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Characterization Techniques for Optical, Structural, Morphological and Biodegradation Properties of Food Films

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ABSTRACT

Objective: To address the main analytical methods used to characterize edible films in their optical, structural, morphological and biodegradation properties.

Design/methodology/approach: It addresses how these techniques contribute to the development of edible films with suitable characteristics according to the specific conditions required by the food. Key methods such as UV-Vis spectrophotometry, colorimetry, optical microscopy, Raman spectroscopy, infrared spectroscopy and scanning electron microscopy are discussed, showing their role in the evaluation of film properties such as transparency, chemical structure, surface morphology and biodegradability.

Findings/conclusions: The findings provided by these characterization techniques are essential for progress in the development of sustainable food packaging that enhances food safety, improves shelf life, and reduces environmental impact.

Keywords: edible films, optical properties, structural properties, morphological properties, biodegradability.

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INTRODUCTION

There is increasing interest in developing edible food films due to the need to replace the use of conventional plastics used as primary food packaging with sustainable alternatives such as biodegradable polymers. These materials are generally made from polysaccharides, proteins and lipids and in addition to serving as a first semi-permeable barrier between the food and its environment, they possess functional properties that contribute to food preservation. Among the various characteristics of edible films that can be evaluated are optical, structural and morphological properties, which are essential to determine the effectiveness of the films in protecting and preserving the food (Olawade *et al.*, 2024).

A comprehensive evaluation of the quality and applicability of edible films requires optical properties such as transparency, color stability, and light absorption. Conventional analytical techniques, including UV-Vis spectrophotometry and colorimetry, offer valuable insights into the films' capacity to impede harmful UV radiation and preserve their aesthetic appeal over time. Furthermore, optical microscopy is a fundamental tool for assessing surface uniformity and the distribution of components within the film matrix (Simona *et al.*, 2021; Nosal *et al.*, 2005).

In addition to optical properties, the structural composition of edible films is essential for understanding their functional behavior. Techniques such as Raman and infrared spectroscopy facilitate the identification of molecular interactions, component compatibility, and chemical stability. These analyses have been instrumental in optimizing film formulations, thereby enhancing their protective and mechanical properties (Hsu *et al.*, 2005; Sucheta *et al.*, 2019; Mohammadi *et al.*, 2018).

The performance of edible films is influenced by morphological properties, which affect parameters such as permeability, mechanical strength, and biodegradation rate. Consequently, advanced imaging techniques, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM), have emerged as instrumental tools for conducting detailed examinations of surface textures, porosities, and overall microstructures. These techniques facilitate a more profound comprehension of the interactions between film components and their impact on food preservation (Farhan and Hani, 2017).

Meanwhile, biodegradation properties are important for evaluating the environmental impact of edible films. A comprehensive understanding of the factors influencing their decomposition, such as polymer structure and environmental conditions, is imperative for the development of packaging solutions that are aligned with sustainability objectives (Agustina and Sasongko, 2023; Jaramillo *et al.*, 2016).

The present review article explores a variety of techniques for optical, structural, morphological, and biodegradation analyses employed for the purpose of characterizing edible films. The article highlights their importance in ensuring the quality, functionality, and environmental sustainability of these materials.

Optical properties

UV-Vis Spectrophotometry

UV-Vis spectrophotometry is an analytical method that quantifies light absorption in the ultraviolet (UV) and visible regions of the electromagnetic spectrum. This method is extensively employed in the assessment of edible films because of its capacity to analyze the optical and functional characteristics of materials. In a particular study, the transparency and UV protection effectiveness of edible films composed of carrageenan and orange essential oil were evaluated through the utilization of UV-Vis spectrophotometry (Simona *et al.*, 2021). The findings indicated that the amalgamation of these components led to a decrease in transmittance across both UV and visible wavelengths, thereby suggesting the potential of these films to function as UV shields (Simona *et al.*, 2021). In a separate study, the addition of calcium alginate and tea polyphenols to films was found to enhance their antioxidant properties, as evidenced by a decrease in optical transmittance (Biao *et al.*, 2019).

A UV-Vis spectrophotometer is employed to quantify the degree of absorption of the dried film subsequent to the preparation of a film-forming solution and the formation of the film through techniques such as casting. A study on edible films composed of potato starch, chitosan, and aloe vera gel employed UV-Vis spectrophotometry to investigate changes in the chemical structure and molecular configuration of the film components after exposure to UV radiation (Bajer, 2020). This technique was also employed to assess the UV barrier qualities of starch films altered through photochemical processes, revealing that exposure to UV-A and UV-C radiation enhanced the films' UV protection capabilities (Shahabi-Ghahfarrokhi *et al.*, 2019).

UV-Vis spectrophotometry is a valuable technique for studying edible films because it provides complete information about the optical and functional properties of materials, which is necessary for better food packaging. A study on casein phosphopeptides in gelatin films demonstrated that these substances reduced the transmission of UV light. This effect was attributed to the enhanced antioxidant and antibiotic properties of the films (Khedri *et al.*, 2021). Furthermore, UV-Vis spectrophotometry evaluates the protective efficacy of films against UV rays, thereby enhancing food safety and prolonging shelf life (Dash *et al.*, 2019).

Colorimetry

Quantifying color in edible films is fundamental to evaluating their visual and commercial potential. The CIELab color system, a widely accepted method for color measurement; is composed of three components: L* (lightness), a* (green to red scale), and b* (blue to yellow scale). This system is frequently utilized for this assessment (Ahmad *et al.*, 2019; Bhatia *et al.*, 2022a). This technique enables precise representation of color, which is of high significance because consumers can easily detect small differences that influence their preferences. Furthermore, in the context of the evolution of transparent edible films, the colorimetric analysis can serve as a reliable indicator of the presence of specific components or alterations in the composition of the film, which may arise from storage conditions or environmental factors such as light and humidity (Zhelyazkov *et al.*, 2021; Arrieta *et al.*, 2014).

This analysis is of particular pertinence in the context of color stability investigations, as it facilitates the observation of alterations over the course of storage duration or under varying temperature exposures. Additionally, it enables the assessment of the impact of specific ingredients on the hue and brightness of the film (Luchese *et al.*, 2018; Homez *et al.*, 2018). The significance of these measurements is that color serves as a primary sensory attribute for the acceptability of a product and may also be indicative of film quality and safety. Color variations may signify lipid component oxidation, alterations in water activity, or interactions among substances (Yildirim-Yalcin *et al.*, 2021); specifically, the coloration of protein and polysaccharide-based edible films can be influenced by ultraviolet light exposure, pH levels, or temperature; hence, diligent monitoring is crucial to maintain a stable, high-quality product (Zhai *et al.*, 2018; Homez *et al.*, 2018).

Optical Microscopy

The optical microscope is a fundamental instrument employed in the analysis of food films that facilitates the observation and description of the surface and shape of these materials. The underlying principle of this method involves the utilization of visible light to illuminate the sample thereby creating a comprehensive representation of its surface characteristics. In the context of analyzing food films, the optical microscope is employed to assess parameters such as uniformity, the presence of flaws, and the distribution of the film's components. In a study of hologram markers in biopolymer films, optical imaging in reflection mode was employed to examine the surface shape and determine the fabrication process of these markers (Podshivalov *et al.*, 2020). This approach ensures that edible films possess a structure that is suitable for their intended use as food packaging materials.

In order to obtain measurements by means of optical microscopy, it is necessary to create a sample of the edible film and subsequently position it beneath the microscope. A light source is employed to illuminate the sample, and the image is observed through the use of mirrors that magnify it. Scanning electron microscopy (SEM) has been employed in conjunction with optical microscopy in numerous studies, including those examining calcium alginate sheets infused with tea polyphenols. This approach yielded comprehensive images of the films' surface and interior architecture (Biao *et al.*, 2019). These metrics facilitate the assessment of film quality and utilization by examining parameters such as thickness, density, and uniformity, among other factors.

The optical microscope is a very useful tool for the analysis of edible films because it provides a comprehensive insight into their structural and surface properties. This profound understanding is vital to the development and improvement of food films that exhibit specific characteristics, such as strength, water vapor permeability, and transparency. For instance, studies employing optical imaging and other characterization methods on chitosan and whey protein films have demonstrated that the incorporation of chitosan enhances the films' strength and reduces their water vapor permeability (Tavares *et al.*, 2020); these alterations are of particular importance for food packaging applications, where edible films must keep food safe and last longer.

Structural Properties

Raman Spectroscopy

Raman spectroscopy is a photonic technique that utilizes monochromatic laser radiation directed at a sample. The scattered light exhibits characteristic frequency changes (Raman bands), which provide chemical and structural information about the material being analyzed (Soler Barrera *et al.*, 2013). Raman spectroscopy is a valuable technique for investigating the composition and structure of edible films thereby verifying their quality and safety. This technique's capability to identify chemical and biological impurities and evaluate the authenticity of components is relevant for ensuring the safety of edible films for human consumption (Neng *et al.*, 2020; Huang *et al.*, 2020). This approach has been shown to detect and quantify particular components, including lipids, proteins and other additives (Du *et al.*, 2019; Jiang *et al.*, 2020); this method does not require sample preparation, thus making it well-suited for real-time and online applications in the food sector (Hu *et al.*, 2019).

Infrared Spectroscopy

Alternatively, infrared spectroscopy is a rapid and simple way to detect polymeric materials using group frequencies and characteristic patterns in the spectral "fingerprint". The underlying principle of this method is the manner in which molecules absorb infrared light; a process that generates vibrations within the chemical bonds. This technique allows for the characterization and study of hydrogen bonds and the identification of specific interactions (Pastor *et al.*, 2003); when polymers are compatible, molecular interactions occur, which makes infrared spectroscopy especially useful for identifying polymer compatibility in polymeric material blends, particularly Fourier transform infrared spectroscopy (FTIR) (M'Bareck *et al.*, 2009).

In a study, the Fourier-transform infrared (FTIR) spectroscopy technique was employed to analyze edible films composed of sodium alginate and casein infused with orange oil. This method enabled the detection of the distinctive peaks of the film components thereby facilitating the assessment of the films' compatibility and structural qualities (Bhatia *et al.*, 2022a). FTIR has been employed to examine edible films composed of gelatin and casein, demonstrating the impact of gallic acid on the films' structure and thermal stability (Bhatia *et al.*, 2022b).

Infrared spectroscopy is imperative for the analysis of edible films as it provides exhaustive insights into their chemical composition and molecular interactions. This is indispensable for the development of edible films with improved properties for specific food applications. Additionally, the capacity of infrared spectroscopy to facilitate rapid and non-destructive evaluation renders it a valuable technology for quality control and research on edible films (Li *et al.*, 2020).

Morphological Properties

The structure of polymers (morphology) can be determined through Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Atomic Force Microscopy (AFM), among other techniques. While SEM generates images in a range of 5 to 10 nm, electron microscopy can achieve magnifications of over 200,000 times (Seymour and Carraher, 1995).

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a technique used to evaluate the surface microstructure of edible polymeric materials. This analysis provides both qualitative and quantitative insights into the organization of the material's components at microscopic detail; its organization and topographical study are done by generating high-resolution, three-dimensional images within a wide range of magnifications (Granada-Restrepo *et al.*, 2014). The images are generated by emitting secondary electrons from the sample when it is hit with a high-energy electron beam. The emitted electrons are detected and produce an electrical signal, which creates the image on a cathode ray tube screen (Vijayalakshmi *et al.*, 2024).

The importance of understanding morphology lies in determining changes in the structural properties of the films. It also helps assess characteristics such as porosity, permeability, flexibility, and resistance (De Paula Herrmann *et al.*, 2004; Giosafatto *et al.*, 2014). Morphology can indicate whether there is good compatibility between the compounds and plasticizers, showing a smooth surface when compatibility exists. Likewise, a compact structure in the cross-section suggests a developed network, while a porous film allows deeper incorporation of the active compound, enabling its prolonged release (Nur Hanani, 2018).

In materials science, SEM is used for the morphological characterization of different materials, studies of adhesion in polymer matrices, fracture analysis, failure analysis, and the morphology of polymer particles, among other applications.

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is a technique that allows for the determination of morphological characteristics, structures (via electron diffraction), the construction of cell images (via high-resolution transmission electron microscopy, HRTEM), as well as the chemical composition of a sample through X-ray fluorescence by energy dispersive X-ray (EDXRF) (Ercius *et al.*, 2020).

In TEM microscopy, a beam of electrons with uniform current density is irradiated onto the sample. The image is generated on a fluorescent screen through the projection of the electron intensity distribution behind the sample, passed through a system of three to eight lenses (Reimer and Kohl, 2008). Depending on the study's goal and the material, information can be collected from individual particles, specific areas, or aggregates. The image can be recorded by directly exposing a photographic emulsion, by exposure to a vacuum plate, or even by using a camera with a coupled charge device (Reimer and Kohl, 2008).

Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM) was developed in 1986 and collects image information through variations in the magnitude of the interaction between the probe and the material's surface being analyzed (Yang *et al.*, 2007). This type of microscopy allows for surface analysis through mechanical scanning using atomic forces, hence the name (Kawai *et al.*, 2014). AFM can be useful in analyzing different types of materials.

Regarding edible polymers (PCs), AFM provides qualitative and quantitative information at the nanoscale for analyzing the morphology of their surfaces, also known as topographic analysis (Zuo *et al.*, 2019). For example, AFM helps understand the interactions between proteins and surfactants in a polymeric composition, among other processes and mechanisms of their molecular interactions (often Van Der Waals forces) (García *et al.*, 2018).

Other applications of AFM in the food area include the qualitative analysis of the molecular structure of important food compounds, especially in the formation of macromolecule networks such as proteins and carbohydrates, and the study of their intermolecular interactions. Moreover, AFM allows the study of the topology (subtle physical properties) of food surfaces, such as roughness, homogeneity, morphology, and fractal analysis (García *et al.*, 2018).

Biodegradation Properties

Biodegradability is defined as the ability of a material to decompose through the enzymatic action of microorganisms, measured under specific storage periods and conditions using standardized methods (López *et al.*, 2010). There are two types of biodegradations: aerobic and anaerobic. The former occurs in the presence of oxygen, while the latter takes place in its absence. In aerobic biodegradation, the waste products generated are biomass, carbon dioxide, water, and inorganic compounds; in anaerobic degradation, biomass, methane, intermediate metabolites, and inorganic compounds are produced (Kyrikou and Briassoulis, 2007). Complete biodegradation implies the destruction of the macromolecular support, resulting in the corresponding by-products (Rubio-Anaya and Guerrero-Beltrán, 2012).

Biodegradation involves various processes, such as the loss of mechanical strength (modifications to surface characteristics), degradation by microorganisms or enzymes, as well as the breaking of the main chain, and subsequent reduction in the molecular weight of the polymers.

Although edible films are considered biodegradable because they are based on natural biopolymers, their degradation rate depends on their molecular links, with a slower degradation observed in films formed through covalent bonds (Li and Chen, 2000). This biodegradation speed is determined by several environmental factors such as soil pH, humidity, temperature, partial oxygen pressure, microbial flora composition, and the presence of other nutrients (Lee, 1996; Kyrikou and Briassoulis, 2007), as well as intrinsic polymer parameters like surface area, monomeric composition, degree of crystallinity or amorphous regions, and molecular weight. It has been reported that higher crystallinity implies a slower biodegradation rate (Nishida and Tokiwa, 1995; Spyros and Kimmich, 1997), and polymers with lower molecular weight degrade more quickly than those with higher molecular weight (Jendrossek *et al.*, 1996).

There are various methods to characterize the biodegradation of a polymer. It can be done by measuring weight loss, changes in tensile strength, or in its physical and chemical properties, according to modifications in the molecular weight distribution or dimensional changes, as well as determining carbon dioxide production or bacterial activity in the soil (Singh and Sharma, 2008).

Research challenges in polymers are focused on developing films with natural biopolymers for making bags or packaging materials that have the shortest biodegradation time based on their intended use (Kean and Thanou, 2010).

Critical analysis of characterization techniques

Ultraviolet-visible (UV-Vis) spectrophotometry, colorimetry, and optical microscopy are essential non-destructive techniques for the optical and structural characterization of materials. These techniques facilitate the analysis of absorption, color, and surface morphology. However, it should be noted that UV-Vis spectrophotometry is restricted to the assessment of transparent substances, and optical microscopy has poor resolution. Raman Spectroscopy and Infrared Spectroscopy both offer detailed insights into the chemical composition and functional groups of materials; however, Raman spectroscopy may demonstrate diminished sensitivity to certain molecules, while infrared spectroscopy necessitates careful sample preparation to avoid interference. Biodegradation analysis is crucial for evaluating environmental impact; nevertheless, testing may be prolonged due to high variability in behavior under uncontrolled conditions.

In essence, structural analysis employing scanning electron microscopy (SEM) and transmission electron microscopy (TEM) yields images at the nanoscale level, thereby facilitating comprehensive insights into the morphology and phase distribution within films. However, it is necessary to note that these methods are costly and require meticulous sample preparation. While Atomic Force Microscopy (AFM) is effective for assessing nanometric topography, it is less adept at analyzing interior morphology. Each of these techniques has specific applications and limitations, and their combination may offer a more comprehensive approach.

The development and application of new technologies in postharvest preservation present various challenges that must be addressed to ensure their effectiveness and feasibility at an industrial level. A primary challenge pertains to scalability and cost, as many of these techniques have demonstrated efficacy in laboratory studies but may necessitate substantial investments in infrastructure for large-scale implementation. Additionally, compatibility with existing industrial processes poses a significant obstacle, as the integration of new technologies into established production lines without compromising operational efficiency is intricate and necessitates technical and logistical adjustments.

Another fundamental challenge is compliance with food safety regulations and standards, which vary by region and may impose restrictions on the use of certain materials or methods. This issue is inextricably linked to environmental sustainability, as certain techniques may generate by-products or require substantial energy consumption, potentially impeding their adoption in industries seeking to minimize their ecological footprint. Furthermore, market and consumer acceptance plays a pivotal role, as public perceptions regarding the utilization of innovative food technologies can sway demand and commercial adoption.

CONCLUSIONS

The characterization techniques examined in this review are instrumental in evaluating the optical, structural, and biodegradation properties of edible films and coatings. These methods yield critical insights into optical transparency, structural integrity, molecular interactions, and degradation behavior, thereby contributing to the optimization of food packaging materials. UV-Vis spectrophotometry and colorimetry facilitate precise measurement of light absorption and color properties, which are required to evaluate film transparency and UV protection. Optical microscopy and SEM provide detailed morphological analysis, revealing surface uniformity, porosity, and structural integrity. TEM and AFM offer nanoscale insights into molecular organization and film composition. Spectroscopic techniques, including Raman and infrared spectroscopy, enhance our understanding of chemical interactions, polymer compatibility, and functional group modifications within the film matrix. Furthermore, biodegradability assessments are crucial for determining the environmental impact of these materials, which is imperative for the development of sustainable packaging solutions. However, these techniques are not without limitations, including the complexity of instrumentation, the time-intensive of sample preparation, and the requirement of specialized expertise. Variability in methodological approaches may also lead to inconsistencies in data interpretation across different studies. To that end, future advancements in this field should prioritize the development of more standardized, rapid, and non-destructive analytical techniques that can be applied efficiently in industrial settings.

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