Hindawi
Journal of Food Processing and Preservation
Volume 2023, Article ID 2848780, 12 pages
https://doi.org/10.1155/2023/2848780

Research Article

An Antioxidant and Intelligent pH-Sensitive Composite Film Based on Gelatin and Persian Gum Using Purple Carrot Extract

Mohammad Sadegh Arab,[1] **Hannan Lashkari**,[2] **Mehrdad Niakousari**,[3] **and Mohammad Hadi Eskandari**[3]

[1]*Department of Food Science and Technology, Sarvestan Branch, Islamic Azad University, Sarvestan, Iran*
[2]*Department of Food Science and Technology, Zarin Dasht Branch, Islamic Azad University, Zarin Dasht, Iran*
[3]*Department of Food Science and Technology, College of Agriculture, Shiraz University, Shiraz, Iran*

Correspondence should be addressed to Hannan Lashkari; hlashkari@gmail.com

Received 30 September 2022; Revised 16 November 2022; Accepted 18 November 2022; Published 31 March 2023

Academic Editor: Charalampos Proestos

The present research is aimed at developing novel intelligent pH-sensitive films based on Persian gum and gelatin using purple carrot extract. To this end, films were developed by mixing polymer solutions containing Persian gum (5%) and gelatin (5%), in equal proportions and partially replacing distilled water of solutions with purple carrot extract at 0, 15, 30, and 45%. Total anthocyanin content, antioxidant activity, color indices, and tensile strength of films were evaluated. Purple carrot extract incorporation in the film caused a slight reduction in the lightness and an increase in the anthocyanin content, DPPH inhibition, yellowness, redness, and tensile strength. The sample containing 45% extract (anthocyanin concentration was 2.39 mg/ml) was selected as the best pH-sensitive film based on mechanical and antioxidant properties and evaluated in terms of moisture content, moisture absorption, pH, heat seal ability, thickness, contact angle, FTIR, X-ray diffraction, and colorimetric tests. The sensitivity of the film to pH changes was assessed in the range of 2-12. The color of the film changed from pink to blue as a function of pH. The results proved that films based on Persian gum, gelatin, and purple carrot extract could be used as novel food grade biomaterials to monitor the freshness/spoilage of food.

1. Introduction

Nowadays, concerns about buying and consuming healthy and natural food have led to particular consumer attention in many areas of the food industry, including packaging. In the food packaging industry, due to the suitable mechanical and physical attributes of synthetic polymers, manufacturers have a great desire to produce and consume them [1]. However, there is an increasing tendency to utilize green and edible polymers to supply the packaging materials for the food industry. Green and biodegradable packaging are prepared from biopolymers including carbohydrates, proteins, and lipids, which results in increasing the stability of the food products. The advantages presented by these biodegradable materials are included water resistance, retaining aromatic compounds, reducing oxidation and discoloration, and antimicrobial activities [2].

Gelatin is an inexpensive biopolymer that presents an excellent performance and good properties as an edible film. However, gelatin has no adhesion ability and hence this property can be achieved with the help of other polymers (starch, Persian gum, CMC, and chitosan). The dispersion of gelatin can form a gel structure upon heating that is heat reversible. The melting point of the gelatin gel (less than 35°C) is lower than human body temperature, which makes it a widely used ingredient in food products to produce a unique taste and organoleptic properties [3].

On the other hand, Persian gum (PG), as an anionic hydrocolloid, is exudated from mountain almond trees [4, 5] which contains a certain amount of protein and natural water-soluble (~30%) and water-insoluble (~70%) fractions [6, 7]. Mahfoudhi, Chouaibi, Donsi, Ferrari, and Hamdi [8] reported that Arabinose, xylitol, galactose and uronic acid (46.8 : 10.9 : 35.5 : 6.0 mass ratio, respectively) with

traces of rhamnose, mannose, and glucose were reported as the monosaccharides present in the polysaccharide chain and some minor protein content. High water holding capacity and emulsification properties, make it a proper choice in food formulations. It has been reported that the water-soluble fraction of PG is dissolved in water at low temperatures and capable to form a fragile film, while the insoluble fraction is unable to dissolve even in hot water and unable to form films due to precipitation during drying of film solution. Protein-based biodegradable films which are produced with gelatin are known as strong translucence films and suitable substitutes for synthetic films [9]. Although PG and gelatin are potential biopolymers to utilize in food packaging, the limited antioxidant activity of these films is a major factor in distinguishing them from active packaging materials. In this context, many attempts have been conducted to develop the functionalities in edible films based on the abovementioned biopolymers by the addition of components with antioxidant characteristics [10].

Anthocyanins are natural and edible pigments produced by plants and found in various parts of the plant such as stems, roots, leaves, flowers, and fruits. They are nontoxic and water-soluble compounds, responsible for the colors blue, purple, and red, and belong to the group of flavonoids [11]. Anthocyanins are glycosides or acyl-glycosides of anthocyanins that are located in cellular vacuoles. The most common anthocyanin types are the glycosides cyanidin, delphinidin, malvidin, polargonidin, peonidine, and petonidine. The application of this anthocyanin was increased due to their physiological, biological, and functional properties including antioxidants, anticancer, and anti-inflammatory activities, heart protection, a barrier against chemicals and antimutation [12]. These compounds are proper additives for the production of active and intelligent packaging and also can change their chemical structure to show different colors at different pHs; therefore, they can be exploited as a pH indicator to track food degradation. Various herbs such as sweet purple potatoes, red cabbage, black soybeans, blackberries, blueberries, and purple or black carrots are good sources of anthocyanins that are used for smart and active packaging [13].

Purple carrots (*Daucus carota* subsp. *Sativus* var. *Atrorubens Alef*) grow in the Middle East and Europe. The acylated anthocyanins in purple carrots are a natural pigment suitable for coloring nectars, beverages, jellies, and pastries. The predominant anthocyanins in purple carrots are cyanidin, which contains various nonacetylated sugars (two of which), or acetylated (three) with sinapic acid, ferulic acid, or coumaric acid [14].

There are a lot of research on the application of anthocyanins in the intelligent films. Wu [15] evaluated the active and intelligent gellan gum-based packaging films for controlling anthocyanins, Chen, Zhang, Bhandari, and Yang [16] studied the pH-sensitive films containing curcumin and anthocyanins, Vedove [17] evaluated smart packaging based on cassava starch and anthocyanin.

This study is aimed at preparing an intelligent and active pH-sensitive film with antioxidant activity based on the combination of Persian gum and gelatin using anthocyanin-loaded purple carrot extract (PCE). Initially, anthocyanins were extracted from the purple carrot. Then, the obtained PCE was incorporated into Persian gum and gelatin film-forming solution. The effects of PCE on the physicochemical and functional characteristics of composite Persian and gelatin film were investigated.

2. Method and Material

2.1. Materials. Bovine gelatin (bloom 200) was purchased from Gelatin Halal Co. (GHC, Tehran, Iran) and Persian gum was obtained from a local market in Shiraz (Fars Province, Iran). Glycerol as the plasticizer was prepared from Emboy Kimya (Turkey). Purple carrots were purchased from a local market in Bandar Abbas (Iran).

2.2. Extraction of PCE as a pH Indicator. The extraction method was carried out according to the method of Barnes, Nguyen, Shen, and Schug [18]. All purple carrot samples were kept in the freezer at -16°C for 24 h, then diced by knife. 10 g of purple carrot samples were added to the centrifugal tube containing 100 ml of a mixture of ethanol, distilled water, and HCl (70 : 30 : 1 v/v/v) and then stirred overnight. Finally, the samples were centrifuged and the supernatant was separated manually and filtered.

2.3. Film Preparation. To prepare film-forming solutions, gelatin (5%, w/v), Persian gum (5%, w/v), and glycerol (3%, w/v) were added to the distilled water. The solutions were mixed in equal proportions by a magnetic stirrer for 6 h. The films were prepared by pouring 20 ml of the mixture into the Petri dishes. In order to make a thin layer, the solutions were spread on the surface by a glass rod with 10 centimeter diameter and then put aside to dry at ambient temperature for 48 h and 24 h in the desiccator. To prepare the active films, 15, 30, and 45% of PCE were replaced with distilled water during the preparation of film-forming solution [19].

2.4. Anthocyanin Content. Total anthocyanins content of films specified by a pH differential method using two buffer systems: KCl buffer, pH 1.0 (0.025 M), and sodium acetate buffer, pH 4.5 (0.4 M). Briefly, 0.4 ml of film samples was blended with 3.6 ml of corresponding buffers and read against water as a blank at 510 nm (A_{510}) and 700 nm (A_{700}). Equation (4) was used to calculate absorbance (A): [20].

$$A = (\mathrm{A510} - \mathrm{A700})\,\mathrm{pH}\,1.0 - (\mathrm{A510} - \mathrm{A700})\mathrm{pH}4.5. \quad (1)$$

Total anthocyanin content (TAC) of samples (mg cyanidin-3-glucoside/100 ml of extract) was evaluated using the following equation:

$$\mathrm{TAC} = \frac{(A \times M \times \mathrm{DF} \times 100)}{\mathrm{MA}}, \quad (2)$$

where A is absorbance, M is the molecular weight of cyanidin-3- glucoside (449.2), DF is dilution factor (10), and MA is the molar absorptivity of cyanidin-3-glucoside (26,900).

2.5. Antioxidant Activity. Antioxidant activity of films was determined based on the radical scavenging assay [21]. For the concentration-dependent antioxidant assay, a pre-weighed amount of the prepared film samples was placed in 5 ml of 10 mM DPPH solution with methanol as solvent. After storing the sample solutions in darkness for 60 min, spectrophotometric methodology for reaction solution was evaluated at 517 nm. DPPH radical scavenging activity was calculated using

$$\text{DPPH radical scavenging activity (\%): } \left[\frac{\text{A0} - \text{A1}}{\text{A0}}\right] \times 100, \quad (3)$$

where A_0 is the absorbance of the blank cell and A_1 is the absorbance of the reaction solution.

2.6. Color Properties. Color variables of the films were determined using a Minolta colorimeter (CR-20, Konica Minolta, Inc., and Tokyo, Japan). The images were cut to 1000 × 1000 pixels and transformed into CIELAB color system, and their corresponding L^*, a^*, and b^* parameters were analyzed using ImageJ 1.47n software (National Institute of Health, USA). The measurements were conducted in triplicates [22].

2.7. Mechanical Analysis. Film tape (40 mm × 5 mm) was fixed between the grips of Instron universal instrument model no. 5543A (Instron Engineering Corp., Norwood, MA, USA). The tensile strength (Pa) was calculated by dividing the maximum peak force (F) by the film area (A) [23, 24].

$$\text{Tensile strength : } \left[\frac{\text{F}}{\text{A}}\right] \times 100, \quad (4)$$

where A obtains by multiplying the film thickness (mm) by film width (mm).

2.8. Moisture Content. The moisture content of films was determined by following the procedure described by Tabatabaei [24] and Muñoz, Aguilera, Rodriguez-Turienzo, Cobos, and Diaz [25]. Films were weighed and dried in oven for 105°C to reach a constant weight. The moisture content of films was calculated using the following equation:

$$\text{MC (\%)} = \left[\frac{Wi - Wf}{Wf}\right] \times 100, \quad (5)$$

where W_i is wet weight and W_f is the dry weight of the film.

2.9. Moisture Absorption. A 2 × 2 cm^2 cut of films was placed in a desiccator at 0% relative humidity by using anhydrous calcium chloride for 6 days at room temperature, weighed, and placed in a vessel containing saturated K_2SO_4 with 97% relative humidity at room temperature, then weighed until the constant weight was gained. Moisture uptake was calculated from [22].

$$\text{Moisture uptake : } \left[\frac{m2 - m1}{m1}\right] \times 100, \quad (6)$$

where m_1 is the initial weight and m_2 is the weight of the film after water absorption.

2.10. pH Test. For pH determination, the film was firstly mixed with 10 ml water and kept to absorb water. Then, the pH of the film solution was evaluated by a digital pH meter (model: Seven Compact S-220, USA) [26].

2.11. Film Thickness. Film thickness was measured by using high accuracy (0.001 mm) digital micrometer (iGaging, san Celemt, California) for at least ten randomized locations of films. This procedure was repeated three times for other independent films [22].

2.12. Heat Sealing Ability. Film samples were cut into a tape shape of 5 × 5 cm. One tape was placed on the top of another and then heat-sealed using an automatic heat sealer (AH26 Mod: Go plus 8, IRAN). The width of the sealing area was equal to 1.5 mm. All sealed films were then conditioned at room temperature with RH = 60% for two days prior to analysis. The heat-sealing ability was measured using the peel and the lap shear tests according to Standard ASTM F-88 [27] with slight modifications, using a texture analyzer (Instron Engineering Corp., Norwood, MA, USA). Each tape of the sealed film was hitched to the clamp, in a manner that each end of the leg was kept perpendicularly to the force direction. The clamps were 50 mm apart and a statistical force of 100 N with a speed of 30 mm/min was applied. The ultimate force in which the tapes were detached was determined as sealing strength (Pa).

2.13. Contact Angle. When a droplet of liquid is placed onto a surface, the angle between the underside of the droplet and the adjacent curvature of the droplet is called the contact angle. The test was conducted using a camera to capture the picture of a placed droplet of liquids (~15 ml) onto the surface of films after 10 s. The contact angle was determined using ImageJ software (1.44p, National Institute of Health, USA). The replications for each liquid and sample were 9 times and 3 times, respectively [28].

2.14. Fourier-Transform Infrared (FTIR) Spectroscopy. FTIR spectra of films were collected in form of transmittance using a FTIR spectrometer (Thermo Nicolet Corp., Madison, WI, USA) at a step of 4 cm^{-1} in a period of 400 to 4000 cm^{-1} wavenumbers. The milled samples were blended with KBr (1 : 100, w/w) and formed into pellets before spectroscopy.

2.15. X-Ray Diffraction (XRD). Crystalline characteristics of films were evaluated using Bruker AXS D8 Advance 191 X-ray diffractometer (Thermo Nicolet Corp., Madison, WI, USA). The operation was at 40 mA and 40 kV using Ni-filtered Cu Kα radiation. The diffraction pattern was obtained from $2\theta = 5 - 80^\circ$ at a scan speed of 0.1°/s.

Anthocyanin (mg/ml)	Test 1	Test 2	Test 3	Average	stdev
0%	0.6	0.5	0.45	0.516667	0.062361
15%	1.35	1.4	1.1	1.283333	0.131233
30%	2.3	2.2	2.15	2.216667	0.062361
45%	2.394	2.391	2.399	2.394667	0.0033

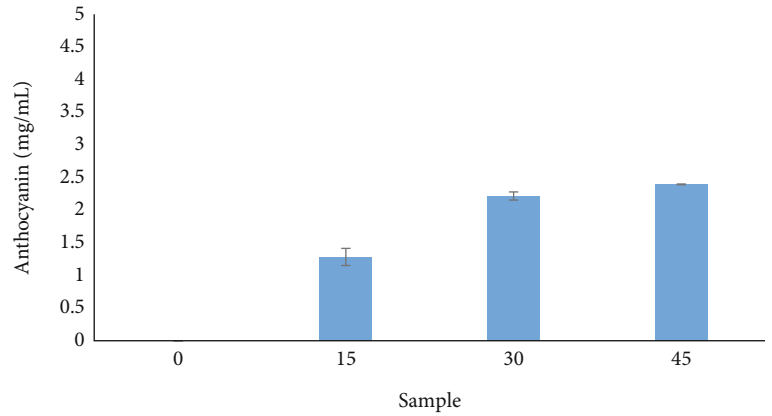


Figure 1: Anthocyanin concentration of control Persian gum-gelatin film and active films at different concentrations of purple carrot extract.

dpph	Test 1	Test 2	Test 3	Average	stdev
0%	0.1	0.15	0.12	0.123333	0.025166
15%	9.86	10.12	10.67	10.21667	0.413562
30%	21.4	20.56	21.43	21.13	0.493862
45%	34.83	33.98	34.47	34.42667	0.426654

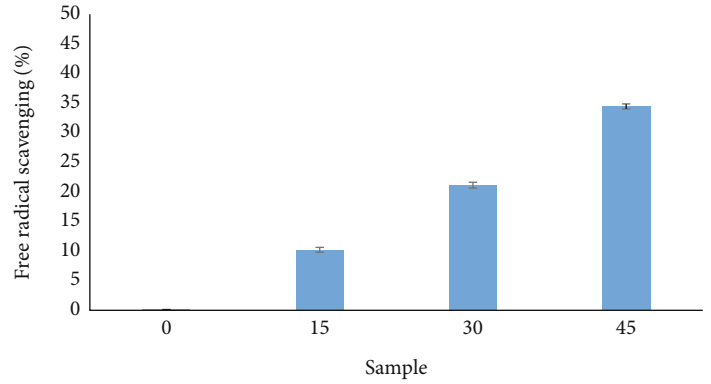


Figure 2: DPPH radical scavenging of control Persian gum-gelatin film and active films at different concentrations of purple carrot extract.

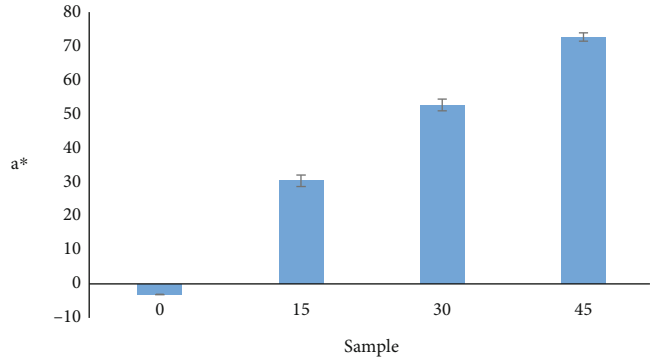
2.16. SEM. The images of the samples were determined by a scanning electron microscope (TESCAN vega3, Czech Republic). SEM images were taken at a 20 kV accelerating voltage.

2.17. Determination of pH-Sensitivity of Film. In order to investigate color changes, the film was flooded with an aqueous media with different pHs from 2 to 12 for about 15 min. The color changes were recorded by Minolta colorimeter (CR-20, Konica Minolta, Inc., Tokyo, Japan). The color difference (ΔE) for the film at different pHs compared to a constant pH was determined through

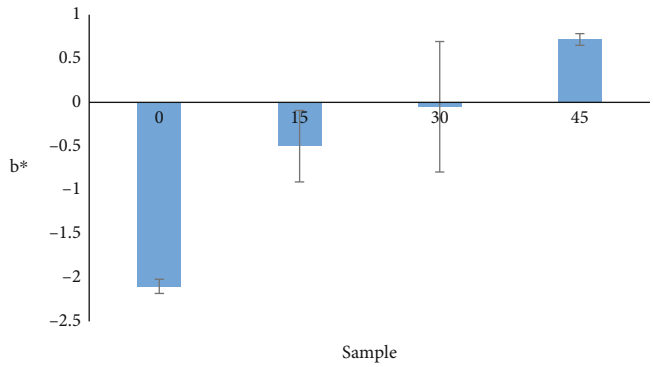
$$\Delta E = \sqrt[2]{(L*-L0)2 + (a*-a0)2 + (b*-b0)2}. \quad (7)$$

2.18. Statistical Analysis. The design of the completely randomized analysis of variance (ANOVA) procedure was conducted using SPSS software (Version 16; SPSS Inc., USA). The comparison of the differences between mean values was also conducted using Duncan's multiple range tests at $P < 0.05$.

*A	Test 1	Test 2	Test 3	Average	stdev
0%	−3	−3.2	−3.1	−3.1	0.08165
15%	32	31	28	30.33333	1.699673
30%	51	55	52	52.66667	1.699673
45%	71	74	73	72.66667	1.247219



*B	Test 1	Test 2	Test 3	Average	stdev
0%	−2	−2.2	−2.1	−2.1	0.08165
15%	0	−1	-0.5	−0.5	0.408248
30%	1	−0.5	−0.65	−0.05	0.744983
45%	0.66	0.68	0.81	0.716667	0.0665



L*	Test 1	Test 2	Test 3	Average	stdev
0%	93	91	93	92.33333	0.942809
15%	89	88	87	88	0.816497
30%	82	85	84	83.66667	1.247219
45%	70.33	68.5	71.1	69.97667	1.090454

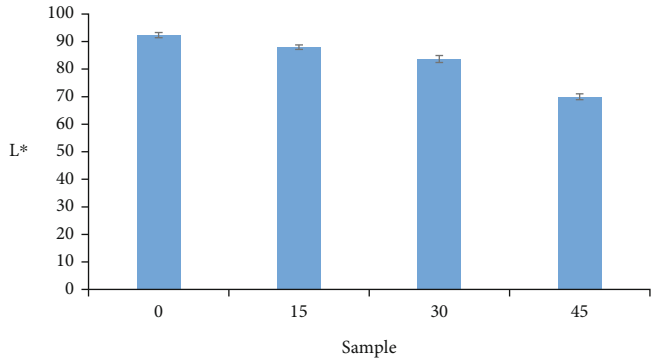


Figure 3: Color measurement of control Persian gum-gelatin film and active film films at different concentrations of purple carrot extract.

Tensile	Test 1	Test 2	Test 3	Average	stdev
0%	64.33	64.2	66.2	65.2	1
15%	68	68	67.4	67.8	0.282843
30%	70	70.2	71.6	70.6	0.711805
45%	76	75.5	78.1	76.53333	1.12645

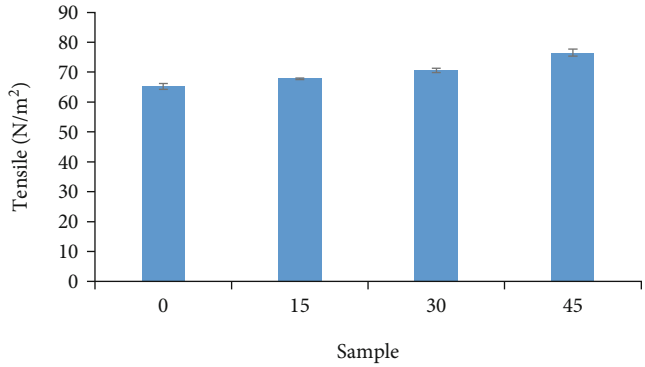


Figure 4: Tensile properties of control Persian gum-gelatin film and active films at different concentrations of purple carrot extract.

Table 1: Physicochemical properties of control Persian gum-gelatin film and active film with 45% purple carrot extract.

Physicochemical properties	Control	Active film
Moisture content (%)	3.90 ± 0.32A	3.10 ± 0.14B
Moisture absorption (%)	6.13 ± 0.24B	12.53 ± 0.44A
pH	6.34 ± 0.03A	6.11 ± 0.08B
Heat-sealing strength (Pa)	81.70 ± 0.60B	83.83 ± 0.25A
Thickness (μm)	80.33 ± 1.69A	79.33 ± 1.24A
Contact angle (°)	66.20 ± 1.72B	76.64 ± 0.77A

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each row indicate significant differences ($P < 0.05$).

3. Result and Discussion

3.1. Anthocyanin Content. Total anthocyanin content in film samples is shown in Figure 1. As expected, the film with 45% of PCE presented higher content in comparison to others.

3.2. Antioxidant Activity. Since free radicals are responsible for nutritional degradation, the antioxidant activity of composite films plays an important role in food packaging [29]. As depicted in Figure 2, the antioxidant activity of film samples was notably improved by increasing PCE ($P < 0.05$). This fact can be attributed to the increase in anthocyanin content in the films. A similar trend in free radical scavenging was reported by Yong, Wang, Zhang, Liu, Qin, and Liu [13] who fabricated chitosan film incorporated with anthocyanin-rich purple and black eggplant extracts.

3.3. Colorimetric. Visual and optical properties are important features of films that are used as packaging or as food coatings. The overall appearance of the food is directly related to the color of the packaging, and this can greatly affect the customer's product friendliness [30]. The values of lightness (L^*), green-red (a^*), and blue-yellow (b^*) parameters of films are shown in Figure 3. The addition of PCE into the film matrix caused a remarkable ($P < 0.05$) effect on this parameter. Control composite film was transparent with more greenness and blueness color, whereas with an increase in the content of anthocyanins, the L^* significantly ($P < 0.05$) decreased, while a^* and b^* significantly ($P < 0.05$) increased. Therefore, PCE resulted in darker and more redness and yellowness composite films which were more intense at higher concentrations. A similar trend was also reported by Kurek [31] for chitosan film incorporated with anthocyanin-loaded blueberry extract. In general, more opacity has been reported with the incorporation of natural active ingredients such as pomegranate rind extract [32] and black eggplant extracts [13] into the film-forming solutions.

3.4. Mechanical Analysis. The mechanical strength and transportation capability play a key role in the selection of a polymer as a packaging material [33]. As shown in Figure 4, the tensile strength of films with PCE with 15, 30, and 45% concentration (67.8, 70.6, and 76.53 Pa, respectively) was higher than control film (65.2 Pa) ($P < 0.05$). The increase in tensile strength of samples incorporated with PCE might be due to H-bonds formed between OH and amine groups of gelatin and Persian gum and polyphenols

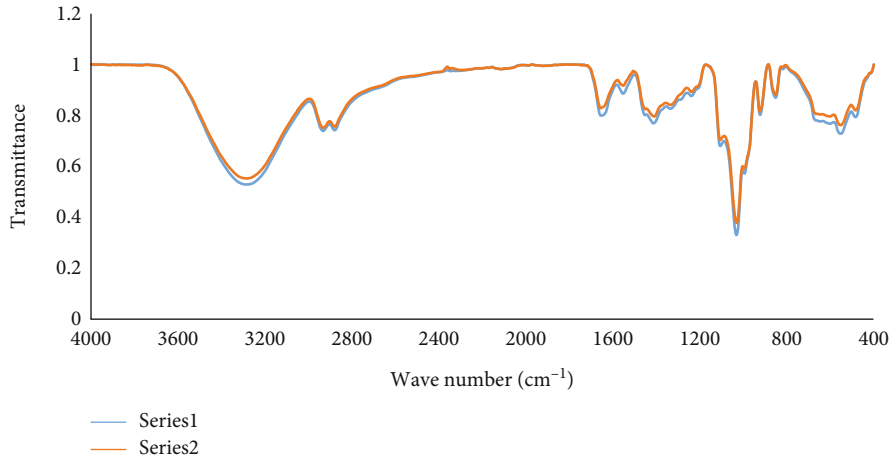


Figure 5: FTIR spectra of control Persian gum-gelatin film (blue) and active film with 45% purple carrot extract (red).

in the extracts which interrelationship and interfacial interaction of polymer chains have a median effect on all mechanical factors [34]. A similar trend of mechanical strength was reported for agar-nanocellulose and zein-pomegranate peel extract films [35]. According to the results of physicochemical and textural properties, the film sample containing 45% extract was selected for more evaluation.

3.5. Moisture Content. As shown in Table 1, significant differences ($P \geq 0.05$) were observed in the moisture content of control film and active film with 45% of PCE. Generally, the relatively low moisture contents in PCE films might be due to the interactions between amine and OH groups of Persian gum, gelatin, and anthocyanins in PCE, which might restrict the interactions of Persian gum and gelatin with water [10]. A similar result was also reported for chitosan film with eggplant extract represented [13], whereas Kurek [31] reported a slightly lower moisture content in blueberry and blackberry pomace extracts-loaded chitosan film. These contradictory results indicate the significant ($P < 0.05$) effect of composition and the concentration of anthocyanins on their degree of bonding to biopolymer matrix and hence the moisture content of final films.

3.6. Moisture Absorption and pH. The water absorption isotherm provides useful information about the microstructure details of the films. The results of the Table 1 showed that PCE-loaded composite film presented more water abortion than control film. This fact might be attributed to higher accessible polar segments due to the presence of anthocyanins which resulted in a higher amount of absorbed water molecules [36]. A similar observation was also reported when grapefruit seed extract was added to chitosan film [37]. On the other hand, the results of pH measurements of film solutions showed a lower value after incorporating PCE which can be related to the presence of the functional acidic group of anthocyanin.

3.7. Heat-Sealing Ability. The sealing ability of films is important for food packaging to manufacture sachets and pouches [38]. As shown in Table 1, PCE-loaded film presented significantly ($P < 0.05$) higher heat-sealing strength (83.33 Pa) than control film (81.7 Pa). Interaction of aldehydes, ketones, and anthocyanin with protein and hydroxyl group, additionally, hydrophobic groups of polyphenols are capable to associate with gelatin chains through hydrogen bonds and hydrophobic interaction. These macromolecules by creating cross-linkage and hydrogen bonds in proteins might result in a strong network in the gelatin matrix, resulting in the better heat-sealing ability of PCE-loaded composite film [39].

Table 2: FTIR peak assignments of control Persian gum-gelatin film and active film with 45% purple carrot extract.

Wavenumber (cm^{-1})	Assignments
3284.47	-OH stretching vibrations
2932.71	-CH2- and > CH-stretching and bending vibrations
1655.46	C = C stretching vibration and C = O stretching vibration
1411.83	C = N stretching vibration
1335.46	HCOO-
1238.48	-OH bending vibration
1106.07	C-N (aliphatic amine)
1031.45	C-O-C stretching vibrations of glycosidic bonds
485.37	C-C-O and C-O-C

3.8. Film Thickness. Mechanical properties, heat-sealing ability, and water vapor/oxygen permeability are predominantly influenced by the film thickness. As illustrated in Table. 1, the thickness of PCE-loaded film and control film were 79.33 μm and 80.33 μm, respectively. Accordingly, no significant ($P < 0.05$) difference was observed in thickness value after the incorporation of PCE. This observation suggested a good distribution of PCE in the film matrix. A similar observation was also reported for pectin/chitosan composite films incorporated with purple sweet potato, blueberry, and cranberry extracts [40]. Since numerous OH- groups are

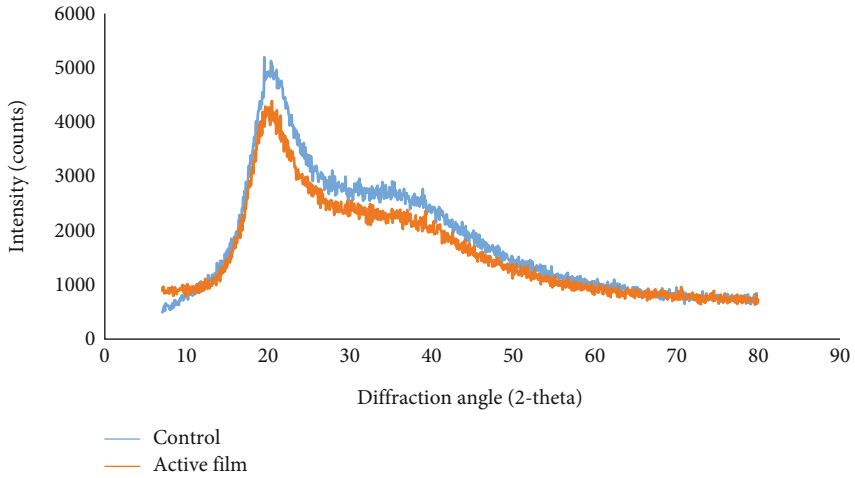


Figure 6: X-ray diffraction pattern of control Persian gum-gelatin film and active film with 45% purple carrot extract.

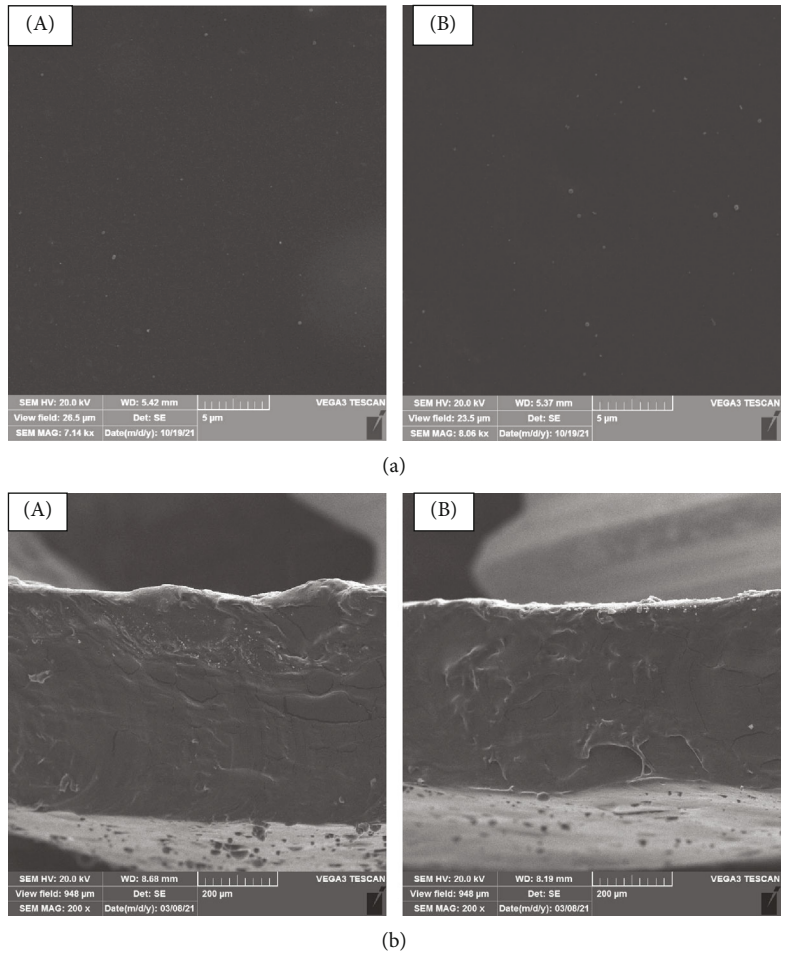


Figure 7: SEM image of surface (a) and cross-section (b) of control Persian gum-gelatin film (A) and active film with 45% purple carrot extract (B).

presented in anthocyanins might result in a firm interaction with gelatin and Persian gum chains in film and a compact network was formed.

3.9. Contact Angle. Contact angle describes the uniformity of surface, wettability, surface free energy, and also the placement of functional groups on the surface which are critical

characteristics in packaging films [41]. As shown in Table 1, the contact angle of PCE-loaded film (76.64°) was significantly ($P < 0.05$) more than the control film (66.2°). All hydrophilic samples have a contact angle of less than 90° and both samples had hydrophilic surfaces. However, less hydrophilicity of PCE-loaded film surface might be due to the dominant anthocyanin content, which led to forming polar sites to interact with Persian gum and gelatin chains, resulting in a lower exposure of biopolymers polar groups for the interaction with water molecules at the surface of films. This phenomenon was also reported with cassava starch-based film with anthocyanin [17].

3.10. FTIR Analysis. FTIR spectra and FTIR peak assignments are presented in Figure 5 and Table 2, respectively. The broad band at 3000-3500 cm^{-1} was attributed to vibrational stretching of hydroxyl groups which reflects the water retention and hydrogen bonding in Persin gum and gelatin [7, 19]. Also, the peak in the range of 2800–3000 cm^{-1} and a peak at 1411 cm^{-1} were mainly ascribed to carbon-hydrogen and C=N bond stretching vibration, respectively [7, 42]. The absorption peak at 1655 cm^{-1} was attributed to the double bond stretching vibrations between carbons (C=C) [24]. The peak around 1031 cm^{-1} might be due to the interaction between hydroxyl groups of plasticizer and film polymers [43]. Moreover, it has been reported that the region in 1600–1700 cm^{-1} is assigned to the amide I spectral region as well as accurate to the secondary structure of proteins which is predominantly a consequence of stretching bonds in C=O of peptide structures [19]. Other peaks at 2932, 1238, and 1020 cm^{-1} might be attributed to carbon-hydrogen and carbon-oxygen single bonds [44]. The peak at 1031 cm^{-1} was also ascribed to the stretching vibration bond of the C–O–C structure [4, 23].

3.11. X-Ray Diffraction (XRD). Figure 6 represents the crystallography patterns obtained from the X-ray diffractometer. The presented broad peak of about 20° implied the amorphous structure of PCE-loaded and control film samples. Since no other significant peak was observed, it can be concluded that no expressive crystalline structure was detected. The peak diffraction of the composite film after incorporating PCE was lower than the control film. This finding was consistent with previous reports in which the diffraction patterns were decreased after the addition of anthocyanins-loaded extract to the film formulations [21, 45]. This fact can be attributed to the disruption of ordered network structures of the biopolymer matrix after incorporating extract [17, 21]. However, some reports showed higher crystallinity of anthocyanin-loaded films [13] which was related to the content and composition of anthocyanins added.

3.12. SEM. Figure 7 shows the surface images of composite films. The surface of the control composite film was relatively compact. Similarly, after the addition of PCE, a uniform/smooth surface without cracks, holes, and insoluble particles was observed, indicating the good compatibility and miscibility of ingredients. The cross-sectional images of two samples show smooth and continuous structure. Also, Gahruie [7] reported homogeneous microstructures of Persian gum/gelatin composite films at pH 4.18 and 5.6.

Figure 8: Color changes of the film containing 45% purple carrot extract at different pH.

3.13. pH Sensitivity of Film. The pH response and ΔE of PCE-loaded film were shown in Figure 8 and Table 3, respectively. Film samples were shown pH-sensitivity and notable color changes as affected by different buffer solutions. By increasing pH values, the color of the film turned from pink to blue/purple. These color changes occur due to the structural changes of anthocyanin at different pHs. Anthocyanins exist basically in cationic form (red or pink flavylium cation) at pH 2–3. The purple color observed at high pH (pH 4–6) due to the generation of carbinol pseudobase and blue-colored observed at pH > 7 due to the formation of quinoidal bases [34]. Other research also showed pH sensitivity of biopolymer-based films incorporated with extracts of purple potato, red cabbage, blackberry pomace, and purple and black eggplant [13]. Changes in color of samples validated by measuring the ΔE of samples (Table 3). The difference ($P < 0.05$) in the amount of ΔE indicates the color change of the film at different pH. The results proved that PCE-loaded film could act as a pH indicator.

Table 3: Color difference's (ΔE) of active Persian gum-gelatin film with 45% purple carrot extract in different pHs against color at pH 2.

pH	L*	a*	b*	ΔE
2	46 ± 1^{a}	35 ± 1^{a}	11 ± 3^{a}	—
3	47 ± 1^{a}	33 ± 1^{b}	5 ± 1^{b}	$6/403^{a}$
4	50 ± 2^{b}	25 ± 2^{c}	4 ± 1^{b}	$8/602^{b}$
5	60 ± 2^{c}	21 ± 1^{d}	3 ± 1^{b}	$10/817^{c}$
6	43 ± 2^{d}	19 ± 1^{e}	2 ± 1^{b}	$17/146^{d}$
7	40 ± 2^{e}	18 ± 1^{e}	0 ± 1^{c}	$3/742^{e}$
8	55 ± 2^{f}	15 ± 1^{f}	-1 ± 1^{d}	$15/330^{f}$
9	57 ± 1^{f}	13 ± 1^{g}	$-1/3 \pm 0/3^{g}$	$2/844^{g}$
10	45 ± 2^{d}	12 ± 1^{h}	$-1/5 \pm 0/2^{g}$	$12/043^{h}$
11	35 ± 2^{g}	11 ± 1^{h}	$-1/8 \pm 0/2^{g}$	$10/054^{i}$
12	38 ± 2^{h}	10 ± 1^{h}	-2 ± 1^{h}	$3/169^{j}$

Different letters in each column indicate significant differences ($P < 0.05$).

4. Conclusion

Since the exploitation of green, nontoxic, and biodegradable packaging materials has been considered a global concern, the design of a proper packaging film to sustain the food product quality and protect it from environmental conditions and degradation is a promising field of study in the food industry. The aim of this study was to prolong the food products shelf-life by the potential of active Persian gum and gelatin composite film incorporated with PCE. The results showed that the antioxidant activity of film was successfully improved by incorporating a high concentration of extract. Moreover, incorporating PCE improved mechanical strength, heat-sealing ability, and moisture absorption. The result suggested that biodegradable films with PCE can be exploited as an assured food packing material with good radical scavenging ability. However, further research is needed to show validate the antioxidant activity and pH sensitivity of PCE-loaded films in real food packaging applications to monitor spoilage.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The financing of this case was provided by the student himself.

References

[1] M. Lotfi, H. Tajik, M. Moradi, M. Forough, E. Divsalar, and B. J. L. Kuswandi, "Nanostructured chitosan/ monolaurin film: preparation, characterization and antimicrobial activity against Listeria monocytogenes on ultrafiltered white cheese," *LWT*, vol. 92, pp. 576–583, 2018.

[2] A. M. Youssef, S. M. El-Sayed, H. S. El-Sayed, H. H. Salama, F. M. Assem, and M. H. Abd El-Salam, "Novel bionanocomposite materials used for packaging skimmed milk acid coagulated cheese (Karish)," *International Journal of Biological Macromolecules*, vol. 115, pp. 1002–1011, 2018.

[3] S. Ahammed, F. Liu, M. N. Khin, W. H. Yokoyama, and F. J. F. H. Zhong, "Improvement of the water resistance and ductility of gelatin film by zein," *Food Hydrocolloids*, vol. 105, article 105804, 2020.

[4] F. Ghiasi, M.-T. Golmakani, M. H. Eskandari, and S. M. H. Hosseini, "A new approach in the hydrophobic modification of polysaccharide-based edible films using structured oil nanoparticles," *Industrial Crops and Products*, vol. 154, article 112679, 2020.

[5] H. Khalesi, B. Emadzadeh, R. Kadkhodaee, and Y. J. F. H. Fang, "Whey protein isolate-Persian gum interaction at neutral pH," *Food Hydrocolloids*, vol. 59, pp. 45–49, 2016.

[6] M. Dabestani, R. Kadkhodaee, G. O. Phillips, and S. Abbasi, "Persian gum: a comprehensive review on its physicochemical and functional properties," *Food Hydrocolloids*, vol. 78, pp. 92–99, 2018.

[7] H. H. Gahruie, A. Mirzapour, F. Ghiasi, M. H. Eskandari, M. Moosavi-Nasab, and S. M. H. Hosseini, "Development and characterization of gelatin and Persian gum composite edible films through complex coacervation," *LWT*, vol. 153, article 112422, 2022.

[8] N. Mahfoudhi, M. Chouaibi, F. Donsi, G. Ferrari, and S. Hamdi, "Chemical composition and functional properties of gum exudates from the trunk of the almond tree (*Prunus dulcis*)," *Food Science and Technology International*, vol. 18, no. 3, pp. 241–250, 2012.

[9] K. Limpisophon and G. Schleining, "Use of gallic acid to enhance the antioxidant and mechanical properties of active fish gelatin film," *Journal of Food Science*, vol. 82, no. 1, pp. 80–89, 2017.

[10] H. Yong, X. Wang, R. Bai, Z. Miao, X. Zhang, and J. J. F. H. Liu, "Development of antioxidant and intelligent pH-sensing packaging films by incorporating purple-fleshed sweet potato extract into chitosan matrix," *Food Hydrocolloids*, vol. 90, pp. 216–224, 2019.

[11] J. Zhang, X. Zou, X. Zhai, X. Huang, C. Jiang, and M. J. F. C. Holmes, "Preparation of an intelligent pH film based on biodegradable polymers and roselle anthocyanins for monitoring pork freshness," *Food Chemistry*, vol. 272, pp. 306–312, 2019.

[12] P. Ongkowijoyo, D. A. Luna-Vital, and E. G. de Mejia, "Extraction techniques and analysis of anthocyanins from food sources by mass spectrometry: an update," *Food Chemistry*, vol. 250, pp. 113–126, 2018.

[13] H. Yong, X. Wang, X. Zhang, Y. Liu, Y. Qin, and J. Liu, "Effects of anthocyanin-rich purple and black eggplant extracts on the physical, antioxidant and pH-sensitive properties of chitosan film," *Food Hydrocolloids*, vol. 94, pp. 93–104, 2019.

[14] S. Akhtar, A. Rauf, M. Imran, M. Qamar, M. Riaz, and M. S. Mubarak, "Black carrot (Daucus carota L.), dietary and health promoting perspectives of its polyphenols: A review," *Trends in Food Science & Technology*, vol. 66, pp. 36–47, 2017.

[15] L. T. Wu, I. L. Tsai, Y. C. Ho et al., "Active and intelligent gellan gum-based packaging films for controlling anthocyanins release and monitoring food freshness," *Carbohydrate Polymers*, vol. 254, article 117410, 2021.

[16] H.-z. Chen, M. Zhang, B. Bhandari, and C.-h. Yang, "Novel pH-sensitive films containing curcumin and anthocyanins to monitor fish freshness," *Food Hydrocolloids*, vol. 100, article 105438, 2020.

[17] T. M. A. R. D. Vedove, B. C. Maniglia, and C. C. Tadini, "Production of sustainable smart packaging based on cassava starch and anthocyanin by an extrusion process," *Journal of Food Engineering*, vol. 289, article 110274, 2021.

[18] J. S. Barnes, H. P. Nguyen, S. Shen, and K. A. Schug, "General method for extraction of blueberry anthocyanins and identification using high performance liquid chromatography-electrospray ionization-ion trap- time of flight-mass spectrometry," *Journal of Chromatography A*, vol. 1216, no. 23, pp. 4728–4735, 2009.

[19] D. Khodaei, K. Oltrogge, and Z. Hamidi-Esfahani, "Preparation and characterization of blended edible films manufactured using gelatin, tragacanth gum and, Persian gum," *LWT*, vol. 117, article 108617, 2020.

[20] H. Lashkari, M. J. Varidi, M. H. Eskandari, and M. Varidi, "Effect of pomegranate juice on the manufacturing process and characterization of feta-type cheese during storage," *Journal of Food Quality*, vol. 2020, Article ID 8816762, 11 pages, 2020.

[21] X. Wang, H. Yong, L. Gao, L. Li, M. Jin, and J. Liu, "Preparation and characterization of antioxidant and pH-sensitive films based on chitosan and black soybean seed coat extract," *Food Hydrocolloids*, vol. 89, pp. 56–66, 2019.

[22] S. M. A. Razavi, A. M. Amini, and Y. J. F. H. Zahedi, "Characterisation of a new biodegradable edible film based on sage seed gum: influence of plasticiser type and concentration," *Food Hydrocolloids*, vol. 43, pp. 290–298, 2015.

[23] H. Dai, S. Ou, Y. Huang, and H. J. C. Huang, "Utilization of pineapple peel for production of nanocellulose and film application," *Cellulose*, vol. 25, no. 3, pp. 1743–1756, 2018.

[24] S. D. Tabatabaei, F. Ghiasi, H. H. Gahruie, and S. M. H. Hosseini, "Effect of emulsified oil droplets and glycerol content on the physicochemical properties of Persian gum-based edible films," *Polymer Testing*, vol. 106, article 107427, 2022.

[25] L. A. Muñoz, J. M. Aguilera, L. Rodriguez-Turienzo, A. Cobos, and O. Diaz, "Characterization and microstructure of films made from mucilage of salvia hispanica and whey protein concentrate," *Journal of Food Engineering*, vol. 111, no. 3, pp. 511–518, 2012.

[26] I. Choi, J. Y. Lee, M. Lacroix, and J. J. F. C. Han, "Intelligent pH indicator film composed of agar/potato starch and anthocyanin extracts from purple sweet potato," *Food Chemistry*, vol. 218, pp. 122–128, 2017.

[27] ASTM, Inernational, *Standard test methods for flexural properties of unreinforced and reinforced plastics and electrical insulating materials*, ASTM D790-07, 2007.

[28] S. Rbihi, A. Aboulouard, L. Laallam, and A. Jouaiti, "Contact Angle Measurements of Cellulose based Thin Film composites: wettability, surface free energy and surface hardness," *Surfaces and Interfaces*, vol. 21, article 100708, 2020.

[29] R. Priyadarshi, B. Kumar, F. Deeba, A. Kulshreshtha, and Y. S. J. F. H. Negi, "Chitosan films incorporated with apricot (*Prunus armeniaca*) kernel essential oil as active food packaging material," *Food Hydrocolloids*, vol. 85, pp. 158–166, 2018.

[30] A. Acevedo-Fani, L. Salvia-Trujillo, M. A. Rojas-Graü, and O. Martín-Belloso, "Edible films from essential-oil-loaded nanoemulsions: physicochemical characterization and antimicrobial properties," *Food Hydrocolloids*, vol. 47, pp. 168–177, 2015.

[31] M. Kurek, I. E. Garofulić, M. T. Bakić, M. Ščetar, V. D. Uzelac, and K. Galić, "Development and evaluation of a novel antioxidant and pH indicator film based on chitosan and food waste sources of antioxidants," *Food Hydrocolloids*, vol. 84, pp. 238–246, 2018.

[32] Y.-Y. Qin, Z.-H. Zhang, L. Li, M.-L. Yuan, J. Fan, and T.-R. Zhao, "Physio-mechanical properties of an active chitosan film incorporated with montmorillonite and natural antioxidants extracted from pomegranate rind," *Journal of Food Science and Technology*, vol. 52, no. 3, pp. 1471–1479, 2015.

[33] S. R. Kanatt, M. S. Rao, S. P. Chawla, and A. Sharma, "Active chitosan-polyvinyl alcohol films with natural extracts," *Food Hydrocolloids*, vol. 29, no. 2, pp. 290–297, 2012.

[34] M. Koosha and S. Hamedi, "Intelligent chitosan/PVA nanocomposite films containing black carrot anthocyanin and bentonite nanoclays with improved mechanical, thermal and antibacterial properties," *Progress in Organic Coatings*, vol. 127, pp. 338–347, 2019.

[35] M. Mushtaq, A. Gani, A. Gani, H. A. Punoo, F. J. I. F. S. Masoodi, and E. Technologies, "Use of pomegranate peel extract incorporated zein film with improved properties for prolonged shelf life of fresh Himalayan cheese (Kalari/kradi)," *Innovative Food Science & Emerging Technologies*, vol. 48, pp. 25–32, 2018.

[36] T. J. Gutiérrez, L. A. Toro-Márquez, D. Merino, and J. R. J. F. H. Mendieta, "Hydrogen-bonding interactions and compostability of bionanocomposite films prepared from corn starch and nano-fillers with and without added Jamaica flower extract," *Food Hydrocolloids*, vol. 89, pp. 283–293, 2019.

[37] J. F. Rubilar, R. M. S. Cruz, H. D. Silva, A. A. Vicente, I. Khmelinskii, and M. C. Vieira, "Physico-mechanical properties of chitosan films with carvacrol and grape seed extract," *Journal of Food Engineering*, vol. 115, no. 4, pp. 466–474, 2013.

[38] N. Samsalee and R. Sothornvit, "Characterization of food application and quality of porcine plasma protein–based films incorporated with chitosan or encapsulated turmeric oil," *Food and Bioprocess Technology*, vol. 13, no. 3, pp. 488–500, 2020.

[39] P. Tongnuanchan, S. Benjakul, T. Prodpran, S. Pisuchpen, and K. Osako, "Mechanical, thermal and heat sealing properties of fish skin gelatin film containing palm oil and basil essential oil with different surfactants," *Food Hydrocolloids*, vol. 56, pp. 93–107, 2016.

[40] B. R. Machado, *Desenvolvimento de filmes de pectina/quitosana/glicerol para produção de embalagens biodegradáveis para alimentos*, Universidade Tecnológica Federal do Paraná, 2019.

[41] A. A. Motedayen, F. Khodaiyan, and E. A. Salehi, "Development and characterisation of composite films made of kefiran and starch," vol. 136, no. 3-4, pp. 1231–1238, 2013.

[42] F. Ghiasi and M.-T. Golmakani, "Fabrication and characterization of a novel biphasic system based on starch and ethylcellulose as an alternative fat replacer in a model food system," *Innovative Food Science & Emerging Technologies*, vol. 78, article 103028, 2022.

[43] K. Nilsuwan, S. Benjakul, and T. J. F. H. Prodpran, "Physical/thermal properties and heat seal ability of bilayer films based on fish gelatin and poly (lactic acid)," *Food Hydrocolloids*, vol. 77, pp. 248–256, 2018.

[44] S. M. Eskandarabadi, M. Mahmoudian, K. R. Farah, A. Abdali, E. Nozad, and M. Enayati, "Active intelligent packaging film based on ethylene vinyl acetate nanocomposite containing extracted anthocyanin, rosemary extract and ZnO/Fe- MMT nanoparticles," *Food Packaging and Shelf Life*, vol. 22, article 100389, 2019.

[45] X. Zhai, J. Shi, X. Zou et al., "Novel colorimetric films based on starch/polyvinyl alcohol incorporated with roselle anthocyanins for fish freshness monitoring," *Food Hydrocolloids*, vol. 69, pp. 308–317, 2017.