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Evaluation of Edible Coatings from Components from *Chlorella vulgaris* and Comparison with Conventional Coatings

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Abstract: The present customer demand for ready-to-eat food items with higher nutritious value and longer shelf life necessitates creative solutions. An edible coating is a sustainable packaging solution that can prevent food deterioration and preserve food quality. Proteins, starch, and the addition of plasticizers are used to create edible coatings. The aim of this study was to develop coating solutions that can best preserve food using isolated starch and proteins from *Chlorella vulgaris*, and then compare them to coatings that comprise conventional ingredients like chitosan and starch. A number of criteria pertaining to the coatings' mechanical, optical, thermal, and physical properties were tested. The alternative coatings performed just as well as the conventional ones, with the protein algal coating exhibiting the best thermal, optical, and physical qualities. The food product that needs to be coated can determine which coating is ideal. In conclusion, edible coatings derived from *Chlorella vulgaris* offer a sustainable solution to preserve ready-to-eat food items, showcasing comparable performance to conventional coatings.

Keywords: edible coating; microalgae; *Chlorella vulgaris*; evaluation

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1. Introduction

Food is a fundamental human need that is vital for both survival and function. In addition to directly affecting humans, food management significantly influences the economy and the environment from production to disposal as waste. The growing demand for processed foods, characterized by a long shelf life, a high nutritional value, and readiness for consumption complicates the food supply chain [1].

The maintenance of quality in food products and the extension of their shelf-life is a major challenge for food companies; therefore, packaging is a crucial consideration [2]. Conventional packaging, primarily based on petroleum polymers, poses significant detrimental consequences on the environment and human health, so there is a need for new eco-friendly packaging solutions. Edible coating is a long-term preservation process, applied directly to the food, which can lead to a long shelf-life, by controlling the transport of moisture and oxygen [3]. Additionally, it enhances resistance against overall fruit quality deterioration, affecting texture, appearance, color, and weight loss [4]. Furthermore, coating is a cost-effective and efficient alternative to modified atmosphere packaging [5]. The concept of edible coating, inspired by natural fruit rinds, provides a thin, edible barrier preventing water and oxygen loss, among other substances [6].

Microalgae, capable of large-scale cultivation and benefiting from genetic engineering, emerge as a renewable and sustainable resource. They may be employed in numerous scientific applications because of their rich biodiversity, including the production of biofuels, medicines, cosmetics, and food, as well as the filtration of wastewater and CO_2 from the air [7]. As a plant source, microalgae have garnered attention for their potential in addressing contemporary challenges and serving as a sustainable source of bioproducts [8].

Chlorella vulgaris in particular is rich in starch [9] and proteins [10], components that are crucial for the development of edible coatings. This microalga has found application in the

food industry as a natural preservative due to its antioxidant and antimicrobial properties. For instance, its addition to meat products like sausages and patties has been shown to extend shelf life by inhibiting microbial growth and lipid oxidation [11]. Similarly, incorporation into bakery products improved freshness while maintaining nutritional value [12]. Moreover, edible coatings containing *Chlorella vulgaris* have been demonstrated to prolong the shelf life of calf fillets [13] and rainbow trout fillets [14] during refrigerated storage.

This study aims to address the urgent need for sustainable methods in food preservation and packaging by investigating the potential of edible coatings sourced from alternative materials, notably *Chlorella vulgaris*, a microalga abundant in biopolymers. The research is significant for its innovative approach to tackling the environmental challenges associated with traditional packaging materials. By harnessing the renewable properties of microalgae, particularly *Chlorella vulgaris*, this study seeks to develop edible coatings capable of prolonging the shelf life of food products while preserving their quality attributes. Additionally, the utilization of biopolymers from microalgae offers versatile applications beyond food preservation, including biofuels, medicines, cosmetics, and wastewater treatment. Through a comparative analysis with conventional coatings, this study aims to underscore the sustainability and efficacy of alternative packaging solutions, thereby contributing to a more environmentally friendly and resource-efficient food industry.

2. Materials and Methods

2.1. Materials

The materials selected for developing edible coatings included chitosan, starch, glycerol, Tween 20, and *Chlorella vulgaris*. Chitosan, starch, glycerol, and *Chlorella vulgaris* were purchased from local markets and Tween 20 was purchased from Sigma-Aldrich (Steinheim, NY, USA).

2.1.1. Extraction of Starch and Proteins from *Chlorella vulgaris*

Proteins were isolated from *Chlorella vulgaris* using a modified aqueous extraction process, using ammonium sulfate salt, according to [15]. The extraction of starch from *Chlorella vulgaris* was conducted with a method of physicochemical cell disintegration according to [16].

2.1.2. Characterization of Protein and Starch

Starch determination was conducted according to [17]. Firstly, a solution of iodine was prepared by mixing appropriate quantities of potassium iodide (KI) and iodine (I2) with deionized water to form a solution containing 0.6% *w/v* KI and 0.254% *w/v* I2. This solution was kept in a dark place due to iodine's sensitivity to light. Standard starch solutions were prepared by heating conventional starch in water to 90 °C for 3 h to create a 0.1% *w/v* solution. Samples for analysis were prepared by mixing the extracted material with deionized water and then subjecting it to centrifugation. The supernatant was then filtered under a vacuum to remove any particles. Subsequently, 120 µL of the iodine solution was added to 4 mL of the sample, producing a dark blue color indicative of starch–iodine complex formation, and photometric measurements were taken at 600 nm. Similar procedures were followed for the standard starch solution, allowing for the construction of a calibration curve for determining starch concentrations in unknown samples.

Protein determination was conducted using the Bradford method [18] to calculate the extraction yield and assess the method's effectiveness. A calibration curve was constructed for the determination of the concentration. In order to confirm the protein measurement performed on the extracts, the Kjeldahl method [19] is employed on the protein powder obtained after lyophilization. This approach ensures accurate protein quantification and validation of the results obtained from the Bradford method.

2.2. Preparation of Edible Coatings

The coatings were prepared using specific formulations tailored for each coating type. A chitosan coating was formulated, as outlined in [20]. Briefly, the coating forming solution was concocted by dissolving 2% *w/v* chitosan in a water solution containing 1% *v/v* acetic acid, serving as a stabilizing agent. To enhance the coating's flexibility and adherence, 1% *w/v* glycerol and 0.2% *w/v* Tween 20 were thoughtfully added as plasticizers. Starch coating solutions were formulated using either conventional potato starch or starch extracted from *Chlorella vulgaris*. In both cases, a blend of 2% *w/v* starch and 4% *w/v* pectin was dissolved in water, providing a stable case for the coating. To further improve texture and resilience, 1% *w/v* glycerol was meticulously added as a plasticizer. Finally, the protein coating solution was formulated according to [21]. The solution comprised 6% *w/v* protein, 3% *w/v* glycerol, and 0.4% *w/v* Tween 20 as a plasticizer dissolved in water. To maintain stability and functionality, the pH was meticulously adjusted to 11. Each coating solution was mixed aseptically using a degassing chamber, ensuring uniformity and quality. Following preparation, the coatings were delicately applied onto glass plates and left to dry under controlled conditions at 30 °C and 50 ± 2% RH for a meticulous 24 h period. They were then hermetically sealed within airtight bags in a cool and dry climate until further analysis could be conducted. The coatings are further mentioned as Chitosan, Starch/Pectin, Starch alg./Pectin, and Protein alg. (alg. stands for components derived from microalgae *Chlorella vulgaris*).

2.3. Film Characterization

2.3.1. Thickness

The physical and mechanical properties of membranes are affected by their thickness. Thickness measurements were taken using an electronic digital caliper, with a minimum measurement of 0.001 mm. By taking measurements at various points and calculating the average, the thickness was determined. This calculated thickness then served as the basis for the subsequent measurements in evaluating the edible coating.

2.3.2. Density

Density of the film was determined from its weight and volume. The volume was calculated according to the area and thickness of the film.

2.3.3. Moisture Content

The moisture content of the coating films was determined through thermogravimetric analysis. The membranes were placed in a reduced pressure oven at 100 °C for approximately 2 h. Their weight was weighed before and after heating and the moisture content (Y) inside the films was calculated by Equation [22].

$$Moisture\ (\%) = \frac{m1 - m2}{m1} \times 100, \quad (1)$$

where $m1$ and $m2$ are the initial and final weights of the samples, respectively.

2.3.4. Color

The color was measured with the Miniscan XE photometer (Hunter Associates Laboratory Inc., Reston, VA, USA) with a 4 mm diameter measuring head aperture. The values were calculated based on the CIELAB color measurement system, with the L* parameter determined by the luminance, the a* parameter by the deviations of red and green, and the parameter b* by the deviations of yellow and blue. The value was obtained by measuring four different points of the membrane and calculating their deviation [23].

2.3.5. Light Transmission and Transparency

A UV–Vis spectrophotometer (UV–Vis Spectrophotometer UV-M51, BEL PHOTONICS, Monza, Italy) was employed to measure the transparency and light transmission of the coatings. The coatings were carefully positioned within the cells, ensuring the entire surface

coverage, and the frequency was adjusted at 600 nm. The cells are placed inside and the $A600$ value was calculated as following:

$$Transparency\ value = \frac{A600}{x}, \tag{2}$$

where $A600$ is the absorbance at 600 nm and x is the thickness (mm) of the film [24].

2.3.6. Water Vapor Permeability

The permeability index of the samples was assessed by gravimetrically incorporating slight modifications to the method outlined in [25]. Silica gel was placed within a glass jar, which was then sealed using a 6 cm diameter piece of coating, covering the jar's opening. The film was secured in place with parafilm, and the jar was placed in an environment maintained at 90% relative humidity (RH) and 20 °C. The weight of the jar was recorded every 24 h until a consistent weight gain was observed [25]. The permeability index was subsequently calculated using the following equation:

$$WVTR\left(\frac{g \cdot s}{m^2}\right) = \frac{\Delta B}{\Delta t \cdot A}, \tag{3}$$

where ΔB is the difference in weight when a steady reduction has been achieved, Δt is the time duration, and A is the surface of the coating.

2.3.7. Thermal Analysis

Thermal analysis was carried out using Differential Scanning Calorimetry (DSC). A heating cycle with a step of 5 °C is performed and simultaneously the heat flow is obtained. The temperature range for the measurements was selected based on research of the literature, with each coating being subjected to a different range. More specifically, for chitosan, a range of 0 to 160 °C was chosen; for starch/pectin, a range of 0 to 250 °C was chosen; for starch algae/pectin, a range of 0 to 250 °C was chosen; and for algae proteins, a range of 0 to 220 °C was chosen. The samples were stored for 24 h at 25 °C and 0% RH in order to minimize moisture content. The glass transition temperature Tg is identified from the heat flow-temperature diagram. The enthalpy of crystallization is derived from the area under the crystallization peak in the thermogram, which is calculated using integration software provided by the DSC instrument. This peak corresponds to the heat released or absorbed during the crystallization process [26]. The enthalpy of crystallization (ΔH_c) is calculated using the following equation:

$$\Delta H_c\ \left(\frac{J}{g}\right) = \frac{Area\ of\ Crystallization\ Peak}{Mass\ of\ Sample}, \tag{4}$$

2.3.8. Mechanical Properties

In order to measure the tensile strength and the elongation at breaking point, an Analyzer Zwick Materials Testing Z2.5/TN15 was utilized. The samples were trimmed to the required dimensions (20 mm × 50 mm). The initial distance between grips was set at 40 mm, with a testing speed of 2 mm/s. A minimum of 10 samples from each film type underwent analysis.

The tensile strength was calculated according to the following equation:

$$TS\ (MPa) = \frac{F}{A}, \tag{5}$$

where F is the force (N) and A is the cross section (mm^2).

The modulus of elasticity was calculated according to Hooke's law:

$$E = \frac{s}{e}, \tag{6}$$

where *s* is the stress (Pa) and *e* is the deformation (%) [24].

2.3.9. Potassium Bromide Fourier Transform Infrared (KBr FTIR) Analysis

Potassium Bromide Fourier Transform Infrared (KBr FTIR) analysis was conducted using an FT/IR-4200 spectrometer from JASCO International Co., Ltd., Hachioji, Japan. This method was employed to investigate complex formations, interactions between components of the inclusion matrix and inclusions, and changes in the molecular arrangement of inclusion products. For the analysis, samples were prepared in potassium bromide (KBr) pellets. Scanning was performed across wavelengths ranging from 4000 to 700 cm^{-1}. Each measurement comprised 32 scans at a resolution of 4 cm^{-1}, providing detailed insights into the structural characteristics and interactions within the studied materials.

2.4. Statistical Analysis

One-way and factorial analysis of variance (ANOVA) were applied in order to analyze the differences. Tukey's range test (a = 0.05) was applied and all the statistical tests were performed with SPSS Statistics 21 software.

3. Results

3.1. Protein and Starch Characterization

The starch content, as determined by the method described above, yielded a measurement of 126.7 mg of amylose/g of starch. This result provides valuable quantitative insight into the composition of the starch samples analyzed. Amylose, a major component of starch, plays a significant role in various food and industrial applications, making its accurate determination crucial for quality control and product development. The precise measurement obtained through this method underscores its effectiveness in quantifying starch content and highlights its utility in research and industry.

Regarding proteins, the extraction yield obtained from the Bradford method is 3.85%. The protein content of *Chlorella vulgaris* generally does not surpass 58%. However, attaining this percentage often involves methods utilizing chemicals like or techniques that use Trichloroacetic Acid (TCA), which is not permissible within the food industry. Thus, this limitation reduces the maximum protein significantly. The yield from the Kjeldahl method resulted in 99.65%. The yield obtained from the Bradford method does not exhibit a significant percentage due to its specificity, as calculated by the Kjeldahl method. This specificity ensures that only proteins are present in both the liquid and dry extracts, thereby excluding other substances that might increase the quantity of the resulting powder but would compromise its protein purity. Consequently, while this specificity may not result in a high extraction yield, it maintains the integrity and purity of the protein powder, which is crucial for subsequent applications and analyses.

3.2. Film Characterization

The results of the film characterization are shown in Table 1.

Table 1. Film characterization of edible coatings.

Physical Characteristics	Chitosan	Starch/Pectin	Starch alg./Pectin	Protein alg.
Weight (g)	0.122 ± 0.002^{b}	0.107 ± 0.004^{c}	0.106 ± 0.004^{c}	0.254 ± 0.003^{a}
Thickness (mm)	0.150 ± 0.019^{a}	0.133 ± 0.038^{a}	0.088 ± 0.015^{a}	0.143 ± 0.029^{a}
Density ($g \cdot cm^{-3}$)	0.506 ± 0.011^{a}	0.497 ± 0.011^{a}	0.768 ± 0.017^{a}	1.123 ± 0.025^{a}
Moisture content (%)	0.271 ± 0.033^{a}	0.018 ± 0.006^{c}	0.043 ± 0.012^{c}	0.115 ± 0.004^{b}
Water solubility (%)	41.75 ± 1.09^{d}	74.74 ± 0.83^{a}	63.50 ± 1.12^{c}	71.50 ± 1.12^{b}
Water vapor transmission rate ($g/(s \cdot m^2)$)	0.0024 ± 0.0000^{b}	0.0041 ± 0.0001^{a}	0.0039 ± 0.0002^{a}	0.0023 ± 0.0000^{b}

Mean value ± standard deviation of four replicates. Values in the same row with different alphabetical letters are significantly different ($p < 0.05$).

The edible films that are analyzed are presented in Figure 1.

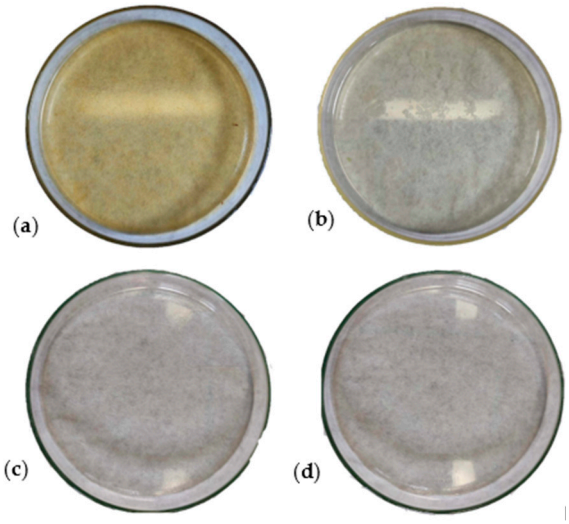

Figure 1. Images of edible films: (**a**) chitosan; (**b**) starch/pectin; (**c**) starch alg./pectin; (**d**) protein alg.

3.2.1. Thickness–Density

The thickness measurements of the four coatings exhibit no statistically significant differences ($p < 0.05$), indicating that the variations are negligible. However, specific trends can still be observed within the data. The starch alginate/pectin coating exhibits the lowest thickness, whereas the chitosan coating emerges as the thickest among the four. Interestingly, the other two coatings display thickness values similar to each other. The standard deviation of the starch coating is higher, indicating a lesser degree of uniformity compared to the others. The thickness does not seem to be affected by the components that create them.

Similarly, the density of the coatings remains consistent across all variations, with no significant differences observed ($p < 0.05$). However, coatings developed from *Chlorella vulgaris* components tend to yield structures with slightly bigger densities. The arrangement of particles or molecules, as well as the presence of additives within the coating, can affect the density. Density stands as a crucial parameter as it can directly impact the barrier properties, mechanical properties, and the overall performance of the coatings [27].

In summary, while thickness and density measurements do not exhibit considerable variation among the different coatings, subtle distinctions exist within the data. These findings underscore the importance of meticulous analysis and understanding of coating properties, as even minor differences can have notable implications for their performance in various applications. Further research and investigation into the factors influencing thickness and density can pave the way for optimized coating formulations tailored to specific requirements and performance criteria.

3.2.2. Moisture Content

Moisture content of coatings is an important factor that influences microbial growth, thereby impacting the overall shelf-life and safety of packaged products. The two starch coatings exhibit lower moisture content compared to their protein and chitosan counterparts. Starch is known to produce dryer films, with lower water activity, which are less likely to support the growth of bacteria [28].

In contrast, the chitosan coating has the highest moisture among the coatings. This phenomenon arises from the production process, as it requires more water to be made than other coatings, and that extra water is trapped in the structure as the coating dries.

Similarly, the protein coating also exhibits a relatively high moisture content. This can be attributed to the presence of glycerol, which is often incorporated into protein-based formulations. Glycerol, which has a high water-holding ability, results in a high moisture content in the protein coating [29].

Understanding these nuances in moisture content across different coating materials is essential for optimizing packaging solutions to mitigate microbial growth and ensure product safety. By tailoring coating formulations to achieve specific moisture levels, manufacturers can effectively enhance the shelf-life and quality of packaged products, meeting consumer expectations for freshness and safety. Furthermore, ongoing research aimed at refining coating formulations holds promise for further advancements in food packaging technology, facilitating improved preservation and microbial control.

3.2.3. Water Vapor Permeability

The rate of food deterioration within packaging is significantly influenced by the water transfer between the internal contents and their external environment. Consequently, the water barrier properties of packaging materials are essential. A thicker film provides increased resistance to mass transmission across its surface, as noted in reference [30], thus enhancing their effectiveness as barriers against water transfer.

Chitosan and protein coatings emerge as standout candidates in terms of their ability to impede the transmission of water vapor when compared to starch coatings. This is primarily attributed to the hydrophobic nature of chitosan, which allows it to serve as an excellent barrier against water vapor [31]. Conversely, the hydrophilic properties of starch and pectin create structures with poor moisture barriers, permitting water vapor to permeate and exit with ease [32].

By providing these detailed insights into the water barrier properties of different coating materials, we gain a deeper understanding of their performance characteristics and their potential impact on food preservation. Leveraging this knowledge, manufacturers can make informed decisions when selecting packaging materials, ensuring optimal preservation of product quality and extending shelf life. Furthermore, ongoing research and development efforts aimed at enhancing the water barrier properties of coatings hold promise for further advancements in food packaging technology.

3.2.4. Water Solubility

Solubility stands as a pivotal attribute of edible films, particularly significant when these films come into contact with water or other liquid environments. Variations in solubility can significantly influence the applicability of these films, depending on the specific requirements of the food products that are intended to coat [33]. Table 1 outlines the water solubility of each coating, shedding light on their respective dissolution characteristics.

Starch coatings exhibit a propensity for high water solubility, due to the presence of amorphous regions within starch molecules. These amorphous areas easily dissolve in water, facilitating the dispersion of the coating material upon contact with aqueous environments [34]. This inherent solubility trait renders starch coatings suitable for applications where rapid dissolution or dispersal is desired, offering versatility in food packaging and preservation.

Conversely, chitosan coatings exhibit lower water solubility owing to their semi-crystalline structure and the presence of acetyl groups, which contribute some hydrophobicity. Chitosan is still considered water soluble under some circumstances, particularly in acidic solutions or after undergoing partial deacetylation [35]. This property renders chitosan-based membranes more resilient against moisture ingress, enhancing their suitability for applications requiring prolonged shelf-life or moisture protection.

Protein-based coatings typically display moderate water solubility, stemming from their complex and highly organized molecular structure [36]. While not as readily soluble as starch coatings, protein-based films offer a balance between solubility and structural integrity, making them suitable for a wide range of applications across the food packaging industry.

Understanding the solubility characteristics of coating materials requires a thorough examination of their molecular composition and structural properties. This knowledge not only guides the selection of materials for specific applications but also facilitates the development of customized coating formulations designed to achieve desired solubility profiles. By tailoring coatings to meet specific requirements, such as rapid dissolution or

moisture resistance, the potential applications of edible films in food packaging and beyond can be significantly broadened.

3.3. Optical Characteristics

The optical characteristics (color and light transmission and transparency) of the coatings are presented in Table 2.

Table 2. Optical characteristics of edible coatings.

Optical Characteristics	Chitosan	Starch/Pectin	Starch alg./Pectin	Protein alg.
L*	$68.70 \pm 4.07^{a,b}$	73.77 ± 1.26^{a}	42.83 ± 1.74^{c}	62.82 ± 1.54^{b}
a*	1.49 ± 1.11^{a}	0.80 ± 0.41^{a}	-0.79 ± 0.70^{b}	-1.43 ± 0.28^{b}
b*	17.30 ± 4.87^{a}	5.98 ± 0.57^{b}	17.69 ± 0.30^{a}	6.12 ± 1.40^{b}
Light Transmission and Transparency	0.386 ± 0.059^{a}	0.536 ± 0.052^{a}	0.521 ± 0.031^{a}	0.001 ± 0.032^{b}

Mean value ± standard deviation of four replicates. Values in the same row with different alphabetical letters are significantly different ($p < 0.05$).

3.3.1. Color

Color and appearance play integral roles in determining the acceptance and appeal of a product among consumers. Consequently, coatings with clear visibility are more often desirable. Table 2 displays the L* (lightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) CIE Lab color values of the films, providing insight into their color characteristics. Visual inspection of the films reveals color variations (Figure 1), with the chitosan film exhibiting a darker shade. However, it is noteworthy that this darker hue does not compromise the film's transparency.

Further analysis indicates color differences among the coatings. Specifically, there is a significant variance in brightness (L*) of the coatings ranging from 42.83 ± 1.74 for starch alg./pectin to 73.77 ± 1.26 for starch/pectin. The substantial difference between the two starch/pectin coatings occurs due to the variations of the starch used and the presence of residual components following the extraction of starch from *Chlorella vulgaris*.

Moreover, the a* values (redness/greenness) suggest that the coatings derived from *Chlorella vulgaris* components exhibit a greener color compared to the other two coatings. This difference in color tone is likely due to the residual green pigment of the microalgae after component extraction.

In elucidating these color characteristics, a deeper understanding of the compositional nuances and processing methods involved in coating formulation is attained. Such insights not only inform the selection of coatings suitable for specific applications but also provide avenues for further optimization to meet the aesthetic preferences and expectations of consumers.

3.3.2. Light Transmission and Transparency

The characterization of light transmission and transparency properties of coatings stands as a crucial aspect in evaluating their suitability for packaging applications [37]. This property, intricately linked to the homogeneity of films, exerts a profound influence on the acceptance of packaging materials within the industry.

After an intriguing observation from Table 2, it is noted that the starch/pectin coatings exhibit higher values compared to the other coatings, resulting in moderately transparent structures. his enhanced transparency can be largely attributed to the presence of pectin, a constituent known to significantly impact the transparency of edible films, as corroborated by previous studies [38,39].

In contrast, the protein coatings stand out for their notably high transparency, leading to superior light transmission and clearer structures. This exceptional clarity can be attributed to the protein's compact and organized molecular structure. The alignment of amino acid chains within proteins facilitates a more uniform. Such alignment minimizes

the light-scattering interfaces [40]. Additionally, the smaller size of protein particles further contributes to the reduction of light scattering, accentuating their suitability for transparent coating materials or packaging applications [41].

Chitosan films, while demonstrating slightly higher light transmittance compared to starch films, present an interesting case. This difference in transparency may arise from the chitosan film's smoother surface and its significantly more amorphous structure [31]. These structural characteristics of chitosan contribute to enhanced light transmission, albeit to a lesser extent compared to protein coatings.

Exploring the intricacies of light transmission and transparency among various coating materials offers valuable insights into their structural and compositional characteristics. This understanding not only aids in selecting the most suitable materials for packaging applications but also drives the development of innovative coatings designed to enhance light transmission and clarity, meeting the diverse requirements of industries and consumer preferences.

3.4. Mechanical Properties

Along with the structure of the food, mechanical properties play a pivotal role in determining the mechanical integrity and longevity of the films, making them critical factors in the evaluation of the membranes.

3.4.1. Tensile Strength

Tensile strength serves as a fundamental parameter in the realm of material science, representing the maximum stress capacity a fil can withstand during tensile testing. This measurement is crucial for the characterization of the mechanical properties of films, offering crucial insights into their structural integrity and performance under stress.

According to Figure 2, the starch/pectin coatings present the highest tensile strength, among the evaluated specimens, with a significant difference. Pectin, with its unique chemical properties, significantly contributes to the strong bonding between the chemical and other substances through interfacial hydrogen and ionic interactions [42]. This intricate network of molecular bonds confers upon the starch/pectin coatings an unparalleled resilience, making them prime candidates for applications demanding superior mechanical durability.

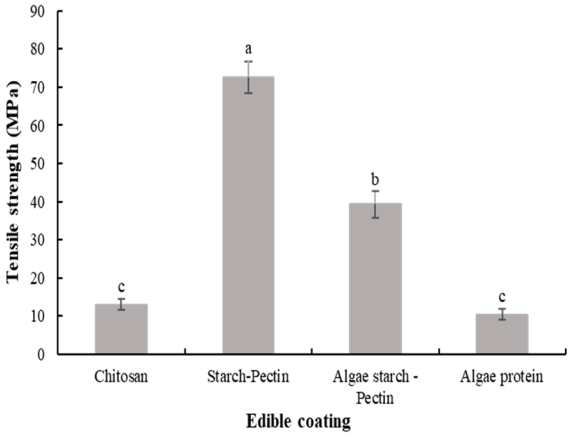

Figure 2. Tensile strength of edible coatings. Mean ± standard deviation with the same letter indicates no significant difference ($p > 0.05$).

A comparison between the starch alg./pectin and the starch/pectin coatings reveals that conventional starch, primarily composed of amylopectin, creates structures with greater durability, due to its ease of gelatinization [43]. This property endows structures formed with conventional starch with significantly heightened durability, thereby elevating their tensile strength. Moreover, residues remaining after the starch extraction from *Chlorella vulgaris* might contribute to the decreased mechanical qualities.

The protein coating presents more modest mechanical properties, attributed to its inherently hydrophilic nature [32]. Similarly, the chitosan coating, while offering certain advantageous properties, showcases a reduction in tensile strength when compared to its starch/pectin counterparts.

In elucidating these intricate relationships between composition, structure, and mechanical performance, a richer understanding of film materials emerges. Such insights not only advance the scientific discourse surrounding film engineering but also pave the way for the development of innovative materials optimized for diverse applications across various industries.

3.4.2. Modulus of Elasticity

The elastic modulus, a fundamental property in materials science, plays a pivotal role in accurately characterizing and developing materials, offering crucial insights into their toughness and deformation behavior [44]. This parameter serves as a barometer of a material's ability to resist deformation under stress, providing valuable information for engineering applications and material selection processes.

The results are presented in Figure 3. Starch-based films stand out for their intriguing combination of properties. They exhibit significantly low elasticity, despite their high tensile strength. This seemingly paradoxical behavior can be attributed to the strong hydrogen bonds between the starch chains [45].

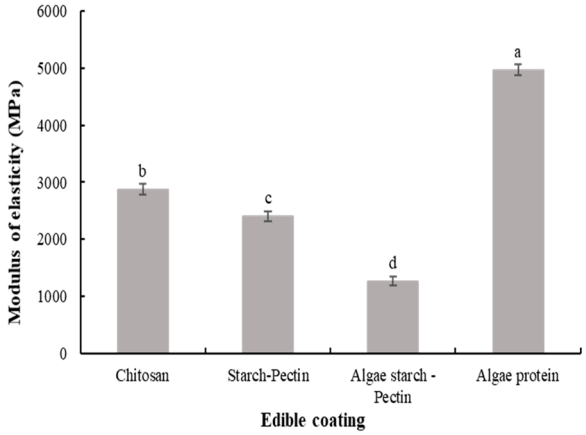

Figure 3. Modulus of elasticity of edible coatings. Mean ± standard deviation with the same letter indicates no significant difference ($p > 0.05$).

In contrast, proteins are renowned for creating coatings with higher modulus of elasticity compared to coatings. This advantage stems from the proteins' unique structural characteristics and the intermolecular forces governing their film formation. The well-organized and densely packed structures of proteins impart them with rigidity and stiffness, rendering them highly elastic [46]. The presence of semi-crystalline regions further enhances the overall stiffness of protein coatings, contributing to their superior elastic modulus.

On the other hand, while chitosan exhibits semi-crystalline areas, it may lack the structural organization and cohesive force of proteins, resulting in comparatively lower elastic modulus [47]. This nuanced understanding underscores the complex interplay between molecular structure, intermolecular interactions, and processing conditions in dictating the stiffness of coatings.

Overall, the stiffness of a coating serves as a multifaceted parameter influenced by a complex relationship between molecule structure, intermolecular interactions, and processing conditions. Proteins, with their intricate and orderly molecular arrangements, are valuable in a variety of applications, including food packaging and biomaterials, owing to their ability to form coatings endowed with commendable mechanical properties.

3.5. Thermal Analysis

Thermal analysis is crucial in the evaluation of edible coatings by providing a precise assessment of their heat resistance. This is essential for ensuring food safety and preserving quality during processing and storage. The results of the thermal analysis are displayed in Figures 4 and 5.

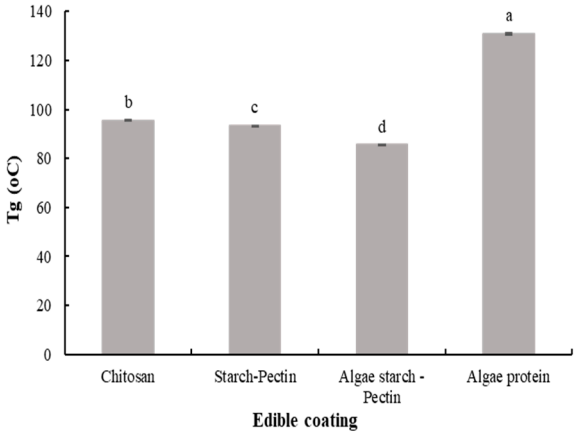

Figure 4. Temperature of glass transition of edible coatings. Mean ± standard deviation with the same letter indicates no significant difference ($p > 0.05$).

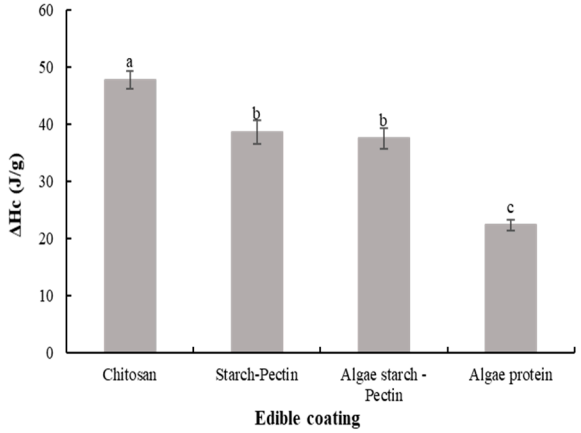

Figure 5. Enthalpy of crystallization of edible coatings. Mean ± standard deviation with the same letter indicates no significant difference ($p > 0.05$).

The thermal stability of the films represents a critical aspect, varying with the composition of the film. This variability not only gives insight into the mechanical strengths of the films but also into their overall stability. Figure 4 presents the value of the glass transition temperature (Tg) derived from DSC analysis, offering a glimpse into the films' response to heat.

The protein film stands out prominently in terms of thermal stability, exhibiting the highest Tg among the evaluated specimens, with significant difference. This phenomenon can be attributed to the intricate and complex structures of protein, which require higher energy inputs to disrupt [48]. The robustness of protein structures against thermal perturbations underscores their suitability for applications requiring resilience to elevated temperatures.

Conversely, the lowest Tg was manifested from the starch alg./pectin coating, with a significant difference, a phenomenon linked to the composition of starch. Starch, featuring both branching and linear amylopectin chains, tends to adopt a more amorphous structure with fewer secondary structures [49]. This inherent molecular arrangement renders the starch alginate/pectin coating more susceptible to thermal transitions at lower temperatures.

Interestingly, despite having similar compositions, the two starch coatings present significant differences in Tg values. This discrepancy can be traced back to contaminants remaining

after the extraction of starch from *Chlorella vulgaris*, which can influence the overall thermal behavior of the coatings. Nevertheless, the glass transition temperatures of all coatings are sufficiently high, ensuring stability after heat treatment without structure compromise.

Moreover, the enthalpy of crystallization (ΔHc) of films is intricately tied to their compositions and materials. The starch coatings exhibit similar values; however, the difference with chitosan coatings is significantly different, even though they are characterized by similar chemical structures. The results of ΔHc of chitosan are at modest levels and in agreement with the work in [50].

However, the protein coating presents a marked departure in terms of thermal behavior. With their distinct molecular structure, proteins typically do not undergo crystallization in the same manner as polysaccharides, leading to significantly lower ΔHc values. This discrepancy underscores the importance of considering the unique thermal properties of proteins in film formulations.

In summary, Tg and ΔHc are crucial parameters for characterizing the thermal properties of coatings, offering valuable insights into their thermal behavior and guiding further research and development endeavors aimed at enhancing their performance and stability across a spectrum of applications. As our understanding of these parameters deepens, avenues for innovation and optimization in film design continue to expand, fostering advancements in materials science and engineering.

3.6. FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR), carried out using Potassium Bromide (KBr) pellets, provided detailed molecular information about the composition of various materials. This analytical technique gave clear information regarding the functional groups characterizing each of the components used in the coatings. The FTIR analysis of the coatings is presented in Figure 6.

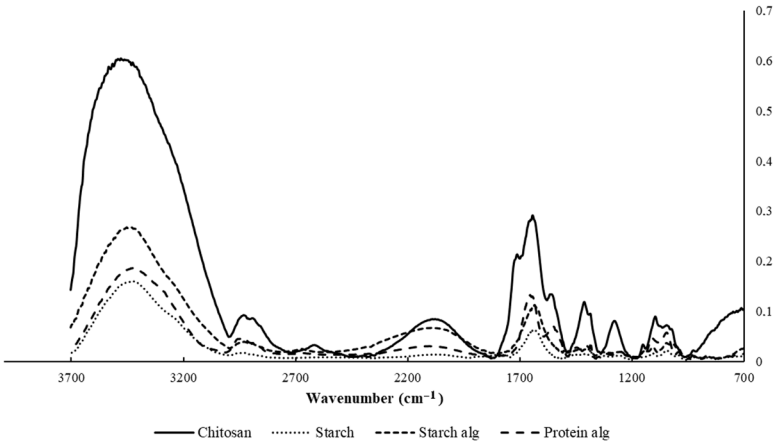

Figure 6. FTIR analysis of coatings: chitosan, starch, starch alg. and protein alg.

The FTIR spectra of the chitosan coating showed a characteristic chitosan spectrum. The broad peak around 3450–3550 cm^{-1} with the presence of O–H and N–H stretching, and sharp peaks at 1655 cm^{-1} and 1590 cm^{-1} for the amide I and amide II bands, respectively, confirmed the fact that chitosan is the principal material [51]. It is very crucial to identify these bands since they are involved in the identification of chitosan and represent the acetylated units and deacetylated amine groups forming part of the core structure of chitosan.

Subsequent coatings of both starch and pectin showed up, distinguished by their distinct spectral fingerprints. On the starch coating, characteristic peaks of starch were observed at about 1047 cm^{-1}, typically for C–O stretching vibrations of glycosidic linkages, with very wide starch O–H stretching, which is normal for regular starch [52]. On the other hand, the starch alg./pectin coating using starch derived from *Chlorella vulgaris* showed relatively similar peaks relating to starch but with minor shifts that were most probably

due to the unique source of the starch. Also, the characteristic peak of pectin at 1730 cm^{-1} is due to the presence of esterified carboxyl groups, hence confirming the incorporation of pectin with starch in both coatings [53].

Finally, the final coating analyzed did indeed contain protein. The presence is evident by the sharp peaks at Amide I and Amide II observed around 1650 cm^{-1} and 1540 cm^{-1}, respectively. These characteristic peaks give information on the peptide bonds of the proteins, and thus, they provide valuable information with respect to the secondary structure of the protein material used in the coating [54].

All these FTIR spectra have shown the presence of each of the specified materials, such as chitosan, starch, pectin, and proteins in the coatings, and have shown the efficiency of the method to specify different structures of polysaccharides and proteins. All of this level of detailed molecular characterization is necessary for tuning the material properties with respect to specific functional requirements of the coating application.

4. Conclusions

The alternative coatings were created using *Chlorella vulgaris* component extracts, specifically starch and proteins. The comprehensive characterization of edible coatings reveals distinct physical, mechanical, and thermal properties that influence their applicability in food packaging. The outcomes demonstrated that the substitute coatings had superior qualities than the conventional ones.

The characterization of extracts from *Chlorella vulgaris* (starch and proteins) provides crucial quantitative data for understanding their composition and potential applications. The precise measurement of starch content, yielding 126.7 mg of amylose per gram of starch, underscores the accuracy of the quantification method. Although the protein extraction yield obtained through the Bradford method is modest at 3.85%, the specificity of the Kjeldahl method ensures the purity and integrity of the protein powder, which is vital for subsequent analyses and applications. These findings highlight the potential of *Chlorella vulgaris* extracts as valuable ingredients in various industries, emphasizing the importance of accurate quantification methods for quality assurance and product development.

The comparative analysis of chitosan, starch/pectin, starch alg./pectin, and protein alg. coatings reveals distinct advantages across various parameters, including weight, density, moisture content, water vapor transmission rate, water solubility, and optical, mechanical, and thermal properties, which are crucial for determining their suitability for specific food packaging applications. Chitosan coatings, with moderate barrier properties and lower water solubility, emerge as suitable candidates for moisture-sensitive applications, combining robust structure with effective moisture barrier capabilities. Starch-based coatings, particularly starch/pectin, exhibit high water solubility and optimal, water vapor transmission rate and light transmission properties, indicating their potential for foods requiring a balance of breathability and clarity, though less ideal for very wet environments. Protein alginate coatings, characterized by the highest weight and density, offer a substantial material presence, with intermediate moisture content that strikes a balance between dryness and moisture retention, alongside moderate water solubility and water vapor transmission rate, suggesting good overall barrier properties with sufficient breathability. The standout feature of Protein alg. coatings is their mechanical strength, evidenced by a higher modulus of elasticity, hinting at superior handling and durability for packaging uses.

The Fourier Transform Infrared Spectroscopy (FTIR) analysis provided a comprehensive elucidation of the composition of the various coatings, each reflecting the unique characteristics of their respective components as introduced in the formulation process. This technique effectively demonstrated its utility in identifying and confirming the molecular signatures of different bio-based materials.

Considering these findings, protein alg. coatings are identified as the most versatile option, offering a blend of solubility, strength, and optical benefits, making them well suited for a variety of food products that require clear, breathable, and moderately moisture-resistant packaging. This comprehensive assessment underlines the necessity of aligning

coating properties with specific food product needs to enhance packaging performance, with protein alg. coatings showing promising versatility for diverse applications. However, the ultimate selection should be guided by the particular requirements of the food product, including sensitivity to moisture, desired shelf life, and visual presentation. These insights pave the way for further research and development in edible coating technologies, emphasizing the importance of material composition in optimizing edible coatings for targeted applications in the food industry.

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