JAES
Journal of Anatolian Environmental and Animal Sciences
(*Anadolu Çevre ve Hayvancılık Bilimleri Dergisi*)
DOI: https://doi.org/10.35229/jaes.1600652
Year: 10, No: 2, 2025 (191-201)
AÇEH
Yıl: 10, Sayı: 2, 2025 (191-201)
ARAŞTIRMA MAKALESİ RESEARCH PAPER

Novel Protein-Fiber Films from Agro-Waste: A Sustainable Approach for Antimicrobial Packaging

Muhammed Zahid KASAPOGLU[1]* **Esra AVCI[2]**
[1]*Istanbul University Cerrahpasa, Institute of Nanotechnology and Biotechnology, Department of Nanotechnology, Istanbul, Turkey.*
[2]*Istanbul Teknokent, Istanbul University-Cerrahpaşa, Avcılar, Istanbul 34320, Turkey.*

Received: *14.12.2024* ***Accepted:*** *14.03.2025* ***Published:*** *25.03.2025*
How to cite: **Kasapoglu, M.Z. & Avci, E. (2025).** Novel Protein-Fiber Films from Agro-Waste: A Sustainable Approach for Antimicrobial Packaging. *J. Anatol. Env. Anim. Sci.*, ***10***(2), 191-201. https://doi.org/10.35229/jaes.1600652
Atıf yapmak için: **Kasapoglu, M.Z. & Avci, E. (2025).** Tarımsal Atıklardan Yeni Protein-Ham Lif Filmler: Antimikrobiyal Paketleme İçin Sürdürülebilir Bir Yaklaşım. *Anadolu Çev. Hay. Bil. Derg.*, ***10***(2), 191-201. https://doi.org/10.35229/jaes.1600652

*: https://orcid.org/0000-0002-2397-6984
: https://orcid.org/0000-0003-0317-2118

***Corresponding author's:**
Muhammed Zahid KASAPOGLU
Istanbul University Cerrahpasa, Institute of Nanotechnology and Biotechnology, Department of Nanotechnology, Istanbul, Turkey.
✉: muhammed.kasapoglu@iuc.edu.tr

Abstract: This study focuses on the development and use potential of antimicrobial edible films, which are among the important topics of recent environmental approaches. In this context, antimicrobial edible films produced with protein and crude fiber obtained from cold-pressed pepper seed oil by-products were examined. Films with protein (P Film) and crude fiber (CF Film) were assessed for structural, mechanical, barrier, and antimicrobial properties. Mechanical tests showed P-CF Film had better flexibility, while P Film achieved the highest elongation at break (33.76%) and tensile strength (0.46 MPa). Barrier properties revealed that films containing both protein and fiber had lower water vapor permeability and significantly higher oxygen permeability than films with only protein or fiber. Antimicrobial tests indicated that both P Film and P-CF Film were effective against *Staphylococcus aureus* and *Escherichia coli*, with P Film showing superior inhibition against *Salmonella typhimurium*. This study highlights the potential of using protein and crude fiber from chili pepper seed oil by-products to develop antimicrobial edible films, offering a sustainable alternative to conventional packaging.

Keywords: Edible films, antimicrobial, protein, crude fiber.

Tarımsal Atıklardan Yeni Protein-Ham Lif Filmler: Antimikrobiyal Paketleme İçin Sürdürülebilir Bir Yaklaşım

Öz: Bu çalışma, son dönemde çevreci yaklaşımların önemli başlıklarından, antimikrobiyal yenilebilir filmlerin geliştirilmesi ve kullanılma potansiyelinin araştırılmasına odaklanmıştır. Bu kapsamda, soğuk sıkım biber çekirdeği yağı yan ürünlerinden elde edilen protein ve ham lif ile üretilen antimikrobiyal yenilebilir filmler incelenmiştir. Protein (P Film) ve ham lif (CF Film) içeren filmler, yapısal, mekanik, bariyer ve antimikrobiyal özellikler açısından değerlendirilmiştir. Mekanik testlerde, P-CF Filmi daha iyi esneklik gösterirken, P Filmi en yüksek kopma uzaması (%33.76) ve çekme mukavemetine (0.46 MPa) ulaşmıştır. Bariyer özellikleri, protein ve lif içeren filmlerin sadece protein veya lif içeren filmlere kıyasla daha düşük su buharı geçirgenliğine ve önemli ölçüde daha yüksek oksijen geçirgenliğine sahip olduğunu göstermiştir. Antimikrobiyal testler, hem P Filmi hem de P-CF Filmi'nin *Staphylococcus aureus* ve *Escherichia coli* üzerinde etkili olduğunu ortaya koymuş, P Filmi'nin ise *Salmonella typhimurium* karşısında üstün inhibisyon gösterdiği belirlenmiştir. Bu çalışma, biber çekirdeği yağı yan ürünlerinden elde edilen protein ve ham lifin antimikrobiyal yenilebilir filmlerin geliştirilmesinde kullanılma potansiyelini vurgulamakta ve geleneksel ambalajlara sürdürülebilir bir alternatif sunmaktadır.

***Sorumlu yazar:**
Muhammed Zahid KASAPOGLU
İstanbul Üniversitesi Cerrahpaşa, Nanoteknoloji ve Biyoteknoloji Enstitüsü, Nanoteknoloji Anabilim Dalı, İstanbul, Türkiye.
✉: muhammed.kasapoglu@iuc.edu.tr

Anahtar kelimeler: Yenilebilir film, antimikrobiyal, protein, ham lif.

INTRODUCTION

Many large-scale food industry byproducts include significant nutrients and chemicals that can be recovered and repurposed. Among these by-products, proteins and fibers are particularly noteworthy due to their potential use in a range of sectors, including the production of edible films. By lowering waste and encouraging recycling, the extraction of these chemicals from waste not only increases the value of the byproducts but also supports sustainable practices (Kumar, Konwar et al. 2023). The waste produced during the manufacturing of cold-pressed hot pepper oil is one prominent example (Sandoval-Castro, Valdez-Morales et al. 2017). Peppers belong to the *Solanaceae* family's *Capsicum genus*. Varieties high in capsaicin are known as hot peppers. Significant waste is produced when peppers are processed into sauces and spices, especially the seeds, which are high in lipids, proteins, and bioactive substances. Because of its significant bioactive content, cold-press extraction of pepper seed oil is becoming more and more popular (Karaaslan, Şengün et al. 2021, Avci, Akcicek et al. 2024).

Edible coated films based on naturally occurring renewable biopolymers have become a revolutionary environmentally friendly food preservation technique as environmental concerns become more pressing (Hadidi, Jafarzadeh et al. 2022). Although biodegradability and edibility are among their best features, edible coatings and films' primary role is to protect food from environmental effects such as microorganisms, oxygen, light and water vapor. When compared to conventional packaging technologies, edible coated films can control the flow of water, oxygen, and solutes, thereby lowering moisture loss and preventing interaction between food and spoiled microorganisms (Wang, Ding et al. 2021, Sood and Saini 2022).

The use of natural polymers, including proteins, polysaccharides, lipids and their mixtures, in food packaging has increased in importance in recent years due to their advantageous qualities such as non-toxicity, thermostability, biodegradability and biocompatibility (Nguyen, Pham et al. 2022). Edible coatings can be made from environmentally benign materials such as starch, chitosan, pectin, alginate, or other mucilaginous seeds (Mohamed, El-Sakhawy et al. 2020, Capar 2023).

Recent research has looked into the usage of protein-based films and fiber-protein composite films as alternatives to traditional synthetic packing materials. These biodegradable and edible films provide a viable alternative for food packaging by combining barrier characteristics and biodegradability (Wang, Ding et al. 2021). Since proteins have good oxygen barrier qualities, functionality, and film-forming ability, there have been a lot of research done on the creation of edible films using proteins (Brito, Carrajola et al. 2019). Nevertheless, the mechanical and water vapor barrier qualities of these coatings are limited. The development and testing of composite edible films derived from biopolymers has garnered significant attention in the field of food packaging research in recent years (Ye, Han et al. 2019, Huang, Xiang et al. 2021, Sani, Geshlaghi et al. 2021, Homthawornchoo, Han et al. 2022). Proteins and fibers can be added to these films to improve their functionality and mechanical properties while also assisting in the creation of environmentally friendly packaging options.

One significant advantage of edible films with antimicrobial properties is their ability to extend the shelf life of food products by inhibiting the development of dangerous and spoiling bacteria (Galus & Kadzińska, 2015). This function is essential to preserving food's safety and quality (Galus and Kadzińska 2015). By lowering the requirement for extra preservatives, antimicrobial edible films can satisfy customer desire for less processed and more natural foods while also promoting the clean label movement (Domínguez, Barba et al. 2018). The antibacterial properties of proteins and fibers in edible films have been the focus of several inquiries (Domínguez, Barba et al. 2018, Kandasamy, Yoo et al. 2021, Mercadal, Picchio et al. 2024). Proteins like soy, whey, and zein have been demonstrated to contain inherent antibacterial characteristics that interact with microbial cell membranes, causing cell breakdown and reducing microbial growth (Ghanbarzadeh and Oromiehi 2008, Alipour, Rahaiee et al. 2023, Jeon, Lee et al. 2023, Mercadal, Picchio et al. 2024). For example, studies have shown that whey protein films containing essential oils have significant antibacterial effect against common foodborne diseases such as *Staphylococcus aureus* and *E. coli* (Seydim and Sarikus 2006). In addition, fibers especially those sourced from plants help edible films retain their antibacterial properties. Fibers such as cellulose, chitosan, and pectin can be added to films to enhance their barrier properties and produce a physical barrier that prevents germs from penetrating (Dai, Huang et al. 2022).

This article primary goal is to determine whether it is feasible to extract protein and fiber from by-products of cold-pressed hot pepper oil in order to produce antimicrobial edible films. By transforming food industry waste into valuable resources, the research supports sustainable practices and waste reduction. The study also demonstrates the feasibility of using protein from these by-products as a key component in edible films due to its natural antimicrobial properties, strong oxygen barrier characteristics, and excellent film-forming ability. By creating a physical barrier that prevents microorganisms from penetrating, the crude fibers taken from hot pepper oil by-products are thought to greatly improve the films' barrier qualities and increase their protective effectiveness. This research fills a gap in the literature by being the first to explore the combination of protein and fiber from hot pepper oil by-products in the production of antimicrobial packaging materials. This study provides a thorough assessment of the mechanical, structural and barrier properties of edible films, revealing that the combination of protein and fiber plays a key role in enhancing water vapor barrier properties. The research also investigates the antimicrobial effectiveness of these films, demonstrating their ability to inhibit *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*. The findings underscore the significant potential of these films in food packaging applications, offering a promising approach to improving food safety through natural, sustainable materials. Overall, this study provides a sustainable, biodegradable alternative to conventional packaging materials, contributing valuable insights into the development of environmentally friendly packaging solutions. The findings also pave the way for future research and optimization of these films for broader applications, aligning with modern environmental and economic goals.

MATERIAL AND METHOD

Material: Pathogenic microorganisms, including *Staphylococcus aureus* ATCC25923, *Escherichia coli* BC1402, and *Salmonella typhimurium* RSSK95091, were received from Beetech Biotechnology Limited Company in Istanbul, Turkey. Cold-pressed hot pepper seed oil byproduct was received from ONEVA Food Co. (Istanbul, Turkey). MRS agar, MRS broth, nutritional agar, and 85% glycerol were supplied by Merck (Darmstadt, Germany).

Protein and crude fiber isolation from cold-pressed hot pepper oil by-product: Crude fiber and protein were isolated from cold-pressed hot pepper oil by-product (HPOB) using methods modified by (Avci, Akcicek et al. 2024). Initially, HPOB was diluted 1:15 (w/v). To enhance protein yield, the diluted samples were ultrasonicated at 301.34 W for 3 minutes. The temperature during ultrasonication was kept at 60°C. The mixture was centrifuged at 10.000 rpm (15 min) at 25°C. To precipitate the crude fibers, the supernatant was combined with 95% ethanol at a ratio of 1:1(w/v) and centrifuged at 10.000 rpm (15 min) at a temperature of 4°C. The crude fibers were then isolated and stored until lyophilization. The pH at which the zeta potential of the remaining liquid decreased to zero was determined. After determining the proteins' isoelectric point as pH 5.5, it was precipitated by centrifugation at 10.000 rpm for 15 minutes at 25 °C. Proteins and crude fibers were lyophilized with a freeze-drying apparatus (Martin Christ GmbH, Beta 1-8 LSCplus, Germany).

Preparing film-forming solutions and films: Three different kinds of film samples were made for this study: protein, crude fiber, and their mixture. The production of films is shown in the flow chart in Figure 1. The P film included 5% protein, the P-CF film contained 5% protein plus 2.5% crude fiber and the CF film contained 5% crude fiber (w/v). These components were combined in distilled water using a magnetic stirrer at 70°C for 1 hour. The combination was then left to sit overnight at +4°C. In addition, 2.5% (w/v) glycerol was added as a plasticizer, and the mixture was agitated for 30 minutes in a sealed container at 70°C in a water bath. The film preparations were placed in 90 × 17 mm sterile Petri dishes and left to dry for 24 hours at 35°C in a fan-assisted incubator. Subsequently, 30 g of the film solution was poured into each sterile Petri dish.

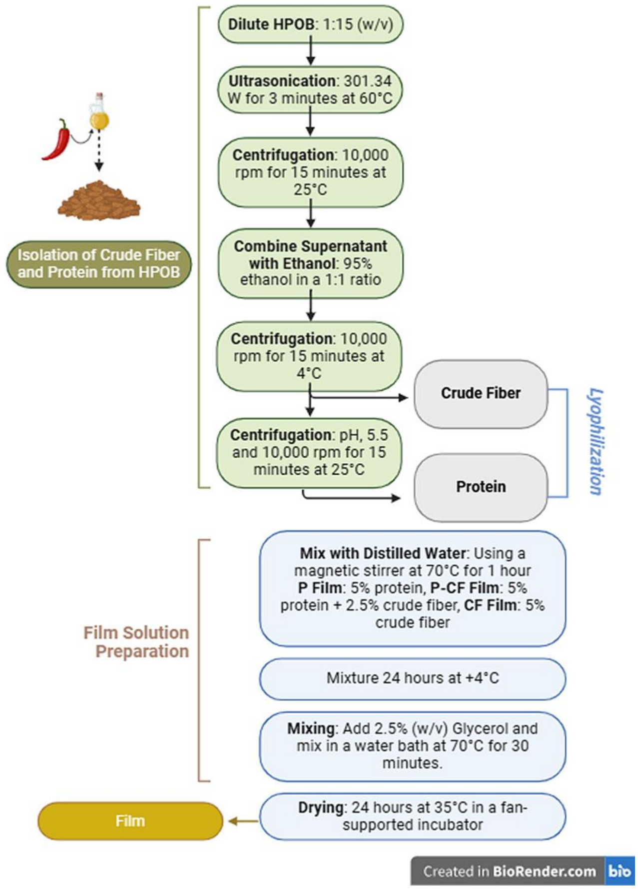

Figure 1. Flowchart of the produced film samples.

Structural characterization:

Thickness and optical properties: A digital micrometer (Fowler Digitrix Mark 2, Chicago, USA) was used to measure the film samples' thickness, and a Konica Minolta Chromameter (CR-400, Minolta Co. Ltd., Osaka, Japan) was used to measure the surface color characteristics (L^*, a^*, and b^*) (Tornuk, Sagdic et al. 2018). Color measurements were taken by placing the film samples on the colorimeter and selecting at least three random places from each sample to measure the color parameters.

Mechanical properties of the films: Tensile strength (Karydis-Messinis, Kyriakaki et al.) and elongation at break (E%) of the film samples were measured using a texture analyzer (TA. XT Plus StableMicro Systems, Surrey, UK) fitted with a 5 kg load cell in accordance with ASTM D882-12 guidelines (Soukoulis, Behboudi-Jobbehdar et al. 2017). Initially, grip separation and crosshead speed were chosen at 50 mm and 4 mm/s for rectangular film specimens (10 cm × 2 cm), respectively. To determine TS and E (%), each test was duplicated at least five times.

Water vapor permeability and oxygen permeability: Water vapor permeability and oxygen permeability were determined according to (Akman, Bozkurt et al. 2021). Glass tubes containing silica gel were heated to 105°C for 24 hours before analysis to remove any moisture. Circular portions of each film sample were cut to seal the tube apertures. These tubes were then weighed and placed in a desiccator with distilled water at 30°C. Glass tubes without film seals served as controllers. The tubes were weighed every two hours for 24 hours. The water vapor permeability (WVP) was determined using the following equation:

$$WVP = \frac{\omega}{t} x \frac{x}{\Delta P x A}$$

where w/t was determined by calculating the amount of water absorbed by the system at steady state using linear regression, and x, ΔP, and A stand for mean film thickness (μm), relative pressure difference (kPa), and film area, respectively, at 25 ºC.

For oxygen permeability, film samples were used to seal conical tubes containing 20 milliliters of sunflower oil, which were then kept at 60°C for nine days. After this time, sodium thiosulfate titration was used to determine the oil samples' peroxide value. The peroxide value was then used to evaluate the films' oxygen permeability.

Scanning electron microscopy (SEM): The morphological characterization of the films was conducted using a field-emission scanning electron microscope (FE-SEM) (Zeiss, EVO® LS 10) under vacuum conditions at an accelerating voltage of 5 kV. Prior to imaging, the samples were carefully prepared by mounting them onto an aluminum stub to ensure stable positioning during analysis. To enhance the conductivity of the samples and obtain high-quality microstructural images, they were uniformly coated with a thin layer of gold using a sputter coater. The imaging was performed under a pressure of 300 Pa, and the surface structures of the films were analyzed at a magnification of 3000×.

Differential scanning calorimetry (DSC): Differential scanning calorimetry (DSC Q20, TA Instruments, Inc., USA) was adjusted to measure the thermal behavior of the films in accordance with the (Abdollahi, Alboofetileh et al. 2013) approach. After weighing the film sample in an aluminum pan, 5 mg was heated at 10 °C per minute from 20 to 300 °C with a 20 mL/min N2 flow. An empty metal pan served as a reference.

Infrared (FTIR) spectroscopic measurements: An FTIR instrument (Bruker Tensor 27 spectrometer with the DLa TGS detector, Bremen, Germany) was used to undertake molecular characterization of the film samples. The wavelength spectral range of 4000–400 cm^{-1} was used for the measurements (Mahcene, Khelil et al. 2020).

Antimicrobial activity: The antimicrobial analysis was carried out using methods modified from previous research by (Kopuz, Akman et al. 2024). The pathogens tested were *Staphylococcus aureus* ATCC25923, *Escherichia coli* BC1402, and *Salmonella typhimurium* RSSK95091. It was activated in TSB broth medium and standardized to around 10^6 CFU/ml. 100 µl of the required bacterial sample concentration was disseminated on TSB agar medium. UV-sterilized film samples (5% protein, 5% crude fiber, and 5% protein + 2.5% crude fiber) were cut into 5x5 mm^2 pieces and placed on inoculated Petri dishes. A disk with 10 µl of 30 µg/ml chloramphenicol was used as a positive control in each Petri plate.

Statistical evaluation: IMB SPSS 25, a Windows-based statistical analysis tool, was used to perform the statistical analysis. The Tukey test was used to assess the statistical differences between the means at the 95% significant level after a one-way analysis of variance (ANOVA) was carried out. Three separate analyses were carried out.

RESULTS AND DISCUSSION

Thickness and color properties of the films: Color and opacity are critical quality criteria that influence the appearance and transparency of a film. These features have a substantial impact on customer perceptions of food products since brightness and color are frequently connected with food quality (Kumar, Ramakanth et al. 2022). The L*, a*, b*, and thickness values for the generated films are shown in Table 1. The color parameters (L*, a*, b*) of the developed films exhibit significant variations depending on their composition. The L* value, representing lightness, was highest in CF Film (74.95±1.06), indicating a brighter appearance, whereas P Film (63.62±2.87) and P-CF Film (61.36±2.53) showed significantly lower values, suggesting a darker coloration due to protein incorporation. The a* value, which indicates the red-green spectrum, was highest in P Film (9.39±2.33), followed by P-CF Film (6.60±1.83), while CF Film had the lowest redness (0.53±0.22), confirming that protein addition enhances the reddish hue of the films, whereas crude fiber reduces this effect. Similarly, the b* value, representing the yellow-blue spectrum, was highest in P Film (43.71±0.50) and lowest in CF Film

(34.65±3.20), demonstrating that protein incorporation intensifies the yellowish tint, while crude fiber addition leads to a moderate reduction in yellow intensity. These results indicate that protein addition darkens the films and enhances red and yellow tones, whereas crude fiber moderates these effects by maintaining a relatively lighter and less intense color profile. Figure 2 depicts the film matrix's structure. It is evident that P-CF Film and P Film are smoother and more consistent than CF Film.

Table 1 Development and characterization of films from protein and crude fiber obtained from cold-pressed hot pepper oil by-product.

Films	Thickness	Color parameters		
		L*	a*	*b**
P Film	0.20±0.01^{a}	63.62±2.87^{b}	9.39±2.33^{a}	43.71±0.50^{a}
P-CF Film	0.17±0.06^{a}	61.36±2.53^{bc}	6.60±1.83^{b}	39.66±0.81^{b}
CF Film	0.23±0.02^{a}	74.95±1.06^{a}	0.53±0.22^{c}	34.65±3.20^{c}

P Film: 5% protein film, P-CF film: 5% protein and 2.5 crude fiber film, CF film: 2.5% crude fiber film, a-c the different lowercases within the same column show that the results are significantly different ($P < 0.05$).

Table 1 reveals that the edible films ranged in thickness from 0.17 to 0.23 mm, and varying concentrations of components such as hot pepper protein isolate and crude fiber resulted in nearly identical film thickness. According to (Rawat and Saini 2024), film thickness is determined by the nature and content of the film. Furthermore, the final thickness of the films is strongly impacted by their preparation and drying techniques (Omar-Aziz, Khodaiyan et al. 2021).

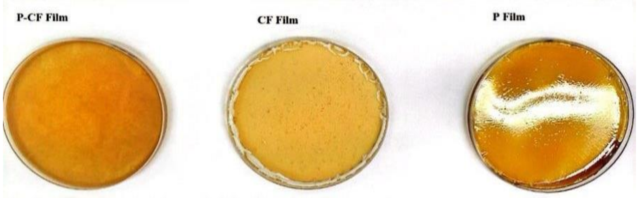

Figure 2. Images of the produced film samples

Mechanical properties of the films

The study's findings clearly show that crude fiber and protein affect the mechanical characteristics of edible films. Table 2 illustrates the weakening effect of polysaccharide fibers and other additives on film mechanical strength. It shows that both P-CF film (0.34±0.01 MPa) and CF film (0.07±0.00 MPa) exhibit significantly lower values compared to the P film (0.46±0.00 MPa). The P-CF film has the largest elongation at break (E) (%34.88±0.17), while other films have lower values. These findings indicate that hot pepper protein and crude fiber can improve the flexibility of film structures, thereby broadening their applications.

Table 2. Mechanical and barrier properties of films

Films	Tensile strength (MPa)	Elongation at break (%)	WVP (g mm h^{-1} m^{-2} kPa^{-1})	PV (meq/kg)
Blank				73.13±5.54^{a}
P Film	0.46±0.00^{a}	33.76±1.88^{ab}	3.94±0.54^{a}	55. 94±4.55^{b}
P-CF Film	0.34±0.01^{b}	34.88±0.17^{a}	2.84±0.27^{b}	23.79±4.48^{d}
CF Film	0.07±0.00^{c}	23.09±0.21^{c}	4.06±0.75^{a}	44.88±6.10^{c}

P Film: 5% protein film, P-CF film: 5% protein and 2.5 crude fiber film, CF film: 2.5% crude fiber film, a-c the different lowercases within the same column show that the results are significantly different ($P < 0.05$.

Tensile properties are generally affected by the components, relative concentration and processing of the film. Films with higher tensile strength typically have lower elongation at break values. According to our research, the film's tensile strength gradually decreased when CF was added. P Film had the maximum tensile strength (0.46 MPa), whereas P-CF Film had the highest elongation value (34.88%). These results suggest that CF functions as a film matrix plasticizer by forming hydrogen bonds with protein molecules (Kamari and Phillip 2018, Yan, He et al. 2023). Thus, crude fiber reduces the tensile strength by expanding the pores in the film matrix. A study showed that the major factors impacting the tensile strength of films were pectin (P), alginate (A), and pectin whey protein (Homthawornchoo, Han et al.) interaction; P and A increased, while P/WP interaction decreased the tensile strength value. Tensile strength was somewhat reduced by interactions between A and WP. Similar results were seen for elastic modulus (E), where the linear interaction between A/WP and P/WP decreased the value while P and A raised it (Chakravartula, Soccio et al. 2019). Similarly, protein alone raised tensile strength and elongation values, whereas crude fiber interaction decreased them.

According to the (Kocira, Kozłowicz et al. 2021, Zhu 2021) adding polysaccharides and bioactive substances to film formulations can have a major effect on the mechanical characteristics of the film, especially lowering its tensile strength. This work is a significant step in improving the mechanical characteristics of edible films and creating more robust and flexible materials for uses like food packaging.

Barrier properties of the films: Water vapor permeability (WVP), one of the film's barrier qualities, should be the lowest (Qin, Liu et al. 2020).Recently, several natural components have been added to edible films in order to improve their physical and functional features and broaden their applications in food packaging (Ghanbarzadeh and Oromiehi 2008, Sani, Geshlaghi et al. 2021, Zhu 2021). At a temperature of 25°C, the WVP values of films supplemented with varying amounts of protein and fiber were measured. The range of WVP values was 2.84 to 4.06 g√mm/m^2 h√kPa. Table 2 demonstrates that films with both protein and fiber had WVP values that were considerably lower than films with either protein or fiber alone ($p<0.05$). The considerable decrease in water vapor permeability in hot pepper protein and fiber combinations is attributed to hydrogen bonding and other intermolecular interactions between the protein and fiber. The hydroxyl groups in the protein and fiber that display hydrophilic qualities may be reduced by newly created hydrogen bonds, which would lower the protein-fiber films affinity for water vapor (Qin, Liu et al. 2019). Several factors influence the permeability and diffusion of gases through polymeric films, including the type of polymer, the film's physical characteristics, film thickness, filler material content, and outside variables such relative humidity and temperature (Kopuz, Akman et al. 2024).

The O2P values of the films are shown as peroxide values (PV) in Table 2. Compared to the blank film with a PV value of 73.13 meq/kg, the PV values of the films ranged from 23.79 to 55.94 meq/kg. The addition of protein and crude fiber significantly improved the oxygen permeability of the films ($P < 0.05$). The increase in oxygen permeability (O2P) by adding only protein and crude fiber to the films may be due to several factors. According to (Kocira, Kozłowicz et al. 2021) is that the incorporation of these components may create a more porous film structure, allowing for greater diffusion of oxygen molecules. Additionally, the hydrophilic nature of proteins and fibers could attract and retain moisture, which in turn can disrupt the film matrix and facilitate the passage of oxygen. In addition, the hydrophilic nature of proteins and fibers may attract and retain moisture, which may disrupt the film matrix and facilitate the passage of oxygen. Furthermore, the increase in oxygen permeability (O2P) may be attributed to the deterioration of the structural integrity of the film due to the heterogeneous distribution of protein and fiber particles, which may create microvoids or channels (Pajak, Fortune et al. 2013).

SEM: The permeability and mechanical properties of the film matrix can be influenced by its structure, shape, and homogeneity. The surface microstructure of the generated films is displayed in Figure 3. Compared to other films, the P-CF Film has a smoother surface. Additionally, the P Film has a continuous matrix, great morphological integrity, and a generally flat surface. The CF and P-CF Films, on the other hand, have a rough surface microstructure with few pores and indentations (Figure 3). As mentioned, the patterns displayed by the P-CF and P films differ from those of the CF films. Specifically, the P Film displays an uneven matrix with irregular wrinkles, indicating protein aggregation due to limited compatibility induced by its more hydrophobic nature, in contrast to the CF Films' very hydrophilic nature. (Jiang, Zou et al. 2020) discovered comparable outcomes for composite films containing carboxymethyl chitosan and whey protein concentrate. SEM analysis revealed that these films exhibited a more uniform and homogeneous structure, likely due to the plasticizing effect of protein, which improved the film matrix. The CF Film, in contrast, showed a rougher and less cohesive surface, likely due to the fibrous nature of crude fiber, which may have led to phase separation.

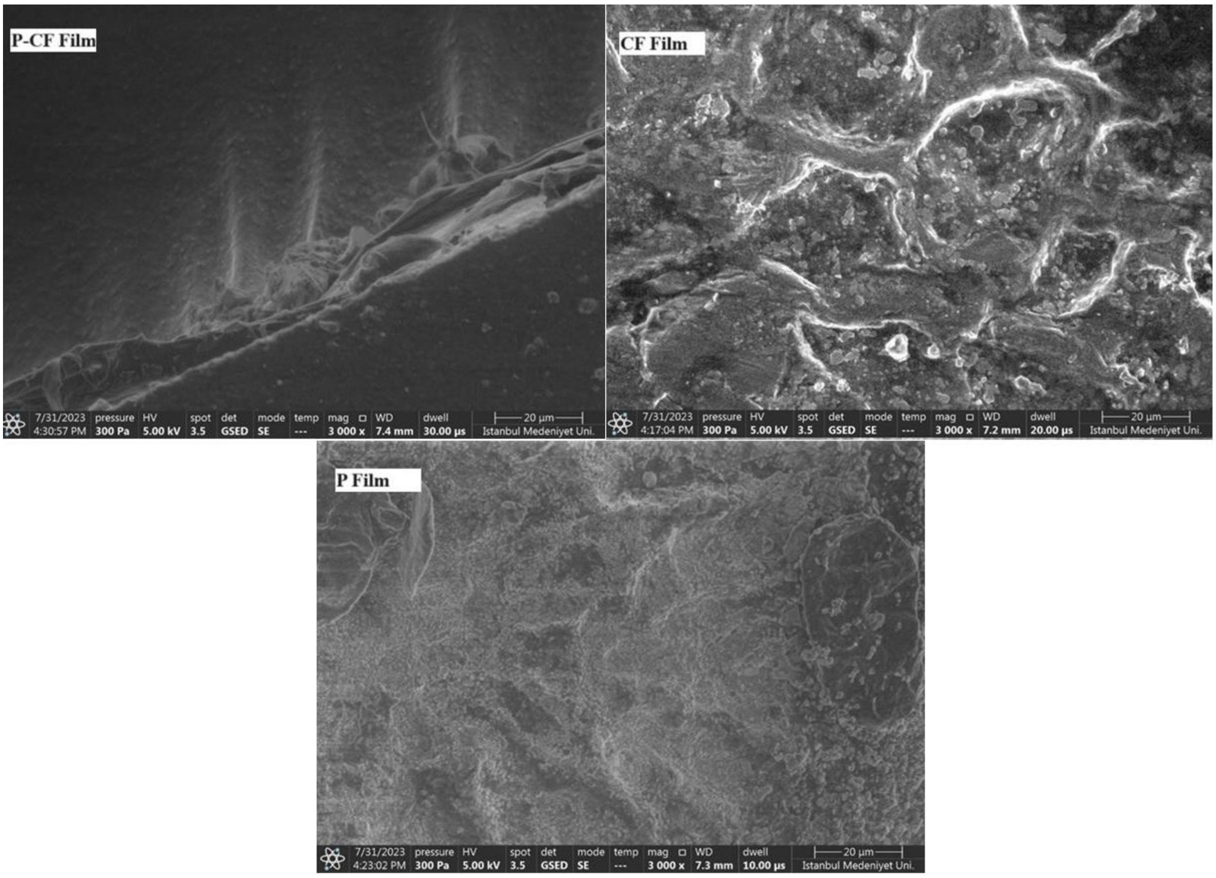

Figure 3 The surface (P Film: 5% protein film, P-CF film: 5% protein and 2.5 crude fiber film, CF film: 2.5% crude fiber film) of the composite films of the scanning electron microscopy morphologies.

DSC

DSC analysis was used to determine the impact of protein interaction with crude fiber on thermal properties. Table 3 illustrates how protein and fiber combinations affect the melting temperatures and fusion enthalpies of the films. The results showed that the melting temperatures of the P Film, P-CF Film, and CF Film were 53.67 °C, 67.42 °C, and 79.78 °C, respectively. These numbers show that the protein and fiber combination considerably changes the films' thermal characteristics. In particular, the P-CF film's lower melting point indicates that protein-fiber molecular interactions weaken the film's stability, which lowers its thermal stability. One crucial factor in assessing the film's thermal stability is its denaturation temperature (Td). The temperature at which a film changes from being brittle to being soft and rubbery is denoted by Td. The material possesses a glassy structure at temperatures below Td and a rubbery state at temperatures over Td. In a related investigation, the Td values for the Zein and SPI (soy protein isolate) films were 77 °C and 86 °C, respectively, while the Td value for the WGP (wheat gluten protein) film was the highest at 106 °C (Duan, Zhou et al. 2023). These results demonstrate that the hydrophilic properties of proteins significantly impact thermal stability. More water molecules may be drawn into the film matrix by a hydrophilic structure, increasing inter-chain mobility and lowering the Td value of the film. Protein and fiber combinations decreased the thermal stability of the films, making them less durable. These findings are important for optimizing the structure and performance of protein films. The "free volume theory", which explains the decrease in hydroxyl groups and hydrogen bonds with the addition of protein, can be used to explain this behavior. Tg and Tm fall as a result of this process, which weakens the biopolymer structure and increases molecular motions. This increases intermolecular free space (Sadeghi-Varkani, Emam-Djomeh et al. 2018).

Table 3. Thermal behavior of the films.

Films	*Tm (°C)*	*ΔHm (J/g)*	*Td (°C)*
P Film	53.67±0.07[b]	82.91±0.05[b]	254.56±0.10[a]
P-CF Film	67.42±0.04[c]	42.41±0.10[c]	234.25±0.14[a]
CF Film	79.78±0.06[a]	231.14±0.02[a]	229.51±0.20[a]

P Film: 5% protein film, P-CF film: 5% protein and 2.5 crude fiber film, CF film: 2.5% crude fiber film, a-c the different lowercases within the same column show that the results are significantly different ($P < 0.05$).

FTIR: FTIR spectroscopy was used to examine the molecular structure of the film samples, and the results are shown in Figure 4. All film samples showed very identical spectrum characteristics. FTIR spectroscopy is well recognized as a valuable method for investigating protein structural changes and the cross-linkages connecting polysaccharides and protein amino groups. The films showed absorption bands between 3000-3600 cm⁻¹, indicating the presence of -OH groups and hydrogen bonding among water molecules crude fiber strands (Karydis-Messinis, Kyriakaki et al. 2024). The absorption bands at 1629 cm⁻¹, 1535 cm⁻¹, and 1400 cm⁻¹ correspond to C=O stretching (amide I), N-H bending (amide II), and N-H bending (amide III), respectively, serving as critical indicators for assessing the secondary structures of proteins.

The FTIR spectra of P-CF films show a modest decrease in peak strength at 1628 cm⁻¹, 1538 cm⁻¹, and 1400 cm⁻¹, indicating the creation of secondary structures in the processed films. This suggests that structural changes occurred during film processing. These results are consistent with findings from other studies in the literature, demonstrating the utility of FTIR spectroscopy in understanding the structural characteristics of films (Fernández, Ausar et al. 2003, Ma, Xin et al. 2017, Karydis-Messinis, Moschovas et al. 2023).

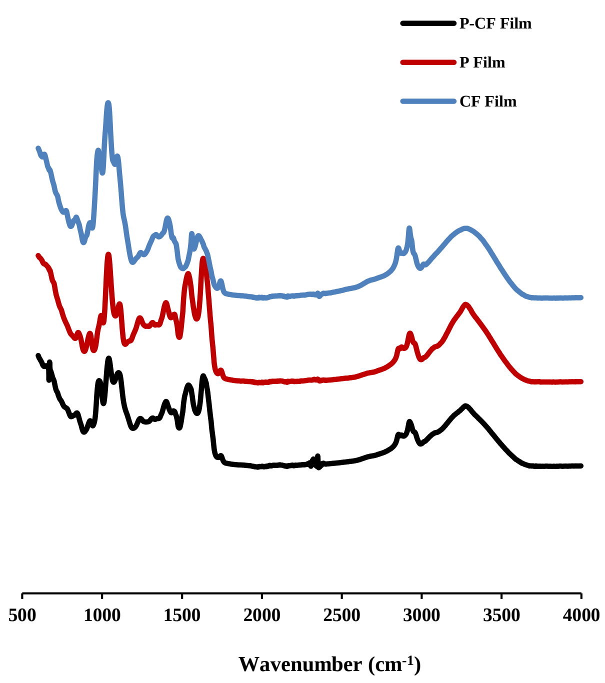

Figure 4. Fourier transform infrared spectra of protein-crude fiber containing composite films. (P Film: 5% protein film, b: CF film: 5% crude fiber film, c: P-CF film: 5% protein and 2.5 crude fiber film).

Antimicrobial activity: The antibacterial activity of the produced films was determined using the disc diffusion method. This approach is extensively used to evaluate the antibacterial characteristics of food packaging materials (Noori, Khanjari et al. 2021). Edible composite films incorporating bioactive chemicals with antibacterial capabilities are more widely used and effective than other packaging materials (Hematizad, Khanjari et al. 2021).

In Table 4, the results show that films containing 5% protein and 5% protein + 2.5% crude fiber have equal inhibitory effects for both *Staphylococcus aureus* ATCC25923 and *Escherichia coli* BC1402. However, against *Salmonella typhimurium* RSSK95091, the film containing 5% protein had a much greater inhibitory effect than the film containing 5% protein + 2.5% crude fiber (Figure 5). The film containing 5% crude fiber did not show antibacterial effects against any of the three pathogens tested. Protein-based films have reduced microbial contamination by interfering with microorganisms' cell division, lowering cell viability and halting proliferation.

Table 4. Antimicrobial activity of the films.

Bacteria	Inhibition circle diameter (Avci et al.)			
	P Film	CF Film	P-CF Film	Chloramphenicol
Staphylococcus aureus ATCC25923	3.13±0.047[b]	0	3.03±0.047[b]	4.13±0.094[a]
Escherichia coli BC1402	3.06±0.124[b]	0	3.16±0.047[b]	4.08±0.062[a]
Salmonella typhimurium RSSK95091	3.50±0.040[b]	0	3.06±0.047[c]	4.06±0.023[a]

P Film: 5% protein film, P-CF film: 5% protein and 2.5 crude fiber film, CF film: 2.5% crude fiber film, a-c different lowercase letters on the same line indicate that the results are significantly different ($P < 0.05$)

The lack of antibacterial activity in crude fiber-based films could be attributed to the absence of specialized antimicrobial processes found in protein-based films. Protein-based films include bioactive chemicals or peptides that can target and disrupt bacterial cell membranes, effectively suppressing bacterial growth (Fiorentini, Suarato et al. 2021, Yan, He et al. 2023).

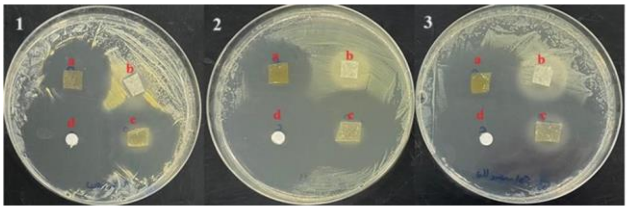

Figure 5. Petri 1: *Staphylococcus aureus* ATCC25923, Petri 2: *Escherichia coli* BC1402, Petri 3: *Salmonella* typhimurium RSSK95091, a: P Film: 5% protein film, b: CF film: 5% crude fiber film, c: P-CF film: 5% protein and 2.5 crude fiber film, d: chloramphenicol.

CONCLUSION

This study introduces an innovative approach for creating antimicrobial edible films using proteins and crude fibers extracted from cold-pressed hot pepper seed oil by-products. The films were categorized into three types: P Film (5% protein), P-CF Film (5% protein and 2.5% crude fiber), and CF Film (5% crude fiber), and were thoroughly evaluated for their structural, mechanical, barrier, and antimicrobial properties. The structural analysis revealed that P and P-CF Films exhibited smoother surfaces compared to CF Film. Mechanical tests indicated that P Film had the highest tensile strength (0.46 MPa) and elongation at break (33.76%), while P-CF Film showed more flexibility. Films with both protein and crude fiber had higher oxygen permeability and lower water vapor permeability than those with either protein or fiber, according to barrier characteristics. Differential scanning calorimetry demonstrated that the combination of protein and fiber resulted in reduced melting temperatures, suggesting diminished thermal stability. Further insights into the films' structures were provided by scanning electron microscopy and Fourier-transform infrared spectroscopy. Antimicrobial testing confirmed that both P Film and P-CF Film were effective against *Staphylococcus aureus* and *Escherichia coli*, with P Film showing superior inhibition against *Salmonella typhimurium*. However, there are limitations to consider. The reduced thermal stability due to the combination of protein and fiber may restrict the films' use in high-temperature applications. While protein-based films exhibited higher tensile strength, the inclusion of crude fiber led to decreased tensile strength, indicating a trade-off that requires further investigation. Additionally, although the films were effective against certain pathogens, their antimicrobial efficacy varied, with the 5% protein film showing particularly strong inhibition against *Salmonella typhimurium*. Future research should focus on enhancing the thermal stability of the films by incorporating stabilizers or exploring alternative processing techniques. Additionally, optimizing the balance between mechanical properties and performance by experimenting with different ratios of protein and fiber or additional additives would be beneficial. Overall, this study successfully demonstrates the potential of using protein and crude fiber from pepper seed oil by-products for antimicrobial edible films, providing a sustainable alternative to conventional packaging materials. Future studies should focus on optimizing the formulation of these edible films to enhance their mechanical and barrier properties while ensuring consumer acceptability, sensory quality, and compatibility with various food products. Additionally, real-time storage studies and consumer perception analysis would be valuable in assessing their commercial viability and practical application in food packaging.

REFERENCES

Abdollahi, M., Alboofetileh, M., Rezaei, M. & Behrooz, R. (2013). Comparing physico-mechanical and thermal properties of alginate nanocomposite films reinforced with organic and/or inorganic nanofillers. *Food Hydrocolloids, **32***(2), 416-424. DOI: 10.1016/j.foodhyd.2013.02.006

Akman, P. K., Bozkurt, F., Dogan, K., Tornuk, F. & Tamturk, F. (2021). Fabrication and characterization of probiotic Lactobacillus plantarum loaded sodium alginate edible films. *Journal of Food Measurement and Characterization, **15***, 84-92.

Alipour, A., Rahaiee, S., Litkohi, H.R., Jamali, S.N., & Jafari, S.M. (2023). Development and optimization of whey protein-Lepidium perfoliatum gum packaging films: An approach towards antimicrobial and biodegradable films.

Industrial Crops and Products, ***196***, 116447. DOI: 10.1016/j.indcrop.2023.116447

Avci, E., Akcicek, A., Tekin Cakmak, Z.H., Kasapoglu, M. Z., Sagdic, O. & Karasu, S. (2024). Isolation of Protein and Fiber from Hot Pepper Seed Oil Byproduct To Enhance Rheology, Emulsionand Oxidative Stability of Low-Fat Salad Dressing. *ACS omega*, ***9***(9), 10243-10252. DOI: 10.1021/acsomega.3c07410

Brito, T., Carrajola, J., Gonçalves, E., Martelli-Tosi, M. & Ferreira, M. (2019). Fruit and vegetable residues flours with different granulometry range as raw material for pectin-enriched biodegradable film preparation. *Food Research International*, ***121***, 412-421. DOI: 10.1016/j.foodres.2019.03.058

Capar, T.D. (2023). Characterization of sodium alginate-based biodegradable edible film incorporated with Vitis vinifera leaf extract: Nano-scaled by ultrasound-assisted technology. *Food Packaging and Shelf Life*, ***37***, 101068.

Chakravartula, S.S.N., Soccio, M., Lotti, N., Balestra, F., Dalla Rosa, M. & Siracusa, V. (2019). Characterization of Composite Edible Films Based on Pectin/Alginate/Whey Protein Concentrate. *Materials*, ***12***(15), 2454. DOI: 10.3390/ma12152454

Dai, Q., Huang, X., Jia, R., Fang, Y. & Qin, Z. (2022). Development of antibacterial film based on alginate fiberand peanut red skin extract for food packaging. *Journal of Food Engineering*, ***330***, 111106. DOI: 10.1016/j.jfoodeng.2022.111106

Domínguez, R., Barba, F.J., Gómez, B., Putnik, P., Kovačević, D.B., Pateiro, M. & Lorenzo, J.M. (2018). Active packaging films with natural antioxidants to be used in meat industry: A review. *Food Research International*, ***113***, 93-101. DOI: 10.1016/j.foodres.2018.06.073

Duan, J., Zhou, Q., Fu, M., Cao, M., Jiang, M., Zhang, L. & Duan, X. (2023). Research on Properties of Edible Films Prepared from Zein, Soy Protein Isolate, Wheat Gluten Protein by Adding Beeswax. *Food and Bioprocess Technology*, ***16***(11), 2443-2454. DOI: 10.1007/s11947-023-03077-2

Fernández, C., Ausar, S.F., Badini, R.G., Castagna, L. F., Bianco, I.D. & Beltramo, D. M. (2003). An FTIR spectroscopy study of the interaction between αs-casein-bound phosphoryl groups and chitosan. *International Dairy Journal*, ***13***(11), 897-901. DOI: 10.1016/S0958-6946(03)00115-8

Fiorentini, F., Suarato, G., Grisoli, P., Zych, A., Bertorelli, R. & Athanassiou, A. (2021). Plant-based biocomposite films as potential antibacterial patches for skin wound healing. *European Polymer Journal*, ***150***, 110414. DOI: 10.1016/j.eurpolymj.2021.110414

Galus, S. & Kadzińska, J. (2015). Food applications of emulsion-based edible films and coatings. *Trends in Food Science & Technology*, ***45***(2), 273-283. DOI: 10.1016/j.tifs.2015.07.011

Ghanbarzadeh, B. & Oromiehi, A. (2008). Biodegradable biocomposite films based on whey protein and zein: Barrier, mechanical properties and AFM analysis. *International Journal of Biological Macromolecules*, ***43***(2), 209-215. DOI: 10.1016/j.ijbiomac.2008.05.006

Hadidi, M., Jafarzadeh, S., Forough, M., Garavand, F., Alizadeh, S., Salehabadi, A. & Jafari, S.M. (2022). Plant protein-based food packaging films; recent advances in fabrication, characterizationand applications. *Trends in Food Science & Technology*, ***120***, 154-173. DOI: 10.1016/j.tifs.2022.01.013

Hematizad, I., Khanjari, A., Basti, A.A., Karabagias, I.K., Noori, N., Ghadami, F., . . & Teimourifard, R. (2021). In vitro antibacterial activity of gelatin-nanochitosan films incorporated with Zataria multiflora Boiss essential oil and its influence on microbial, chemicaland sensorial properties of chicken breast meat during refrigerated storage. *Food Packaging and Shelf Life*, ***30***, 100751. DOI: 10.22059/jvr.2022.337641.3231

Homthawornchoo, W., Han, J., Kaewprachu, P., Romruen, O. & Rawdkuen, S. (2022). Green tea extract enrichment: mechanical and physicochemical properties improvement of rice starch-pectin composite film. *Polymers*, ***14***(13), 2696. DOI: 10.3390/polym14132696

Huang, Y., Xiang, X., Luo, X., Li, X., Yu, X. & Li, S. (2021). Study on the emulsification and oxidative stability of ovalbumin-pectin-pumpkin seed oil emulsions using ovalbumin solution prepared by ultrasound. *Ultrasonics Sonochemistry*, ***78***, 105717. DOI: 10.1016/j.ultsonch.2021.105717

Jeon, Y.J., Lee, H. & Min, S.C. (2023). Effects of in-package atmospheric dielectric barrier discharge cold plasma treatment on the antimicrobial efficacy of whey protein isolate-based edible films that incorporate malic acid against Salmonella in chicken breast processed meat. *Innovative Food Science & Emerging Technologies*, ***85***, 103339. DOI: 10.1016/j.ifset.2023.103339

Jiang, S., Zou, L., Hou, Y., Qian, F., Tuo, Y., Wu, X. & Mu, G. (2020). The influence of the addition of transglutaminase at different phase on the film and film forming characteristics of whey protein concentrate-carboxymethyl chitosan composite films. *Food Packaging and Shelf Life*, ***25***, 100546. DOI: 10.1016/j.fpsl.2020.100546

Kamari, A. & Phillip, E. (2018). Chitosan, gelatin and methylcellulose films incorporated with tannic acid for food packaging. *International Journal of Biological Macromolecules*, ***120***, 1119-1126. DOI: 10.1016/j.ijbiomac.2018.08.169

Kandasamy, S., Yoo, J., Yun, J., Kang, H.B., Seol, K.H., Kim, H.W. & Ham, J.S. (2021). Application of whey protein-based edible films and coatings in food industries: An updated overview. *Coatings*, ***11***(9), 1056. DOI: 10.3390/coatings11091056

Karaaslan, M., Şengün, F., Cansu, Ü., Başyiğit, B., Sağlam, H. & Karaaslan, A. (2021). Gum arabic/maltodextrin microencapsulation confers peroxidation stability and antimicrobial ability to pepper seed oil. *Food Chemistry*, ***337***, 127748. DOI: 10.1016/j.foodchem.2020.127748

Karydis-Messinis, A., Kyriakaki, C., Triantafyllou, E., Tsirka, K., Gioti, C., Gkikas, D. & Avgeropoulos, A. (2024). Development and Physicochemical Characterization of Edible Chitosan–Casein Hydrogel Membranes for Potential Use in Food Packaging. *Gels*, ***10***(4), 254. DOI: 10.3390/gels10040254

Karydis-Messinis, A., Moschovas, D., Markou, M., Gkantzou, E., Vasileiadis, A., Tsirka, K. & Murphy, C. (2023). Development, physicochemical characterization and in vitro evaluation of chitosan-fish gelatin-glycerol hydrogel membranes for wound treatment applications. *Carbohydrate Polymer Technologies and Applications*, ***6***, 100338. DOI: 10.20944/preprints202402.0173.v1

Kocira, A., Kozłowicz, K., Panasiewicz, K., Staniak, M., Szpunar-Krok, E. & Hortyńska, P. (2021). Polysaccharides as Edible Films and Coatings: Characteristics and Influence on Fruit and Vegetable Quality-A Review. *Agronomy*, ***11***(5), 813. DOI: 10.3390/agronomy11050813

Kopuz, S., Akman, P.K., Tekin-Cakmak, Z.H. & Karasu, S. (2024). Development and characterization of antimicrobial films from gums obtained from cold-pressed flaxseed oil by-product. *Polymer Bulletin*, *81*(2), 1767-1787. DOI: 10.1007/s00289-023-04793-7

Kumar, L., Ramakanth, D., Akhila, K. & Gaikwad, K.K. (2022). Edible films and coatings for food packaging applications: A review. *Environmental Chemistry Letters*, 1-26. DOI: 10.1007/s10311-021-01339-z

Kumar, S., Konwar, J., Purkayastha, M.D., Kalita, S., Mukherjee, A. & Dutta, J. (2023). Current progress in valorization of food processing waste and by-products for pectin extraction. *International Journal of Biological Macromolecules*, ***239***, 124332. DOI: 10.1007/s10311-021-01339-z

Ma, Y., Xin, L., Tan, H., Fan, M., Li, J., Jia, Y. & Hu, X. (2017). Chitosan membrane dressings toughened by glycerol to load antibacterial drugs for wound healing. *Materials Science and Engineering: C*, ***81***, 522-531. DOI: 10.1016/j.msec.2017.08.052

Mahcene, Z., Khelil, A., Hasni, S., Akman, P.K., Bozkurt, F., Birech, K. & Tornuk, F. (2020). Development and characterization of sodium alginate based active edible films incorporated with essential oils of some medicinal plants. *International Journal of Biological Macromolecules*, ***145***, 124-132. DOI: 10.1016/j.ijbiomac.2019.12.093

Mercadal, P.A., Picchio, M.L. & González, A. (2024). Food-protecting films based on soy protein isolate and natural deep eutectic solvents: Antimicrobial and antioxidant properties. *Food Hydrocolloids*, ***147***, 109414. DOI: 10.1016/j.foodhyd.2023.109414

Mohamed, S.A., El-Sakhawy, M. & El-Sakhawy, M.A.M. (2020). Polysaccharides, protein and lipid-based natural edible films in food packaging: A review. *Carbohydrate polymers*, ***238***, 116178. DOI: 10.1016/j.carbpol.2020.116178

Nguyen, T.T., Pham, B.T.T., Le, H.N., Bach, L.G. & Thuc, C.H. (2022). Comparative characterization and release study of edible films of chitosan and natural extracts. *Food Packaging and Shelf Life*, ***32***, 100830. DOI: 10.2139/ssrn.3931690

Noori, N., Khanjari, A., Rezaeigolestani, M., Karabagias, I.K. & Mokhtari, S. (2021). Development of antibacterial biocomposites based on poly (lactic acid) with spice essential oil (Pimpinella anisum) for food applications. *Polymers*, ***13***(21), 3791. DOI: 10.3390/polym13213791

Omar-Aziz, M., Khodaiyan, F., Yarmand, M.S., Mousavi, M., Gharaghani, M., Kennedy, J.F. & Hosseini, S.S. (2021). Combined effects of octenylsuccination and beeswax on pullulan films: Water-resistant and mechanical properties. *Carbohydrate Polymers*, **255**, 117471. DOI: 10.1016/j.carbpol.2020.117471

Pajak, P., Fortune, T. & Przetaczek-Roznowska, I. (2013). Protein and Polysaccharide Based Edible Packagings: Profile and Applications. *Zywnosc-Nauka Technologia Jakosc*, ***20***(2), 5-18. DOI: 10.15193/zntj/2013/87/005-018

Qin, Y., Liu, Y., Yuan, L., Yong, H. & Liu, J. (2019). Preparation and characterization of antioxidant, antimicrobial and pH-sensitive films based on chitosan, silver nanoparticles and purple corn extract. *Food Hydrocolloids*, ***96***, 102-111. DOI: 10.1016/j.foodhyd.2019.05.017

Qin, Y., Liu, Y., Zhang, X. & Liu, J. (2020). Development of active and intelligent packaging by incorporating betalains from red pitaya (Hylocereus polyrhizus) peel into starch/polyvinyl alcohol films. *Food Hydrocolloids*, ***100***, 105410. DOI: 10.1016/j.foodhyd.2019.105410

Rawat, R. & Saini, C.S. (2024). A novel biopolymeric composite edible film based on sunnhemp protein isolate and potato starch incorporated with clove oil: Fabrication, characterizationand amino acid composition. *International Journal of Biological Macromolecules*, ***268***, 131940. DOI: 10.1016/j.ijbiomac.2024.131940

Sadeghi-Varkani, A., Emam-Djomeh, Z. & Askari, G. (2018). Physicochemical and microstructural properties of a novel edible film synthesized from Balangu seed mucilage. *International Journal of Biological Macromolecules*, ***108***, 1110-1119. DOI: 10.1016/j.ijbiomac.2017.11.029

Sandoval-Castro, C.J., Valdez-Morales, M., Oomah, B.D., Gutiérrez-Dorado, R., Medina-Godoy, S. & Espinosa-Alonso, L.G. (2017). Bioactive compounds and antioxidant activity in scalded Jalapeño pepper industrial byproduct (Capsicum annuum). *Journal of Food Science and Technology*, ***54***, 1999-2010. DOI: 10.1007/s13197-017-2636-2

Sani, I.K., Geshlaghi, S.P., Pirsa, S. & Asdagh, A. (2021). Composite film based on potato starch/apple peel pectin/ZrO2 nanoparticles/microencapsulated Zataria multiflora essential oil; investigation of physicochemical properties and use in quail meat packaging. *Food Hydrocolloids*, ***117***, 106719. DOI: 10.1016/j.foodhyd.2021.106719

Seydim, A. & Sarikus, G. (2006). Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food Research International*, ***39***(5), 639-644. DOI: 10.1016/j.foodres.2006.01.013

Sood, A. & Saini, C. (2022). Red pomelo peel pectin based edible composite films: Effect of pectin incorporation on mechanical, structural, morphological and thermal properties of composite films. *Food Hydrocolloids*, ***123***, 107135. DOI: 10.1016/j.foodhyd.2021.107135

Soukoulis, C., Behboudi-Jobbehdar, S., Macnaughtan, W., Parmenter, C. & Fisk, I.D. (2017). Stability of Lactobacillus rhamnosus GG incorporated in edible films: Impact of anionic biopolymers and whey protein concentrate. *Food Hydrocolloids*, ***70***, 345-355. DOI: 10.1016/j.foodhyd.2017.04.014

Tornuk, F., Sagdic, O., Hancer, M. & Yetim, H. (2018). Development of LLDPE based active nanocomposite films with nanoclays impregnated with volatile compounds. *Food Research International*, ***107***, 337-345. DOI: 10.1016/j.foodres.2018.02.036

Wang, H., Ding, F., Ma, L. & Zhang, Y. (2021). Edible films from chitosan-gelatin: Physical properties and food packaging application. *Food Bioscience*, ***40***, 100871. DOI: 10.1016/j.fbio.2020.100871

Yan, J., He, S., Chen, L., Chen, H. & Wang, W. (2023). Characterization, antioxidant and antibacterial activities of gelatin-chitosan edible coated films added with Cyclocarya paliurus flavonoids. *International Journal of Biological Macromolecules*, ***253***, 127664. DOI: 10.1016/j.ijbiomac.2023.127664

Ye, Q., Han, Y., Zhang, J., Zhang, W., Xia, C. & Li, J. (2019). Bio-based films with improved water resistance derived from soy protein isolate and stearic acid via bioconjugation. *Journal of cleaner production*, *214*, 125-131. DOI: 10.1016/j.jclepro.2018.12.277

Zhu, F. (2021). Polysaccharide based films and coatings for food packaging: Effect of added polyphenols. *Food Chemistry*, *359*, 129871. DOI: 10.1016/j.foodchem.2021.129871