment.

Light transmission of protein films can vary depending on several factors, including the specific protein used, film thickness, processing methods, and any additives or modifications applied. Whey protein films typically exhibit good transparency and light transmission properties. They can allow a significant amount of light to pass through, resulting in relatively high transmittance values. However, the exact level of light transmission can be influenced by factors such as film thickness and the presence of any plasticizers or crosslinking agents (Galus *et al*., 2016). Soy protein films can have varying degrees of light transmission depending on the specific type of soy protein used and the film formulation. In general, soy protein films tend to be less transparent compared to whey protein films. The presence of soy protein aggregates or other impurities can contribute to reduced light transmission. Gelatin films are known for their excellent light transparency. They can allow a significant amount of light to pass through, resulting in high transmittance values. Gelatin films are often used as edible coatings or packaging due to their clarity and visual appeal (Nor Adilah *et al.*, 2021). Casein films generally tend to have lower light transmission compared to whey or gelatin films (Matías *et al.*, 2018).

Protein-based films have been found to exhibit superior UV light blocking properties compared to PVC wrap films. This is attributed to the high content of aromatic amino acids in the protein-based structure, which enables them to effectively absorb UV light. As a result, protein-based films are considered more transparent than PVC films and are suitable for use in food packaging (Jariyasakoolroj *et al.*, 2020).

The protein-based films exhibit a visual appearance similar to that of PVC films, characterized by uniform transparency. These films are flexible, homogeneous, and possess smooth surfaces devoid of any observable pores or cracks. Placing them over a white background allows the background color to remain clearly visible, highlighting the transparency of the protein-based films (Nor Adilah *et al.*, 2021).

Tensile Strength

Various methods are employed to measure the mechanical properties of protein films.

Tensile testing involves subjecting a film sample to controlled tension until it breaks. The applied force and resulting deformation are measured, allowing for the determination of properties such as tensile strength, elongation at break, Young's modulus, and toughness. Tensile testing provides valuable information on the film's ability to withstand stretching or pulling forces (Jariyasakoolroj *et al.*, 2020).

Compression testing involves applying a compressive force to a film sample and measuring the resulting deformation. It is particularly useful for assessing the ability of protein films to withstand pressure or impacts (Winotapun *et al.*, 2019).

Flexural testing, also known as three-point or four-point bending, measures the film's resistance to bending. This method provides information on the film's flexural strength, modulus of elasticity, and flexibility (Yu *et al.*, 2021).

Puncture testing assesses the film's resistance to penetration by measuring the force required to puncture the film with a sharp probe (Xiao *et al.*, 2021).

Nanoindentation is a technique used to measure the mechanical properties of thin films at a micro- or nanoscale level. A small probe is indented into the film, and the resulting force-displacement curve is analyzed to determine properties such as hardness, elastic modulus, and viscoelastic behavior. Nanoindentation provides detailed information about the film's mechanical response at small scales (Winotapun *et al.*, 2019).

Dynamic mechanical analysis (DMA) involves subjecting a film sample to oscillatory forces or deformations over a range of frequencies and temperatures. It measures the film's viscoelastic properties, including storage modulus, loss modulus, and damping behavior. DMA is particularly useful for studying the film's response to dynamic or cyclic loading conditions (Velaga *et al.*, 2018).

The mechanical properties of protein films can vary depending on factors such as the protein source, film formulation, processing conditions, and any additives or modifications applied.

Tensile strength measures the maximum stress a film can withstand before breaking. Whey protein films have been reported to exhibit relatively high tensile strength, indicating good resistance to stretching or pulling forces. The high tensile strength (TS) of WPC film at 6.14 MPa indicated a brittle nature that makes it unsuitable for industrial applications. This brittleness can be attributed to intermolecular chain interactions, including disulfide bonding, hydrogen bonding, hydrophobic interactions, and electrostatic forces between protein chains (Galus *et al.*, 2016). Soy protein films generally have lower tensile strength compared to whey protein films (Xu *et al.*, 2017). Gelatin films also have moderate tensile strength but can be improved with crosslinking agents or plasticizers (Nor Adilah *et al.*, 2021). Casein films typically have lower tensile strength compared to other protein films (Matías *et al.*, 2018).

Elongation at break measures the ability of a film to stretch before breaking. The low elongation at break (EAB) value of whey protein films indicated good flexibility. This could be attributed to the unfolded structure and covalent disulfide bonding present in the film, which contribute to its higher strength and ability to withstand greater deformations. Soy protein films usually have lower elongation at break compared to whey protein films. Gelatin films can have moderate elongation at break, which can be enhanced with plasticizers. Casein films generally have lower elongation at break compared to other protein films (Pengbo *et al.*, 2023).

Young's modulus is a measure of the stiffness or rigidity of a film. Whey protein films typically have lower Young's modulus values, indicating good flexibility. Soy protein films can have moderate to high Young's modulus, indicating higher stiffness. Gelatin films generally have lower Young's modulus compared to soy protein films. Casein films can have moderate to high Young's modulus depending on the specific casein type and film formulation (Kaewprachu *et al.*, 2016).

Toughness measures the ability of a film to absorb energy before breaking. Whey protein films have been reported to have good toughness, indicating resistance to fracture. Soy protein films generally have lower toughness compared to whey protein films. Gelatin films can have moderate toughness, which can be improved with plasticizers or crosslinking agents. Casein films typically have lower toughness compared to other protein films (Hadidi *et al.*, 2022).

Thermal Properties

Several methods are used to measure thermal properties of protein films. Differential Scanning Calorimetry (DSC) is a widely used method that measures the heat flow into or out of a sample as it undergoes temperature changes. This technique can provide information about the thermal transitions of protein films, such as denaturation or melting temperatures, enthalpy changes, and heat capacity. DSC is particularly useful for studying the thermal stability and transitions of protein films (Wang *et al.*, 2017).

Thermogravimetric Analysis (TGA) measures the weight changes of a sample as it is subjected to controlled temperature conditions. This method can determine the thermal stability and degradation behavior of protein films. TGA provides information about the onset, rate, and extent of thermal decomposition or mass loss, which can be used to assess the thermal stability and decomposition kinetics of protein films (Winotapun *et al.*, 2019).

DMA used to measure the viscoelastic properties of protein films as a function of temperature. This technique applies a controlled oscillatory force or deformation to a sample while subjecting it to a temperature ramp. DMA can provide information about the glass transition temperature (Tg) of protein films, as well as changes in storage modulus, loss modulus, and damping behavior with temperature (Wang *et al.*, 2017).

Infrared Spectroscopy (IR) can be used to analyze the molecular vibrations and structural changes in protein films as a result of temperature variations. This technique can provide information about changes in protein secondary structure, hydrogen bonding, and conformational changes induced by temperature (Xiao *et al.*, 2021).

Thermal Conductivity Measurement can be used to determine the ability of protein films to conduct heat. These methods involve measuring the heat transfer through a film sample under controlled temperature conditions, providing information about the thermal insulation or conductivity properties of protein films (Jensen *et al.*, 2015).

Comparing the DSC results of different protein films can provide insights into their thermal behavior and stability.

Denaturation temperature (Td): Td is the temperature at which protein molecules undergo structural changes, leading to unfolding or denaturation. Comparing the Td values of different protein films can indicate their thermal stability. Films with higher Td values are generally more stable and resistant to heat-induced structural changes.

Denaturation enthalpy (ΔH): ΔH represents the amount of heat absorbed or released during protein denaturation. Comparing the ΔH values of protein films can provide information about the energy required for denaturation. Higher ΔH values indicate a higher degree of protein unfolding or denaturation.

Glass transition temperature (Tg): Tg is the temperature at which an amorphous material, such as a protein film, transitions from a glassy to a rubbery state. Comparing the Tg values of different protein films can indicate their molecular mobility and flexibility at different temperatures. Films with higher Tg values tend to be more rigid and have lower molecular mobility.

Melting temperature (Tm): Tm is the temperature at which a protein film transforms from a solid to a liquid state. Comparing the Tm values of different protein films can provide insights into their thermal stability and the presence of ordered secondary structures, such as alpha-helices or beta-sheets. Higher Tm values indicate a higher degree of thermal stability (Lee *et al.*, 2010).

The Tm values of protein films can vary depending on the source of the protein. Different proteins have different amino acid compositions, secondary structures, and molecular interactions, which can influence their melting behavior. For example, proteins with predominantly alpha-helical structures may have higher Tm values compared to those with predominantly beta-sheet structures. Also, the Tm values of protein films can be influenced by factors such as film formulation and processing conditions. Parameters like film thickness, protein concentration, plasticizers, crosslinking agents, and drying methods can affect the Tm values. It is important to ensure consistent film formulation and processing conditions when comparing Tm values. Protein modifications, such as chemical crosslinking or enzymatic modifications, can alter the Tm values of protein films. These modifications can affect the protein's structural stability and interactions, leading to changes in the Tm values. Comparisons should be made between films with similar modification methods or with appropriate controls. The way by which protein films are prepared and handled can impact their Tm values. Factors such as sample purity, hydration level, and sample handling can affect the protein's structural stability and, consequently, the Tm values. It is crucial to ensure consistent and appropriate sample preparation methods for accurate Tm comparisons (Winotapun *et al.*, 2019).

Additionally, it is important to interpret the Tm values in conjunction with other thermal and structural characterization techniques to gain a comprehensive understanding of the protein films' properties.

Comparing the melting temperature (Tm) values of whey protein, soy protein, gelatin, and casein films can provide insights into their thermal stability and structural characteristics. Whey protein films typically exhibit Tm values in the range of 60-75°C. Whey proteins, derived from milk, are known to have a relatively lower Tm compared to proteins with predominantly alpha-helical structures (Galus *et al.*, 2016). Soy protein films generally have Tm values in the range of 80-100°C. Soy proteins, derived from soybeans, often contain proteins with a higher proportion of beta-sheet structures, which can contribute to higher Tm values (Xiao *et al.*, 2021). Gelatin films typically have Tm values in the range of 25-40°C. Gelatin, derived from collagen, is composed mainly of random coil structures. As a result, gelatin films have relatively lower Tm values compared to proteins with more ordered secondary structures like alpha-helices or beta-sheets (Li *et al.*, 2021). Casein films generally exhibit Tm values in the range of 40-60°C. Casein, derived from milk, consists of a mixture of proteins with varying secondary structures (Matías *et al*., 2018).

Oxygen Permeability

The oxygen permeability of protein films can be measured using various techniques.

Oxygen Transmission Rate (OTR) Measurement: involves measuring the rate at which oxygen passes through a protein film. It typically utilizes a permeation cell, where the film is placed between two chambers. One chamber is filled with oxygen, while the other is maintained at a lower oxygen concentration or a vacuum. The flow of oxygen through the film is measured over time using sensors or detectors, providing the OTR value (Bagheri *et al*., 2019).

Gas Permeation Testing: involves exposing the protein film to a known concentration of oxygen and measuring the oxygen permeating through the film. This can be done using techniques such as gas chromatography or mass spectrometry, which analyze the oxygen concentration before and after passing through the film (Acquah *et al*., 2020).

Dynamic Headspace Analysis: involves placing the protein film as a barrier between a sample containing oxygen and a detector. The oxygen permeates through the film into the detector, and the change in oxygen concentration over time is measured. This technique is often used for films with specific applications, such as food packaging (Velaga *et al*., 2018).

Electrochemical sensors, such as oxygen sensors or oxygen electrodes, can be used to measure the oxygen permeability of protein films. These sensors measure changes in electrical signals or current caused by the oxygen permeating through the film (Wang *et al*., 2017).

Optical techniques, such as fluorescence quenching or phosphorescence quenching, can be used to measure the oxygen permeability of protein films. These methods rely on the change in fluorescence or phosphorescence intensity of a dye or probe embedded in the film, which is affected by the presence of oxygen (Velaga *et al*., 2018).

Whey protein films generally have relatively high oxygen permeability. This is due to the presence of hydrophilic regions in whey proteins that can facilitate the diffusion of oxygen molecules. Soy protein films typically exhibit moderate to low oxygen permeability. Soy proteins have a more compact structure compared to whey proteins, which can restrict the movement of oxygen molecules through the film. Additionally, the presence of hydrophobic regions in soy proteins can further limit oxygen permeability. Gelatin films generally have high oxygen permeability. Gelatin, derived from collagen, consists of random coil structures that create pathways for oxygen molecules to pass through the film easily. The high oxygen permeability of gelatin films can be advantageous for certain applications, such as wound dressings, where oxygen diffusion is desirable. Casein films typically have moderate to low oxygen permeability. Casein proteins have a complex structure, consisting of different subunits and a combination of alpha-helices and beta-sheets. This structure can hinder the movement of oxygen molecules through the film, resulting in lower oxygen permeability compared to whey protein films (Kaewprachu *et al*., 2016).

Microstructure of Protein Films

The scanning electron microscope (SEM) is commonly used to study the microstructure of protein films. The protein film sample needs to be properly prepared for SEM analysis. This typically involves fixing the film onto a suitable substrate, such as a glass slide or a metal stub. The sample may also need to be dehydrated or freeze-dried, depending on the nature of the protein film and the desired analysis. To enhance the conductivity of the sample and prevent charging effects during SEM imaging, a thin conductive coating is often applied to the protein film. Common coating materials include gold, gold-palladium, or carbon. The coating can be applied using techniques like sputter coating or carbon evaporation. The prepared sample is then loaded into the SEM chamber, and the instrument is set up accordingly. This involves adjusting the vacuum level, optimizing the electron beam parameters (e.g., accelerating voltage, beam current), and selecting the appropriate imaging mode (e.g., secondary electron imaging, backscattered electron imaging). Once the instrument is properly set up, the protein film sample can be imaged using the SEM. The electron beam scans the sample surface, and interactions between the beam and the sample generate signals that are detected and translated into an image. Secondary electron imaging provides high-resolution surface morphology information, while backscattered electron imaging can provide compositional contrast (Pengbo *et al*., 2023).

The acquired SEM images can be analyzed to study the microstructure of the protein film. This may involve measuring film thickness, observing surface topography, evaluating pore size and distribution, or assessing the presence of cracks, defects, or other structural features. Quantitative analysis can be performed using specialized software to obtain statistical data on various parameters. It allows researchers to visualize and understand the microstructural features of the films, aiding in the development and optimization of protein film-based materials for various applications.

Chemical Properties

The Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy method is commonly used to study the chemical structure of protein films.

The protein film sample needs to be properly prepared for FTIR analysis. This typically involves depositing the film onto a suitable substrate, such as a potassium bromide (KBr) pellet or a specialized FTIR sample holder. The sample should be sufficiently thin and uniform to allow for accurate spectral analysis. The FTIR instrument is set up for analysis. This involves calibrating the instrument, purging the system to remove any interfering gases, and ensuring that all components are properly aligned. The instrument is typically equipped with an infrared light source, an interferometer, and a detector. The prepared protein film sample is placed in the FTIR instrument, and the measurement is conducted. The instrument emits infrared light that passes through the sample, and the resulting transmitted or reflected light is detected. The instrument records the intensity of the light at different wavelengths across the infrared spectrum. The recorded infrared spectrum is analyzed to obtain information about the chemical structure of the protein film. Protein films exhibit characteristic absorption bands in the infrared region, which correspond to specific functional groups and molecular vibrations. These absorption bands provide information about the types of chemical bonds present in the film, such as amide bonds (peptide bonds) and functional groups like -OH, -NH, and -C=O. The FTIR spectrum is interpreted by comparing it to reference spectra or using spectral libraries to identify specific functional groups and molecular vibrations. The positions and intensities of absorption peaks in the spectrum can provide information about the protein secondary structure, presence of specific amino acid residues, and any chemical modifications or interactions within the film. This information is valuable in understanding the structure-function relationship of protein films and can aid in optimizing their performance for various applications, such as food packaging, biomedical materials, or sensors (Yu *et al.*, 2021).

The FTIR results of whey protein film, soy protein film, and gelatin film can provide insights into their chemical structure and composition. Here is a comparison of the FTIR characteristics of these protein films (Galus *et al.*, 2016).

The whey protein film typically exhibits a strong and broad amide I band in the range of 1600-1700 cm^{-1}. This band is associated with the stretching vibrations of the C=O bonds in the protein backbone, providing information about the secondary structure of the protein film. The amide II band, located around 1500-1600 cm^{-1}, represents the bending vibrations of the N-H bond and the stretching vibrations of the C-N bond. It provides information about the presence of various secondary structures, such as alpha-helices and beta-sheets, within the whey protein film (Galus *et al*., 2016).

Additional characteristic bands may appear in the whey protein film FTIR spectrum, such as the amide III band (1200-1300 cm^{-1}) and the amide A band (3000-3500 cm^{-1}), which provide information about the presence of specific amino acid residues and hydrogen bonding interactions. On the other hand, the soy protein film typically shows a narrower and less intense amide I band compared to whey protein. The position and shape of this band can provide information about the secondary structure of the soy protein film, such as the presence of beta-sheets or random coil structures. The amide II band in the soy protein film FTIR spectrum typically exhibits multiple peaks or shoulders, indicating the presence of different secondary structures and molecular vibrations. Similar to whey protein, the soy protein film FTIR spectrum may also show characteristic bands related to specific amino acid residues and hydrogen bonding interactions. The gelatin film typically exhibits a broad and intense amide I band, similar to whey protein (Han *et al*., 2016). The position and shape of this band can provide information about the secondary structure of the gelatin film, which is influenced by the presence of collagen in the gelatin. The amide II band in the gelatin film FTIR spectrum typically shows multiple peaks or shoulders, similar to soy protein. This indicates the presence of various secondary structures and molecular vibrations within the gelatin film (Alak *et al*., 2019). The FTIR spectrum of the gelatin film may also show characteristic bands related to specific amino acid residues and hydrogen bonding interactions, similar to whey protein and soy protein. It's important to note that the FTIR results may vary for different studies and experimental setups.

The X-ray Diffraction (XRD)

XRD method is commonly used to study the crystalline structure and orientation of materials, including certain types of protein films that exhibit crystallinity.

The protein film sample needs to be properly prepared for XRD analysis. This typically involves depositing the film onto a suitable substrate, such as a glass slide or a specialized XRD sample holder. The sample should be sufficiently thin and uniform to allow for accurate X-ray diffraction measurements. The XRD instrument is set up for analysis. This involves calibrating the instrument, aligning the X-ray source, and positioning the detector. The instrument typically uses a high-energy X-ray source, such as a rotating anode generator or a synchrotron radiation source, to produce X-rays. The prepared protein film sample is placed in the XRD instrument, and the measurement is conducted. The X-rays are directed onto the sample, and the resulting diffracted X-rays are detected. The instrument records the intensity of diffracted X-rays at different angles. The recorded XRD pattern is analyzed to obtain information about the crystalline structure of the protein film. XRD patterns consist of a series of sharp peaks that correspond to the scattering of X-rays by crystal planes within the material. The positions and intensities of these peaks provide information about the arrangement of atoms within the crystal lattice. The XRD pattern is interpreted by analyzing the positions and intensities of the diffraction peaks. This analysis can provide information about parameters such as the lattice spacing, crystal symmetry, crystal orientation, and crystallite size of the protein film. By comparing the XRD pattern to known crystal structures or using crystallographic software, researchers can identify the specific crystalline phases present in the film (Balaguer *et al*., 2013).

It's important to note that not all protein films exhibit crystallinity, and XRD analysis may not be suitable for samples that are amorphous or have low crystallinity. In such cases, other techniques like FTIR or solid-state NMR spectroscopy may be more appropriate for studying the chemical structure of protein films.

Whey protein films are typically amorphous or semi-crystalline, meaning they may not exhibit distinct crystalline peaks in the XRD pattern. Soy protein films and gelatin films can also be amorphous or semi-crystalline, but they may exhibit some level of crystallinity depending on their preparation methods and processing conditions (Galus *et al*., 2016).

If any crystalline peaks are observed in the XRD patterns, the crystal structure can be analyzed. Whey protein films are primarily composed of globular proteins like β-lactoglobulin, which do not typically exhibit well-defined crystal structures. Soy protein films, on the other hand, may contain proteins like glycinin and β-conglycinin, which can form crystalline structures. Gelatin films, derived from collagen, may also exhibit some degree of crystallinity in the form of collagen fibrils (Alak *et al*., 2019).

Antimicrobial Properties

Gelatin films have been extensively studied for their antimicrobial properties, making them valuable for applications in food packaging and biomedical materials. These films exhibit antimicrobial activity against a wide range of microorganisms, including bacteria, fungi, and some viruses. The presence of natural antimicrobial peptides and proteins, such as collagen-derived peptides, in gelatin contributes to this effect. These peptides disrupt microbial cell membranes and interfere with essential cellular processes, inhibiting the growth and proliferation of microorganisms (Li *et al*., 2020).

Gelatin films have demonstrated effectiveness against both Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, and *Listeria monocytogenes*. They have also exhibited inhibitory effects on various fungi, such as *Candida albicans* and *Aspergillus niger*. The mechanisms underlying the antimicrobial properties of gelatin films can vary. Some studies suggest that the release of antimicrobial peptides from the gelatin matrix disrupts microbial membranes, while other mechanisms involve chelation of essential metal ions required for microbial growth or the alteration of pH levels, creating unfavorable conditions for microbial survival (Alak *et al*., 2019).

Incorporating antimicrobial agents such as essential oils, plant extracts, or nanoparticles into the gelatin matrix enhances their antimicrobial properties (Table 1). Modifying the film structure, such as through cross-linking or blending with other polymers, can also affect the release rate and efficacy of antimicrobial compounds (Karimi *et al*., 2022).

Gelatin films with antimicrobial properties find potential applications in food packaging to extend the shelf life of perishable products by inhibiting microbial growth. They can also be utilized in wound dressings or biomedical devices to prevent infection. However, it is important to note that the effectiveness of gelatin films may vary depending on the specific application and the targeted microorganisms. Further research and optimization of gelatin film formulations and processing techniques hold promise for enhancing their antimicrobial efficacy and expanding their applications in diverse fields (Alak *et al*., 2019)

Whey protein films have demonstrated antimicrobial activity against various microorganisms, including bacteria and fungi. The presence of bioactive peptides derived from whey proteins, such as lactoferrin, lactoperoxidase, and immunoglobulins, is responsible for this antimicrobial effect. These peptides possess antimicrobial properties and can inhibit the growth and proliferation of microorganisms through various mechanisms. They can disrupt microbial cell membranes, leading to cell leakage and death. Additionally, they may interfere with microbial enzyme systems or nutrient uptake, inhibiting their growth. Some peptides exhibit antimicrobial activity by inducing oxidative stress or modulating the immune response of the host (Galus *et al*., 2016).

Notable bacteria that have been shown to be affected by whey protein films include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, and *Listeria monocytogenes*. Whey protein films have also exhibited inhibitory effects on various fungi, such as *Candida albicans* and *Aspergillus niger* (Kaewprachu *et al*., 2016).

Casein protein films have also demonstrated antimicrobial properties. Casein-derived peptides, such as lactoferricin and casocidin, exhibit antimicrobial activity against various bacteria and fungi. These peptides can disrupt microbial membranes or interfere with microbial enzyme systems, leading to cell death or growth inhibition (Matías *et al*., 2018).

Soy peptides, such as soybean trypsin inhibitors and lunasin, have shown antimicrobial activity against bacteria and fungi. Corn zein films have shown potential antimicrobial properties. Zein is a prolamin protein found in corn, and studies have reported its inhibitory effects against bacteria and fungi. The antimicrobial activity of corn zein films is attributed to the hydrophobic nature of zein, which can disrupt microbial cell membranes (dos Santos Paglione *et al*., 2019).

Table 1- Incorporation of different essential oils in protein film as antimicrobial food packaging

Protein film	Added essential oil	The type of food applied for	reference
Gelatin	Clove	Fish	(Karimi *et al*., 2022)
Whey protein isolate	Garlic, rosemary, and oregano	Fresh beef	(Hadidi *et al*., 2022)
Casein	Origanum Volgare L.	Cherry tomato	(Matías et al., 2018)
Soy protein	Oregano and thyme	Fresh ground beef	(Martelli-Tosi *et al*., 2018)
Gluten	Star anise	Snacks	(Bagheri *et al*., 2019)
Zein	Cinamon and mustard	Tomatoes	(Sayadi *et al*., 2021)

Antioxidant Properties

Gelatin protein films have been investigated for their antioxidant properties. Gelatin protein films have demonstrated radical scavenging activity, meaning they can neutralize free radicals and prevent oxidative damage. Free radicals are highly reactive molecules that can cause cellular damage and contribute to various diseases. Gelatin films containing natural antioxidants, such as phenolic compounds or flavonoids, have shown effective radical scavenging activity, reducing the levels of harmful free radicals (Table 2). Gelatin protein films have been found to possess metal-chelating properties. Certain metal ions, such as iron and copper, can catalyze free radical formation through Fenton and Haber-Weiss reactions, leading to oxidative stress. Gelatin films containing chelating agents can bind to these metal ions and inhibit their ability to generate free radicals, thereby reducing oxidative damage. These films have shown the ability to inhibit lipid oxidation, which is a major cause of food spoilage and deterioration. Lipid oxidation can lead to the formation of harmful compounds and rancidity in food products. Gelatin films with antioxidant properties can prevent or delay lipid oxidation by scavenging free radicals or inhibiting the initiation and propagation of lipid oxidation reactions. These films can also serve as protective barriers, preserving the antioxidant activity of incorporated compounds. By encapsulating antioxidants within the gelatin matrix, the films can shield them from degradation caused by environmental factors, such as oxygen or light (Table 2). This helps to maintain the stability and efficacy of antioxidants, ensuring their long-term antioxidant activity (Hadidi *et al*., 2022).

Whey protein films have demonstrated antioxidant properties due to the presence of bioactive peptides, such as lactoferrin and lactoperoxidase. These peptides exhibit radical scavenging activity and can effectively neutralize free radicals, providing protection against oxidative stress. Whey protein films have also shown hydrogen peroxide scavenging and metal-chelating properties, further contributing to their antioxidant activity (Yu *et al*., 2021).

Soy protein and corn zein films have been investigated for their antioxidant properties. Antioxidant compounds present in soy, such as isoflavones and phenolic compounds, contribute to the antioxidant activity of soy protein films. These compounds exhibit radical scavenging activity and can inhibit lipid oxidation. Soy protein films also possess metal-chelating properties, further enhancing their antioxidant potential (Jensen *et al*., 2015).

Table 2- Different antioxidant types used in protein packaging film for food products

	Type of antioxidants	Classifications	Examples of antioxidants	Examples of films applied in	Food product	Reference
Antioxidants	Primery	Synthetic	BHA, BTA	Corn Zein	Turkey	(Sahraee *et al*., 2019)
		Natural	Plant extracts and essential oils	Gelatin	Fish oil	(Sahraee *et al*., 2019)
	Secondary	Chelators	EDTA	Whey protein	Turkey frankfurter	(Galus *et al*., 2016)
			Polylactic acid	Zein	Fish fillet	(Sayadi *et al*., 2021)
		UV absorbers	Nanoparticles	Gelatin	Bakery products	(Milani *et al*., 2015)
		Single oxygen quenchers	Carotenoids	Gelatin	Porks	(Alak *et al*., 2019)
			Polyphenols	Casein	Cheese	(Salajegheh *et al*., 2020)
		Oxygen scavengers	Metal oxide	Gelatin	Cake	(Karimi *et al*., 2022)
			Ascorbic acid	Whey protein	Roasted peanuts	(Garavand *et al*., 2020)

Protein Sources for Food Packaging

Gelatin

Gelatin possesses a range of properties which makes it highly suitable for packaging film applications. Firstly, gelatin exhibits excellent film-forming properties, allowing it to be easily processed into films. These films are thin, flexible, and transparent, while also possessing good mechanical strength. This combination of properties makes gelatin an ideal material for packaging films, as it can provide a protective barrier while maintaining the integrity of the packaged product.

In addition to its film-forming capabilities, gelatin is a natural protein derived from collagen, making it biodegradable. This means that gelatin can be broken down by microorganisms, enzymes, and natural processes over time, resulting in eco-friendly disposal. The biodegradability of gelatin is a highly desirable property for packaging films, as it helps to reduce environmental impact and waste accumulation.

Furthermore, gelatin films exhibit good water vapor barrier properties, enabling them to effectively control the moisture content of packaged products. This is crucial in maintaining the freshness and quality of perishable goods, as it helps prevent moisture loss or gain, thereby extending the shelf life of the products.

While not as effective as synthetic materials, gelatin films also provide a certain level of oxygen barrier properties. This property is important for packaging applications, as it helps slow down the oxidation process and preserve the quality and shelf life of oxygen-sensitive products.

Moreover, gelatin is compatible with various active ingredients, allowing for the incorporation of functional additives into gelatin films. This enhances their performance and offers additional benefits such as antioxidant or antimicrobial properties. Gelatin can also be modified or blended with other materials to enhance its properties and tailor it to specific packaging needs.

Lastly, gelatin is widely used in the food industry and is considered safe for consumption. This makes gelatin films suitable for direct food contact applications, such as food packaging or edible films and coatings or encapsulation (Alak *et al*., 2019).

While gelatin offers numerous desirable properties for packaging film, it is important to consider its disadvantages as well. Gelatin films can be sensitive to moisture, which can result in changes to their mechanical properties, such as increased brittleness or reduced strength. This sensitivity limits their use in high-humidity environments or for products with high moisture content. Additionally, gelatin has a relatively low melting point, making it unsuitable for heat-resistant packaging applications like microwaveable or hot-fill packaging.

Furthermore, gelatin films have limited resistance to water and fat, which can cause them to soften, lose structural integrity, or interact unfavorably with the packaged product. This limitation poses challenges for packaging products with high water or fat content. While gelatin films provide some barrier properties, they may not match the performance of synthetic materials in terms of oxygen or moisture barriers. Thus, their use may be restricted in certain packaging applications requiring higher barrier properties.

Another consideration is the limited shelf life of gelatin films, particularly those derived from animal sources, due to their biodegradability and susceptibility to microbial growth. This can be problematic for long-term storage or products requiring extended shelf life. Additionally, gelatin, derived from animal sources like collagen, can potentially trigger allergic reactions in individuals with specific allergies to animal proteins. This restricts the use of gelatin films in certain applications, particularly in allergen-sensitive industries or for products targeting specific consumer groups (Karimi *et al*., 2022).

Ongoing research and development efforts aim to address these limitations and enhance the overall performance of gelatin films. Moreover, alternative sources of gelatin, such as plant-based or modified gelatins, are being explored to overcome some of these disadvantages.

Gelatin for film packaging is primarily derived from animal sources, particularly from the collagen found in the skin, bones, and connective tissues of animals. Gelatin derived from bovine sources, such as cows or cattle; gelatin derived from porcine sources, such as pigs; and gelatin from poultry sources, including chicken and turkey, are widely used in the food and packaging industries.

It's important to note that gelatin derived from animal sources may pose challenges related to religious or dietary restrictions, allergenic potential, or ethical considerations for certain individuals or industries.

In recent years, there has been increasing interest in exploring alternative sources of gelatin to address these concerns. Some alternative sources being explored include fish gelatin derived from fish skins and scales, as well as plant-based gelatin substitutes, such as modified starches or proteins from sources like seaweed or legumes. These alternative sources offer potential solutions for individuals or industries seeking non-animal-derived options for gelatin-based packaging films.

To improve the properties of gelatin as packaging film, various additives can be incorporated. These additives can enhance specific characteristics or address limitations of gelatin films. Here are some commonly used additives (Winotapun *et al*., 2019).

Common plasticizers used in gelatin film include glycerol, sorbitol, polyethylene glycol, and propylene glycol. These additives help reduce brittleness and increase the film's ability to conform to different shapes or packaging requirements.

Crosslinking agents like glutaraldehyde, genipin, and transglutaminase can be added to promote crosslinking between gelatin molecules, resulting in improved film properties.

Natural antioxidants, such as vitamin E, ascorbic acid, or plant-derived extracts, can be added to enhance the film's stability and prolong the shelf life of the packaged product (Table 2).

Colorants, such as natural or synthetic pigments or dyes, can be added to gelatin films to provide visual appeal, branding, or product differentiation. These additives help create attractive packaging designs or enhance the appearance of the film.

Antimicrobial agents can be incorporated into gelatin films to inhibit the growth of microorganisms and extend the shelf life of the packaged product. Examples of antimicrobial additives include essential oils, chitosan, or silver nanoparticles (Table 1).

Various additives can be used to enhance the barrier properties of gelatin films. For example, montmorillonite clay can be added to improve oxygen barrier properties, while lipids or waxes can enhance moisture barrier properties.

Fillers, such as cellulose fibers or nanoparticles, can be added to improve the mechanical strength, thermal resistance, or barrier properties of gelatin films. These additives help enhance the overall performance and durability of the film.

Montmorillonite clay nanoparticles, such as organically modified montmorillonite (OMMT), have been studied to enhance the barrier properties of gelatin films. These nanoparticles can improve the oxygen and water vapor barrier properties, mechanical strength, and thermal stability of gelatin films (Wang *et al*., 2017).

Metal oxide nanoparticles, such as titanium dioxide (TiO2) or zinc oxide (ZnO), have been explored for their UV-blocking properties in gelatin films. These nanoparticles can enhance the film's resistance to UV radiation, thereby protecting the packaged product from photochemical degradation (Garavand *et al*., 2020).

Silver nanoparticles (AgNPs) have gained attention for their antimicrobial properties. When incorporated into gelatin films, they can inhibit the growth of microorganisms, extending the shelf life of the packaged product and providing an additional protective barrier against contamination (Kim *et al*., 2020).

Cellulose nanocrystals (CNCs) derived from cellulose fibers have been investigated as reinforcing agents in gelatin films. These nanoparticles can enhance the mechanical properties, such as tensile strength and elasticity, of gelatin films, making them more suitable for packaging applications (Kao *et al*., 2014).

Chitosan nanoparticles have been studied for their potential as antimicrobial agents in gelatin films (Garavand *et al*., 2020).

Hybrid nanoparticles combining the antimicrobial properties of silver nanoparticles with the barrier-enhancing properties of nanoclays have been investigated. These hybrid nanoparticles can provide both antimicrobial and barrier functionalities to gelatin films, making them suitable for food packaging applications (Jariyasakoolroj *et al*., 2020).

Several essential oils have been studied and used in gelatin films to improve their packaging properties. Rosemary essential oil is known for its antioxidant and antimicrobial properties. When incorporated into gelatin films, it can help extend the shelf life of packaged products by inhibiting the growth of microorganisms and reducing oxidative degradation (Velaga *et al*., 2018).

Thyme, oregano, cinnamon, lemongrass, tea tree essential oils have strong antimicrobial properties, making them a valuable additives for gelatin films. It can help inhibit the growth of bacteria and fungi, thereby enhancing the safety and shelf life of the packaged product (Farhan *et al*., 2017).

Gelatin film is used as packaging for a variety of foods. Gelatin films are commonly used to package confectionery items such as gummy candies, jelly beans, marshmallows, and fruit snacks. The film provides a protective barrier, enhances the product's appearance, and helps maintain freshness. Gelatin films can also be used for packaging dairy products like yogurts, puddings, and custards. The film helps prevent moisture loss, maintains product texture, and extends shelf life. Gelatin films are sometimes used for packaging meat and poultry products such as deli meats, sausages, and pâtés. The film can help improve product presentation, prevent moisture loss, and provide a protective barrier against contaminants. Gelatin films can be utilized for packaging bakery items like cakes, pastries, and cookies. The film helps retain moisture, preserve freshness, and protect the product during transportation. Gelatin films are also used for packaging pet foods, including treats and wet pet food products. The film helps maintain product quality, prevent spoilage, and provide an attractive presentation (Alak *et al*., 2019).

Whey Protein

Whey protein, derived from milk during the cheese-making process, possesses a range of favorable properties that render it suitable for packaging film applications. With excellent film-forming properties, whey protein can be processed into thin films or coatings, serving as a base material for packaging films. Moreover, whey protein films are biodegradable, ensuring they naturally break down over time without causing harm to the environment. This makes them a sustainable alternative to conventional plastic packaging materials (Galus *et al*., 2011).

Additionally, whey protein films display robust mechanical strength, enabling them to withstand handling and transportation without tearing or breaking easily. This characteristic makes them suitable for packaging a diverse range of products.

Considering whey protein's safety for human consumption and its common usage in food products, whey protein films are generally deemed safe for direct food contact, making them an ideal choice for food packaging.

Notably, whey protein is a byproduct of the dairy industry, rendering it a renewable and sustainable resource. By utilizing whey protein for packaging films, waste can be reduced, and a circular economy can be promoted (Pengbo *et al*., 2023).

While whey protein offers several favorable properties for packaging film, it is essential to consider its drawbacks. Whey protein films can be sensitive to moisture, resulting in reduced mechanical strength and barrier properties. This limits their use in applications requiring high moisture resistance. Additionally, whey protein films have lower heat resistance compared to synthetic films, restricting their suitability for high-temperature processes like sterilization or hot filling.

Moreover, whey protein films exhibit solubility in water, which can be considered disadvantageous in certain packaging applications. The processing of whey protein into films can be challenging due to its high viscosity and tendency to form gels.

Furthermore, whey protein as a valuable food ingredient may increase the production costs of packaging films compared to conventional plastics. This cost factor can limit the widespread adoption of whey protein-based films in certain applications. The availability of whey protein as a raw material for film production may also be constrained by the supply and demand dynamics of the dairy industry, impacting the scalability and commercial viability of whey protein-based packaging films.

Additionally, whey protein films may be sensitive to ultraviolet (UV) light, leading to discoloration and degradation over time. With advancing technology, these limitations may be mitigated, making whey protein films more viable for a broader range of packaging applications (Matías *et al*., 2018).

Whey protein concentrate (WPC) and whey protein isolate (WPI) are both forms of protein derived from whey, a byproduct of the cheese-making process. The main difference between whey protein concentrate (WPC) and whey protein isolate (WPI) lies in their protein content and processing methods. WPC typically contains around 70-80% protein, while WPI has a higher protein content, usually around 90% or higher. This is because WPI undergoes additional processing steps to remove more non-protein components, such as lactose and fats.

Whey protein concentrate is produced by filtering and drying whey, resulting in a powder that retains a portion of the non-protein components naturally present in milk. On the other hand, whey protein isolate goes through further processing, including additional filtration or ion exchange, to remove more of the non-protein components, resulting in a more pure protein powder. WPI is generally more expensive than WPC due to the additional processing required to achieve higher protein content and remove more non-protein components (Chaudhary *et al.*, 2020).

Various additives have been utilized to enhance the properties of whey protein as a packaging film. Plasticizers, Cross-linking agents, Antioxidants and fillers or reinforcements like cellulose nanofibers, chitosan fibers, and starches can be incorporated to enhance mechanical properties, barrier properties, and stability. Coating agents such as lipids (e.g., fatty acids, waxes) and polysaccharides (e.g., chitosan, alginate) are employed to improve water vapor resistance and barrier properties. Additionally, colorants and flavorings can be added for visual appeal or to provide specific taste or aroma to the packaged product.

Blending with polymers like starch, alginate, or chitosan, can improve the mechanical strength, barrier properties, and stability of whey protein films (Galus *et al.*, 2016).

Various essential oils have been studied for their potential to improve the properties of whey protein films. Oregano oil has been investigated for its antimicrobial properties when incorporated into whey protein films. It has shown potential in inhibiting the growth of bacteria and fungi, thereby extending the shelf life of packaged products. Thyme oil, cinnamon, clove, rosemary, and tea tree oil have been explored for their antimicrobial and antioxidant activities. It has been used as an additive in whey protein films to improve their barrier properties, mechanical strength, and resistance to oxidation.

Whey protein films have shown promise as edible coatings for fruits and vegetables, offering potential to extend their shelf life, maintain quality, and reduce post-harvest losses. Additionally, these films have been investigated as coatings for meat and poultry products, aiming to improve quality, prevent moisture loss, and enhance shelf life. By acting as barriers against oxygen and microbial growth, whey protein films contribute to maintaining the freshness and safety of packaged meat.

Moreover, whey protein films have been studied for packaging dairy products such as cheese, yogurt, and milk. These films offer potential benefits in terms of preserving freshness, preventing moisture migration, and enhancing the overall quality and shelf life of these products. Furthermore, whey protein films have been explored for coating bakery and confectionery products like bread, cookies, chocolates, and candies. By utilizing these films, the freshness and quality of these products can be preserved while preventing moisture-related issues.

Leguminaceae Proteins (Soy Beans, Pea, etc.)

Proteins derived from legumes, specifically those belonging to the Leguminaceae family such as soybeans, lentils, and peas, possess several advantageous properties that make them well-suited for packaging films. These proteins exhibit excellent film-forming abilities, enabling the creation of thin and flexible films suitable for packaging applications. Moreover, they have the capability to form cohesive and continuous films, providing a protective barrier for packaged products.

Leguminaceae protein films demonstrate commendable barrier properties, including resistance to moisture, oxygen, and UV light.

Additionally, these proteins enhance the mechanical strength of packaging films, improving tensile strength, elasticity, and puncture resistance (Rani *et al*., 2020).

One notable advantage of Leguminaceae proteins is their plant-based origin, rendering them biodegradable and renewable. In addition, Leguminaceae proteins can be modified or blended with other ingredients to enhance their functional properties. For instance, blending these proteins with plasticizers or other polymers can improve film flexibility, elongation, and moisture resistance. This versatility allows for customization and adaptation to specific packaging needs (Sani *et al*., 2021).

Several members of the Leguminaceae family have been studied for their potential use in packaging film making.

Extensive research has been conducted on the use of soy protein films derived from Glycine max for various packaging applications. Soy protein isolates or concentrates have been utilized to create films with commendable mechanical strength, barrier properties, and biodegradability.

Studies have examined the impact of soy protein films on food quality, sensory attributes, and shelf life extension. Applications include coating fruits, vegetables, and meat products, as well as packaging bakery goods, snacks, and dairy products (Han *et al*., 2018).

Researchers have explored surface modification techniques to improve the properties of soy protein films. Surface treatments, such as plasma treatment, chemical modification, or coating with other materials, have been investigated to enhance film functionality, such as water resistance, antimicrobial properties, and controlled release of active compounds.

Antioxidants such as tocopherols (vitamin E), ascorbic acid (vitamin C), and polyphenols have been incorporated into soy protein films to enhance their oxidative stability. Natural antimicrobial compounds like essential oils, plant extracts, and bacteriocins have been used to improve the antimicrobial properties of soy protein films.

Glycerol and sorbitol are commonly used plasticizers in soy protein films. They help reduce film brittleness and enhance film elongation and tensile strength.

Nanomaterials such as silver nanoparticles, zinc oxide nanoparticles, and cellulose nanofibers have been studied for their potential to improve soy protein film quality.

Emulsifiers like lecithin or other food-grade surfactants can improve the emulsion stability and film-forming properties of soy protein films. They can help create more uniform films with improved mechanical strength and barrier properties (Martelli-Tosi *et al*., 2018).

Pea protein films, derived from Pisum sativum, have emerged as a promising alternative to soy protein films. By utilizing pea protein isolates or concentrates, films with excellent film-forming ability, barrier properties, and mechanical strength have been developed.

To further enhance film properties, pea protein can be blended with other polymers. Blending pea protein with chitosan, alginate, or starch, for instance, can significantly improve film flexibility, water resistance, and barrier properties (Du *et al*., 2018).

Pea protein films exhibit moderate barrier properties, characterized by low water vapor permeability (WVP) and oxygen permeability (OP) values. Moreover, the thermal stability of these films has been found to be satisfactory for packaging applications.

Lentil protein films possess a range of properties that make them well-suited for packaging applications. Notably, they exhibit impressive mechanical strength and flexibility. With desirable tensile strength and elongation at break, these films can withstand handling and packaging processes without easily breaking or tearing.

In terms of barrier properties, lentil protein films fall within the moderate to excellent range. Over time, these films naturally degrade, offering a more sustainable alternative to conventional plastic films (Acquah *et al*., 2020).

However, it is important to note that lentil protein films can be sensitive to moisture. Without proper protection or modification, they have the potential to absorb water and compromise their mechanical properties. To address this issue, strategies such as cross-linking or incorporating hydrophobic additives like fatty acids, waxes, lipids, and silanes can be employed to enhance the water resistance of lentil protein films.

Another advantageous characteristic of lentil protein films is their good transparency. This allows for visual inspection of the packaged products, ensuring quality control. They are compatible with a diverse range of food products, including fruits, vegetables, bakery goods, snacks, and meat products (Ortiz *et al*., 2018).

Chickpea (Cicer arietinum) protein films have been investigated as a potential packaging material. Researchers extracted protein from chickpea flour and prepared film-forming solutions by dissolving the protein in a suitable solvent. Further optimization of the formulation and processing conditions could enhance the performance of chickpea protein films for broader applications in food packaging (Du *et al*., 2018).

Graminacea Protein Films

Several members of the Graminaceae family, also known as the grass family, have been studied for their filmmaking capability (Balaguer *et al*., 2013).

Rice protein, wheat gluten, corn zein, and barley protein have been extensively studied for their film-forming properties, making them a promising material for the development of edible films (Hager *et al*., 2019).

Wheat proteins possess valuable elastic and cohesive properties that make them suitable for non-food applications, including the production of garbage bags. Additionally, wheat proteins can be utilized as edible coatings in various food applications. For example, when used as coatings on shell eggs, degradable polymers and oils derived from wheat proteins can effectively inhibit microbial invasion and enhance shell strength. The development of degradable films and coatings using wheat gluten opens up new marketing opportunities for this versatile ingredient (Bagheri *et al*., 2019).

Zein, a type of alcohol-soluble prolamine storage protein found in corn, was divided into four classes (α-zein, β-zein, γ-zein, and δ-zein) based on solubility and sequence homology. Among these classes, α-zein constitutes the majority, accounting for 70-85% of the total zein mass, while γ-zein is the second most abundant, making up 10-20% of the fraction. All zein classes are rich in hydrophobic and neutral amino acids like leucine, proline, and alanine, with some polar amino acid residues such as glutamine also present. α-Zein is composed of highly similar repeat units and has a high α-helix content (Fazeli *et al*., 2022). Notably, zein differs from other proteins in that it is almost devoid of lysine and tryptophan, and contains only a small amount of arginine and histidine residues. These unique amino acid compositions contribute to its distinct solubility, which is primarily limited to acetone, acetic acid, aqueous alcohols, and aqueous alkaline solutions. Due to Zein protein unique features, such as filmmaking, significant thermal stability and gas barrier, it seems suitable for food packaging. Zein edible films and coatings create a transparent yellowish, brilliant, and elastic appearance and show exceptional preservative features in food packaging (Sayadi *et al*., 2021).

Barley, a key grain in brewing, is used in both raw and malted forms, often combined with other grains. The endosperm is vital for plant growth due to its nutrient content. Brewers' spent grain, a major by-product of brewing, has sparked interest in sustainable utilization of its protein content. This aligns with the industry's goal to reduce waste, which includes significant liquid and solid by-products per beer production. Harnessing barley proteins, valued for their nutritional quality, presents promising opportunities in the food sector (Yang *et al*., 2015).

By combining other film base materials with barley protein, such as gelatin, it is possible to achieve improved characteristics like tensile strength and percent elongation (Karimi *et al*., 2022).

Advances in Protein-based Film Technology

Film Preparation through Thermoplastic Method

Edible films offer a means to effectively regulate the movement of substances within food or between the food and its surroundings. The wide variety of proteins, with their countless arrangements of amino acids, enables a range of interactions and chemical reactions to occur as proteins denature and link together during heat processing. Two primary processes can be employed to create edible films (Fig. 1). The "wet process" involves dispersing or dissolving biopolymers in a film-forming solution (known as solution-casting), followed by the evaporation of the solvent. On the other hand, the "dry process" relies on the thermoplastic properties exhibited by certain proteins and polysaccharides at low moisture levels, using compression molding and extrusion.

The thermoplastic method for film making involves the use of heat and pressure to transform a polymer or biopolymer into a pliable and moldable state, allowing it to be shaped into a film.

A suitable thermoplastic polymer or biopolymer is chosen based on its desired properties, such as film strength, flexibility, and barrier properties. The polymer is typically combined with other ingredients, such as plasticizers, to improve its processability and final film properties. These additives help to reduce the glass transition temperature of the polymer, making it easier to shape and mold. The mixture is heated to a temperature above the glass transition or melting point of the polymer. This causes the polymer chains to become mobile and allows for the formation of a viscous, molten state. The molten polymer is then subjected to pressure and shaped into a thin film using various techniques, such as compression molding or extrusion. Compression molding involves placing the molten polymer between two plates and applying pressure to form a film, while extrusion involves forcing the molten polymer through a die to create a continuous film. Once the desired film shape is achieved, the film is rapidly cooled to solidify the polymer chains and lock them into place. This solidification process can be achieved by cooling the film using chilled rollers or immersing it in a cooling bath. After solidification, the film may undergo additional treatments, such as annealing or stretching, to further enhance its mechanical properties and performance (Velaga *et al*., 2018).

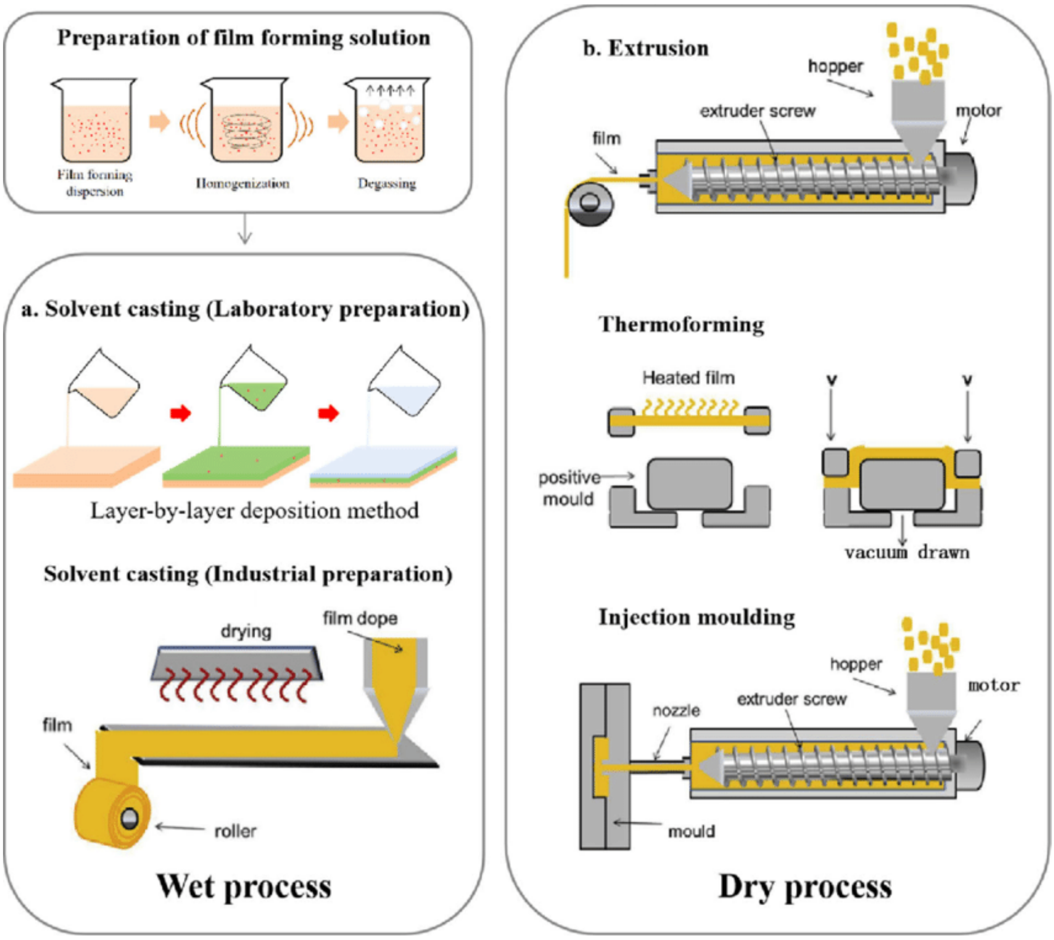

Fig. 1. Schematic of Film preparation through casting and thermoplastic method (Chen et al., 2021)

Wheat gluten, corn zein, soy protein, myofibrillar proteins, and whey proteins have all been successfully transformed into films using methods like compression molding and extrusion. These thermoplastic processes present a highly efficient way to manufacture edible films on a large scale, thanks to the use of low moisture levels, high temperatures, and short processing times. The addition of water, glycerol, sorbitol, sucrose, and other plasticizers allows the proteins to undergo transition into a glassy state, making them easier to shape and process without losing their integrity (Cheng *et al.*, 2021).

The specific ways in which proteins interact during thermoplastic processing are not fully understood. The reactivity of proteins is influenced by their physicochemical environment and the thermomechanical treatment they undergo. The type and quantity of plasticizer used can also impact protein reactivity and the properties of the extruded material. For instance, a study examining the effect of different plasticizers on gluten protein reactivity found that octanoic and lactic acids hindered protein aggregation, despite the high mixing temperatures employed, which typically promote aggregation. The aggregation of gluten proteins is believed to involve reactions between sulfhydryl and disulfide groups. However, under acidic conditions, these reactions are less favorable as sulfhydryl acts as a catalyst. Whey protein films that were compression-molded with water as a plasticizer resulted in brittle and insoluble films, whereas those plasticized with glycerol were flexible, and their solubility depended on the temperatures used during film formation (Yang *et al.*, 2015).

In the case of corn zein, it was extruded into sheets along with fatty acids. Stable extrusion runs were achieved due to the binding of the fatty acids to the zein surface, as indicated by differential scanning calorimetry (DSC). This binding phenomenon prevented the separation of lipid phases and the sticking of zein onto the extruder barrel (Salajegheh *et al.*, 2020).

Nanocomposite Protein Films

The use of biodegradable packaging materials has certain limitations as they cannot completely replicate the characteristics of petroleum-based materials. However, the incorporation of nano-sized materials allows for the modification of these materials, providing similar properties to traditional packaging materials. A nanocomposite film is created by blending a biopolymer with nanofillers to form a solid material system with multiple phases. When producing nanocomposites through the casting method, it is essential to first disperse the nanoparticles (NPs) in water, as they are typically sold in agglomerated microscopic forms. By incorporating various nanomaterials (ranging from 1 to 100 nm) into plant protein-based films, their barrier, optical, mechanical, and antimicrobial properties can be enhanced. This modification alters the physicochemical and functional characteristics of the films, opening up possibilities for their use as multifunctional ingredients in food products. Nanomaterials possess unique chemical, biological, electrical, thermal, mechanical, optical, and magnetic properties that differ from those of bulk materials (Garavand *et al*., 2020).

There are various examples of nanocomposite protein films that have been developed by incorporating nanomaterials into protein-based matrices.

Gelatin can be combined with nanoclays to create composite films. The addition of nanoclays improves the mechanical properties and barrier performance of the gelatin film. The addition of nanoclays can significantly increase the tensile strength, stiffness, and toughness of gelatin films. The clay particles create a reinforcing network within the gelatin matrix, improving its mechanical performance. Nanoclays can enhance the barrier properties of gelatin films, particularly against gases, such as oxygen and carbon dioxide, and water vapor. The presence of the nanoclays creates tortuous paths for gas and moisture molecules, slowing down their permeation through the film. Nano clay particles can improve the thermal stability of gelatin films. They act as heat barriers, reducing the rate of heat transfer through the film and enhancing its resistance to high temperatures. Depending on the type and concentration of nanoclays used, they can also influence the transparency of gelatin films. In some cases, the addition of nanoclays can lead to a slight reduction in film transparency, which may be desirable for certain applications. The improved barrier properties of nanoclay-reinforced gelatin films can help extend the shelf life of food products by reducing oxygen and moisture permeation. This can help in preserving the quality and freshness of the packaged goods (Winotapun *et al*., 2019).

Gelatin can be combined with silver nanoparticles to create antimicrobial composite films. The silver nanoparticles provide antimicrobial properties to the film, making it suitable for food packaging applications. Soy protein isolate can be combined with graphene oxide, a two-dimensional nanomaterial, to create composite films. The addition of graphene oxide improves the mechanical strength and barrier properties of soy protein film. Whey protein isolate can be blended with zinc oxide nanoparticles to create nanocomposite films. The incorporation of zinc oxide nanoparticles enhances the antimicrobial and barrier properties of whey protein film. Corn zein can be combined with nanoclays to create composite films. The addition of nanoclays improves the mechanical properties and barrier performance of corn zein film (Yu *et al*., 2021).

Non-thermal Techniques for Improving Film Properties

Protein films, due to their limited mechanical strength, tendency to rupture, and poor water resistance, are not suitable for use in the food industry. However, there are various non-thermal and thermal techniques available to address these limitations and modify the properties of protein films. Techniques such as microwave, ultrasound, cold-plasma, irradiation, and high-pressure processing can be employed to enhance the mechanical strength, water resistance, and overall performance of protein films (Jariyasakoolroj *et al*., 2020).

These techniques work by reducing particle size, promoting intermolecular electrostatic repulsion between protein molecules, and facilitating cross-linking. By modifying the protein films using these methods, their properties can be significantly improved. This is particularly important in the food industry, as modified protein films with excellent barrier properties can help reduce food waste and environmental pollution. Additionally, they contribute to enhancing the quality, safety, and security of food products. Non-thermal and thermal techniques offer effective ways to overcome the limitations of protein films, making them more suitable for use in the food industry. These modifications not only enhance the mechanical strength and water resistance of the films but also improve their barrier properties, leading to a range of benefits for food preservation and sustainability.

Non-thermal techniques encompass a range of methods from physics, chemistry, and biology that do not require heating to induce changes in a system. These techniques, such as microwave, ultrasound, high-pressure processing, irradiation, and cold plasma, have been widely utilized to improve the properties of protein-based edible films (Kao *et al*., 2014).

The drying process for whey protein isolate (WPI) edible films involved the use of microwave drying. It took approximately 5 minutes to dry the films in a microwave oven. Interestingly, both microwave drying and drying at room conditions yielded comparable results in terms of water vapor permeability (WVP) for the WPI films. Additionally, the application of microwave drying led to increased values in elongation and tensile strength for the films (Galus *et al*., 2016).

Ultrasound was used to unfold the pea protein isolate and expose hydrophobic groups on the protein surface. This resulted in both dissociation of PPI and formation of larger aggregates. Additionally, FE-SEM analysis showed that ultrasound reduced cracks and protein aggregates on the surface of the PPI film. The film structure was examined using FTIR, which revealed shifts in peak positions in the amide I and II regions, indicating changes in the protein's secondary structure due to ultrasound. These structural modifications led to improvements in the properties of the PPI films. Specifically, ultrasound greatly enhanced film transparency, significantly increased film tensile strength (while not affecting elongation at break), and reduced moisture content and water vapor permeability of the film (Moosavi *et al*., 2020).

A study was conducted to explore the effects of various cold plasma treatments on the modification of whey and gluten protein film properties. Atomic force microscopy (AFM) images showed that whey protein films became significantly rougher after plasma treatment, while the roughness of treated gluten films decreased dramatically. Additionally, the tensile strength of the films improved significantly after 10-minute treatment. Fourier-transform infrared spectroscopy (FTIR) revealed the introduction of functional groups such as C-O and O=C bonds, as well as the creation of cross-links, which could potentially lead to changes in various film properties. Furthermore, both edible polymers exhibited a significant decrease in gas permeability (Yang *et al*., 2015).

Conclusion

Biodegradable protein-based packaging offers a promising solution to address the environmental concerns associated with traditional plastic packaging materials. The physical properties of protein-based packaging films, such as flexibility, strength, and barrier properties, make them suitable for various packaging applications. Additionally, their chemical properties, including biodegradability and compatibility with food products, further enhance their appeal. The antimicrobial and antioxidant properties exhibited by certain protein sources add an additional layer of functionality to these films, making them ideal for extending the shelf life of perishable goods. With a wide range of protein sources available, including plant-based and animal-based, there is ample opportunity for customization and optimization of protein-based packaging films. Furthermore, recent advancements in the field, such as the incorporation of bioactive compounds and the use of nanotechnology, have opened up new possibilities for improving the performance and functionality of protein-based packaging. However, challenges still exist, including the need for standardized production processes, scaling up manufacturing, and ensuring cost-effectiveness. Continued research and development efforts are essential to overcome these challenges and fully realize the potential of biodegradable protein-based packaging as a sustainable alternative in the packaging industry. Overall, protein-based packaging films represent an exciting and promising avenue for environmentally friendly packaging solutions with desirable physical and chemical properties, antimicrobial and antioxidant capabilities, and potential for further advancements and innovation.

Author Contributions

Samar Sahraee: Conceptualization, research and review, validation, writing-original draft, **Jafar M. Milani:** Project management, supervision, writing-reviewing and editing

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بسته‌بندی زیست‌تخریب‌پذیر ساخته‌شده از پروتئین

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چکیده

فیلم‌های پروتئینی به‌دلیل ویژگی‌های منحصر به فرد و قابلیت‌های تطبیق‌پذیری خود، توجه زیادی در زمینه توسعه مواد بسته‌بندی پایدار جلب کرده‌اند. این فیلم‌ها به خاطر ممانعت از نفوذپذیری گازها، ویژگی‌های مکانیکی خاص و قابلیت‌های اتصال بین مولکولی، نسبت به سایر پلیمرهای زیستی بیشتر مورد توجه قرار گرفته‌اند. در سال‌های اخیر، محققان به بررسی روش‌های جدیدی برای بهبود خواص فیلم سازی، افزایش استحکام مکانیکی و کاهش نفوذپذیری به گازها در فیلم‌های پروتئینی پرداخته‌اند. منابع پروتئینی مختلفی مانند ژلاتین، پروتئین آب پنیر، پروتئین سویا، زئین ذرت، گلوتن گندم و کازئین برای ساخت این فیلم‌ها مورد بررسی قرار گرفته‌اند. تکنیک‌هایی مانند ترکیب افزودنی‌ها، استفاده از عوامل اتصال‌دهنده عرضی و به‌کارگیری نانومواد، برای بهبود خواص این فیلم‌ها در حال اکتشاف هستند. همچنین، فیلم‌های کامپوزیتی بر پایه پروتئین با ترکیب پروتئین‌ها با دیگر پلیمرهای زیستی یا مواد مصنوعی به‌منظور دستیابی به عملکرد بهبود یافته تولید شده‌اند. پیشرفت‌ها در فناوری‌های تولید مانند قالب‌ریزی فیلم، اکستروژن و الکتروریسی، کنترل دقیق ضخامت، مورفولوژی و ویژگی‌های ساختاری فیلم‌های پروتئینی را امکان‌پذیر کرده است. این فیلم‌ها نه تنها ویژگی‌های نفوذپذیری بسیار خوبی دارند، بلکه زیست تخریب‌پذیری و تجدیدپذیری نیز از دیگر مزایای آن‌هاست که با افزایش تقاضا برای تولید بسته‌بندی‌های سازگار با محیط‌زیست هم‌راستا است. بهبود و تهیه فیلم‌های پروتئینی دارای پتانسیل قابل توجهی برای متحول کردن صنعت بسته‌بندی و کمک به ساخت دنیایی سبزتر و محیط زیست‌دوستانه تر می‌باشد. این مقاله به بررسی تحقیقات و پیشرفت‌های کنونی در این حوزه می‌پردازد و منابع مختلف پروتئین، تکنیک‌های اصلاح فیلم، روش‌های تولید، چالش‌ها و چشم‌اندازهای آینده را مورد بررسی قرار می‌دهد.

واژه‌های کلیدی: بسته‌بندی مواد غذایی، پروتئین، تجزیه‌پذیر، خواص فیزیکوشیمیایی، نانوتکنولوژی

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