Journal of Food Quality

Organic Film and Coating for Improving Food Quality

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Research Article

Preparation and Characterization of Biodegradable Composited Films Based on Potato Starch/Glycerol/Gelatin

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The use of plastics is resisted worldwide. Therefore, finding alternatives to plastic packaging products is an urgent issue. This work was dedicated to the preparation of biodegradable composited films with potato starch, glycerol, and gelatin. The formulation of the biodegradable film was first optimized via response surface methodology combined with the multi-index comprehensive evaluation method that considered physical properties (thickness, water solution (WS), tensile strength (TS) and elongation at break (*E*%)) and barrier property (light transmittance (*T*%)). Results indicated that the optimal conditions were 2.5% starch, 2.0% glycerol, and 1.5% gelatin (based on water). The optimized film presented excellent properties with TS of 4.47 MPa, *E*% of 109.91%, WS of 43.64%, and *T*% of 41.21% at 500 nm, and the comprehensive evaluation score of the composite film was 28.68. Moreover, a model verification experiment was further conducted, which proved that the predicted value highly matched experimental values, indicting the credibility and accuracy of the model. The resulting films were further characterized on the basis of rheological measurements, Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The rheological measurements proved that the film-forming solution exhibited low shear viscosity and non-Newtonian fluid behavior. FTIR and SEM revealed excellent compatibility among starch, glycerol, and gelatin. Hence, the resulting optimized film may be expected to provide theoretical basis and technical support for the food packing industry.

1. Introduction

In the food industry, food packaging is required to enhance food protection and guarantee shelf life on the basis of their appropriate physicochemical features [1, 2]. Petroleum-based plastic packaging is still widely used due to its cost-effectiveness. However, with the worsening of environmental issues caused by nonrenewable and nondegradable plastic packaging and the serious harm to human health due to the accumulation of plastic waste [3, 4], some prohibitions about petroleum-based plastic packaging have been enforced. Internationally, the European Chemicals Agency has limited the use of conventional synthetic plastic polymers for food packaging [5]. In the domestic setting, plastic waste materials have been prohibited from importation due to their toxicity [6, 7]. Therefore, to overcome these issues, replacing traditional plastic packaging with renewable, biodegradable materials has attracted considerable attention in recent decades. Generally, biodegradable packaging films were produced by natural biodegradable materials included polysaccharides, proteins, lipids, and their complexes, which make up for the drawbacks of plastic and solve those root environmental issues caused by plastic waste. Among these natural biodegradable films, starch-based films have incomparable advantages included abundant, low-cost, high film transparency, over other food packaging materials, which has been proved that starch-based film has exceed 60% of the total biodegradable film [8].

Starch is a highly annually renewable and high-molecular-weight polymer material that is composed of two types of α-glucan, namely amylose and amylopectin [9, 10]. Amylose is a linear polysaccharide that is connected by α-1,4-glycosidic bonds, and that presents excellent film-forming property similar to fibers [11]. Moreover, the α-1,4-glycosidic bonds and α-1,6-glycosidic bonds present in amylopectin are conducive to the thickening effect and freeze-thaw stability [12]. The proportion of amylose and amylopectin in various sources of starch is also different and directly affects film-forming properties [13, 14]. Therefore, increasing efforts have been devoted to the research of various resources of starch-based films [9, 15, 16]. The maize starch matrix film was produced and it was found that the films possessed low tensile strength (TS) and water vapor permeability (WVP), which could be enhanced by zein blended, and great compatibility with maize starch matrix [10]. Chollakup et al. [17] investigated cassava starch film, who proved the hydrophobic interactions and hydrogen bonding present in cassava starch and whey protein blended films to explain their barrier mechanisms at the molecular level. Lin et al. [18] utilized cellulose nanocrystals and PVA (polyvinyl alcohol)/glycerol to modify the formability of oxidized starch films with excellent WVP, surface free energy, and oxygen resistance. The characteristic properties of starch–PLA (polylactic acid) composite films [19] significantly improved with the use of cellulosic strengthening agents (cellulosic fibers and cellulose nanocrystals) and coffee husk extracts to enhance tensile properties, reduced barrier permeability, and endowed additional antioxidant capacity to food packaging systems. These results indicated that the research on starch-based film from various resources had been continued, and the contribution of physicochemical and mechanical properties by a single starch is limited and needed to blend with other components to acquire desirable performances for improving product texture and resistance to various physical changes.

Furthermore, as potato staple food strategy initiated, potatos have been the largest noncereal food crop worldwide [20], and potato planting area expanded 6.7 million hectares (2020) [21]. The yield of potato starch also increased up to 96 million tonnes (2016) [22], which has been a promising natural carbohydrate for food packing material. The size of potato starch granules is relatively large (25–100 μm), and exists in typile B-type crystallinity [23]. Also, in the presence of plasticizers and under high temperature and shearing conditions, potato starch would acquire thermoplastic property which was suitable for preparing biodegradable film [24]. However, the performance of potato starch-based films still needs to be improved to extend the application in food package because of their poor water resistance. To address such issue, plasticizing agents and other active ingredients were incorporated to endow various advantages to films. Glycerol has been reported as the most starch-compatible plasticizing agent that can endow polymers with flexibility and resilience [25, 26]. Moreover, gelatin, a protein of animal origin, exhibits excellent thermoreversible capability and thermal stability, presenting possible barrier performance [27, 28].

Although a range of various composite films has been investigated, few pieces of research were conducted about the optimized formulation of the composite film via response surface methodology (RSM) combined with the multi-index comprehensive evaluation method that considered physical properties (thickness, water solution (WS), tensile strength (TS), and elongation at break (E%)) and barrier property (light transmittance (T%)). Thus, this study aimed to prepare a durable biodegradable film with excellent comprehensive performance based on potato starch, glycerol, and gelatin. The optimized films were further characterized by scanning electron microscopy (SEM), rheological measurements, and Fourier transform infrared spectroscopy (FTIR).

2. Materials and Methods

2.1. Materials. Potato starch was purchased from a local market in Baoding, China. Glycerol was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China). Edible gelatin, extracted from pig skin, was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Preparation of Complex Films. Potato starch was dispersed in 100 mL of distilled water and stirred for 30 min at 80°C until completely gelatinized. Glycerol and gelatin were successively added to the film-forming solution. The mixtures were degassed for another 30 min by using an ultrasonic device (SK5200H, Ningbo Scientz Biotechnology Co., Ltd. Zhejiang, China), then casting onto a plate, and dried at 40°C for 36 h in an incubator [29]. After this period, the films were peeled for further analysis.

2.3. Comprehensive Score Calculation. Membership degree and principal component analysis (PCA) were used to evaluate the films comprehensively. In accordance with importance, the indexes of the films were normalized to optimize the production process. Each index weight was determined. Indexes with high membership, such as TS and elongation at break (E%), were calculated using equation (1). WS (water solution) and light transmittance (T%) were calculated with equation (2):

$$P = \frac{A_i - A_{\min}}{A_{\max} - A_{\min}}, \tag{1}$$

$$P = \frac{A_{\max} - A_i}{A_{\max} - A_{\min}}, \tag{2}$$

where P is the index membership degree; A_i is the actual value; $A_{\min}$ is the minimum of the same index; $A_{\max}$ is the maximum of the same index.

The comprehensive score (Y) was calculated with the following equation:

$$\begin{aligned} Y = {} & a_1 \times P_1 + a_2 \times P_2 + a_3 \times P_3 \\ & + a_4 \times P_4 + a_5 \times P_5, \end{aligned} \tag{3}$$

where a_1, a_2, a_3, a_4, and a_5 are the weights of thickness, TS, E%, WS, and T%, respectively, and P_1, P_2, P_3, P_4, P_5 are membership degrees of thickness, TS, E%, WS, and T%, respectively.

2.4. Design of Experiments. Film concentration ratios were optimized by using RSM to investigate the synergistic effect of the variables. Three-level (starch concentration X_1 [1–6 g/100 mL], glycerol concentration X_2 [0.5–3 g/100 mL], and gelatin concentration X_3 [0.5–3 g/100 mL]) factorial design was applied to investigate the effects of the parameters on WVP, TS, T%, and E%. After preliminary tests, the appropriate levels of the independent variables needed to obtain the optimal characteristic properties of the biodegradable composite film were measured.

A total of 17 formulas with five replicates at the center point were evaluated to ensure the repeatability of the RSM model. These formulas are listed in Table 1, which clearly records the coded and uncoded levels of starch, glycerol, and gelatin concentrations. The response for each formulation was independently evaluated with three replicates. A random experimental design was used to eliminate the effects of possible uncontrolled factors.

2.5. Verification Test. Verification procedures were applied to determine the optimal level of composite films with excellent properties. The experimental data and fitted value predicted by the model were compared to verify the validity and adequacy of the RSM model.

2.6. Acquisition of Light Transmission Curves. UV-vis spectrophotometer (α-1500, Shanghai, China) was used to acquire the light transmittance spectra of the complex films. Briefly, the film was cut into strips (1 × 4 cm) and placed closely to the inner surface of the cuvette to record the T% with three replicates ranging from 400 nm to 800 nm.

2.7. Rheology of Film-Forming Solutions. The static rheological properties of the film-forming solutions were determined by using a rotational rheometer (RheolabQC, Anton Paar Instruments Ltd., Japan) equipped with a concentric cylinder (CC 39, 40 mm diameter, Austria) and temperature control system (TEZ 150P-C). The shear rate was varied from 1 s^{-1} to 300 s^{-1} with the Ramp log procedure at 25°C and the flow behavior was recorded every 30 s.

The rheological properties of films were further stimulated by the Cross model [30]:

$$\eta = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{1 + (K\gamma)^{\text{Þ}}}, \quad (4)$$

where η, η_0, and η_{∞} represented viscosity, the initial shear viscosity, and infinite shear viscosity, respectively. γ indicated shear rate (s^{-1}), and K and Þ were time constant and dimensionless exponent, respectively.

2.8. Physical Properties. The thicknesses of the films was recorded by using an electronic thickness gauge (EC770, Shanghai, China) at 10 random positions. An autotensile tester (PARAM XLW-PC, Jinan, China) was used to measure the TS and elongation at break (E%) of the films at a cross-head speed of 300 mm/s [31]. T% was measured with a UV-vis spectrophotometer at 500 nm.

WVP of the film was measured by the gravimetric method [32]. The film (8 cm × 8 cm) was sealed in a circular cup with 20 g anhydrous $CaCl_2$ (0% RH (relative humidity)). The cups were placed inside of desiccator with distilled water (100% RH). The difference in RH promoted the transport of water vapor, which caused weight changes.

WS was tested according to the modified weight loss method [33] which was performed with three replicates to determine the WS of films. Briefly, at room temperature, a film (2 cm × 2 cm) was weighed prior to dissolution in distilled water for 24 h and then removed and dried at 90°C to a constant weight. WS was calculated by the following equation:

$$\text{WS} = \frac{M_1 - M_2}{M_1} \times 100\%, \quad (5)$$

where M_1 and M_2 are the initial and terminal weights of the films (dry basis), respectively.

2.9. Morphology of Films. The microstructures of cross-sections and surfaces were measured by using a scanning electron microscope (JSM-7900F, Japan) with an accelerating voltage of 12.5 kV. Before the observation, the films were coated with a thin layer of gold.

2.10. FTIR Spectroscopy. FTIR spectra were acquired over the range of 500–4000 cm^{-1} in attenuated total reflection mode with a resolution of 4 cm^{-1} by using an FTIR spectrometer (Shimadzu Instrument Co. Ltd., Japan).

3. Results and Discussion

3.1. Light Transmission Analysis. The light transmittance of the film is an important index to directly affect consumer acceptance. And the light transmission curve of a resulting optimized film (starch + glycerol + gelatin) is shown in Figure 1(a). The remarkable UV barrier capability shown by the film over the UV range of 200–280 nm would protect food from oxidative deterioration [32]. This characteristic was due to the intermolecular hydrogen bond between starch and glycerol during the film-forming process, resulting in good miscibility [34]. The optical property of film is significantly reinforced with wavelength increasing. Moreover, over the visible range (at 500 nm), the T% of films reached the maximum value then remained constant at approximately 90%, indicating excellent transparency. Such high transmittance indicated the uniform of film. Perfectly clear food packaging would not adversely affect the consumer experience. The picture covered by these films could be clearly observed (Figure 1(b)), and further proved good transmittance.

Table 1: Experiments design with coded and uncoded independent variables.

Run	Code variables			Uncoded variables		
	X_1	X_2	X_3	Starch concentration	Glycerol concentration	Gelatin concentration
1	1.000	0.000	1.000	4	2	2
2	0.000	1.000	−1.000	3	2.5	1
3	0.000	0.000	0.000	3	2	1.5
4	0.000	1.000	1.000	3	2.5	2
5	−1.000	0.000	1.000	2	2	2
6	0.000	0.000	0.000	3	2	1.5
7	−1.000	0.000	−1.000	2	2	1
8	−1.000	1.000	0.000	2	2.5	1.5
9	−1.000	−1.000	0.000	2	1.5	1.5
10	0.000	0.000	0.000	3	2	1.5
11	0.000	0.000	0.000	3	2	1.5
12	1.000	0.000	−1.000	4	2	1
13	0.000	−1.000	−1.000	3	1.5	1
14	1.000	−1.000	0.000	4	1.5	1.5
15	0.000	0.000	0.000	3	2	1.5
16	0.000	−1.000	1.000	3	1.5	2
17	1.000	1.000	0.000	4	2.5	1.5

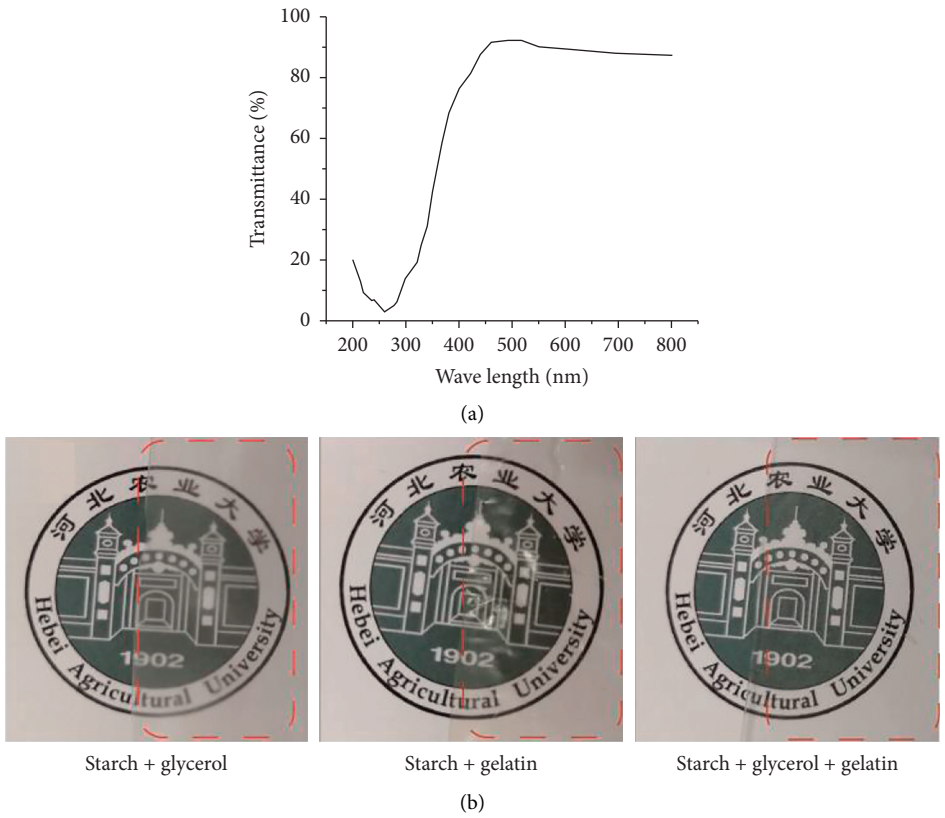


Figure 1: (a) The light transmission curve. (b) The pictures of the films.

3.2. Comprehensive Evaluation of the Composite Film. PCA was performed by using SPSS19.0 software on six randomly selected sets of data (Table 2) to evaluate film comprehensive performance. According to the criterion of eigenvalues exceeding 1, the results of PCA (Table 3) showed that the three principal components (PC1, PC2, and PC3) are extracted and the variance contributions of PC1, PC2, and PC3 were 40.425%, 29.065%, and 20.892%, respectively. In addition, the cumulative variance of the three components reached 90.382%, indicating that PC1, PC2, and PC3 could contain most of the information (more than 90%) of all indexes (TS, *E*%, WS, thickness, and *T*%) and reflect the comprehensive performance of the films. Therefore, PC1, PC2, and PC3 could replace the original complex indexes.

Table 2: Test data randomly selected for principal component analysis.

Indexes	Thickness (mm)	*E*%	TS (MPa)	*T*%	WS %
1	0.071	40	3.350	0.284	0.2674
2	0.060	198.95	4.130	0.414	0.4343
3	0.066	102.15	11.640	0.298	0.3256
4	0.073	66.17	1.270	0.439	0.4331
5	0.070	166.2	1.987	0.123	0.3496
6	0.066	198.95	2.967	0.361	0.3197

Table 3: Eigenvalues and variance contribution rates of related components.

Principal components	Eigenvalue	Variance contribution rate (%)	Cumulative variance contribution rate (%)
PC1	2.021	40.425	40.425
PC2	1.453	29.065	69.489
PC3	1.045	20.892	90.382
PC4	0.388	7.766	98.148
PC5	0.093	1.852	100.000

Table 4 lists the factor loading matrix and the contribution rate of thickness, TS, E%, and WS to PC1, PC2, and PC3 (eigenvalue > 1). As shown in Table 4, thickness, *E*%, and WS mainly affected PC1; TS mainly affected PC2; *T*% and TS mainly affected PC3. After normalization, the percentages of thickness, TS, *E*%, WS, and *T*%, were 13%, 34%, 18%, 30%, and 5%, respectively. The comprehensive score (Y) was calculated by using the following equation in accordance with equations (1)–(3):

$$Y = 13\% \times P_1 + 34\% \times P_2 + 18\% \times P_3 + 30\% \times P_4 + 5\% \times P_5. \quad (6)$$

3.3. RSM Analysis. RSM was applied to investigate the linear, quadratic, and interaction effects of each independent variable (starch, glycerol, and gelatin concentrations) on response values. All the response experimental data are listed in Table 5.

The different evaluation indexes, including the F value, the lack-of-fit value, and the correlation coefficient R^2, are presented in Table 6 to verify the model's adequacy and accuracy. The predictive model was extremely significant ($p < 0.01$). A low p value indicates the high significance and applicability of the regression model. Moreover, the p values of the linear (X_1), quadratic (X_{13}), and interactive (X_{12}, X_{13}, and X_{23}) models were all less than 0.05, which indicated that the model fits well with the measured data. R^2 and adj-R^2 were used to prove the adequacy and goodness of fit of the applied model. A high R^2 value ($R^2 > 0.9$) indicated the high reliability of the models and acceptable error between the calculated and experimental results. The insignificance between R^2 and R^2-adj indicated the adequate accuracy and applicability of the selected polynomial model. The response comprehensive score (Y) was fitted with the regression equation as shown in the following equation:

$$Y = 50.96 - 7.92\,X_1 + 4.12\,X_2 + 2.71\,X_3 + 0.012\,X_1X_2 - 5.48\,X_1X_3 + 0.95\,X_2X_3 - 8.20\,X_{12} - 10.53\,X_{22} - 8.70\,X_{32}. \quad (7)$$

3D response surfaces were used (Figure 2) to further interpret the effects of interactions among independent variables (starch, glycerol, and gelatin concentrations) on response value. The closer the bottom curve, which is the projection of the 3D response surface, to the ellipse, the more obvious the interaction effect and vice versa. In accordance with Table 5, the figure shows significant interaction between X_1 and X_2 and no significant interaction between X_1 and X_2 and between X_2 and X_3. Such results further indicated that the RSM model was reliable and veritable.

3.4. Verification Experiments. Verification experiments on the optimal variable concentrations (2.40% starch, 2.11% glycerol, and 1.68% gelatin) and experimental concentrations (2.5% starch, 2.0% glycerol, and 1.5% glycerin) were performed to validate the reliability and validity of the model equations. The results of the verification experiments are shown in Table 7. The acceptable percentage error between the predicted value and experimental value further demonstrated the reasonable adequacy of the response surface equations for responses and the reliability of the experiments.

Figure 3(a) depicts the variation curve of viscosity with the shear rate of 3 film solutions. Film thickness and uniformity are affected by the fluidity (viscosity) of film-forming solutions. The figure shows that the viscosity of the film-forming solutions decreased with the increase in shear rate, exhibiting non-Newtonian fluid behavior that might be attributed to the rearrangement of entangled molecules under high-speed shear [35, 36]. Such a result was in accordance with the finding of Glusac et al. [37], who proved that potato starch and zein proteins exhibited shear-thinning behavior. And the rheological behavior of the three films was successfully fitted by the Cross model (Table 8). The p value of all films was lower than 1, which further proved the pseudoplastic fluids. K-value was related to the destruction of the molecular structure inside the fluid [30]. And the three-component film showed the smallest K-value, which indicated the least time needed to form a new network structure and hydrogen bonding [38].

Table 4: The factor loading matrix of three components.

Index	Principal components			Percentage (%)
	1	2	3	
T%	0.514	−0.538	0.575	13
Thickness(mm)	0.844	0.475	0.062	34
E%	0.772	0.313	−0.520	18
TS (MPa)	0.038	0.705	0.662	30
WS (%)	0.668	−0.586	0.041	5

– indicated negative correlative.

Table 5: Experiments data of the responses.

Runs	Response					
	Thickness (mm)	*E*%	TS(MPa)	*T*% (500 nm)	Water solubility%	Comprehensive scores (*Y*)
1	0.076	87.633	9.873	36.4	52.6	25.90
2	0.057	121.033	4.630	44.9	60.7	33.04
3	0.071	138.300	2.217	38.0	51.3	36.77
4	0.073	109.800	9.223	31.1	52.2	31.46
5	0.079	101.733	10.800	33.1	58.5	29.85
6	0.096	115.433	5.517	35.5	58.1	31.73
7	0.084	66.900	9.573	36.1	46.7	20.41
8	0.097	88.133	11.207	31.8	31.8	26.39
9	0.056	157.400	2.327	52.8	35.8	41.77
10	0.096	169.600	6.800	34.9	55.8	46.30
11	0.079	195.300	18.230	38.7	60.2	56.35
12	0.095	184.300	7.917	32.3	52.1	50.44
13	0.082	60.867	22.400	32.2	42.3	22.68
14	0.101	201.700	6.045	37.3	65.1	54.44
15	0.084	173.200	6.907	35.5	53.6	47.26
16	0.096	144.250	5.985	50.5	46.1	39.47
17	0.056	197.733	3.750	48.9	48.7	52.70

Each value represents the mean value of three determinations.

Table 6: ANOVA study for the model fitting.

Source	SS	Df	*F* value	*p* value	Significant
Model	2010.48	9	11.31	0.0021	**
X_1	501.49	1	25.39	0.0015	**
X_2	135.80	1	6.88	0.0343	*
X_3	58.54	1	2.97	0.1288	
X_1X_2	0.000625	1	$3.16E-05$	0.9957	
X_1X_3	120.01	1	6.08	0.0431	*
X_2X_3	3.59	1	0.188	0.6826	
X_{12}	282.79	1	14.32	0.0069	**
X_{22}	466.45	1	23.62	0.0018	**
X_{32}	318.35	1	16.12	0.0051	**
Residual	138.25	7			
Lack-of-fit	61.41	3	1.07	0.4571	
Pure error	76.84	4			
Cor. total	2148.73	16			
		$R^2 = 0.9357$	$R^2_{Adj} = 0.8529$		

*represented significant ($p < 0.05$), **represented extremely significant ($p < 0.01$).

The different viscosities of the film-forming solutions indicated different entanglement networks [30]. In principle, film-forming solutions become viscous with the increase in solute concentration. However, Figure 3(a) indicates that the film solution of the three mixed matrixes (starch + glycerol + gelatin) showed low viscosity. This result was consistent with Hussain et al. [39], who believed that the viscosity decrease could be attributed to the alteration in the macromolecular organization of the blended gelatin at a high shear rate.

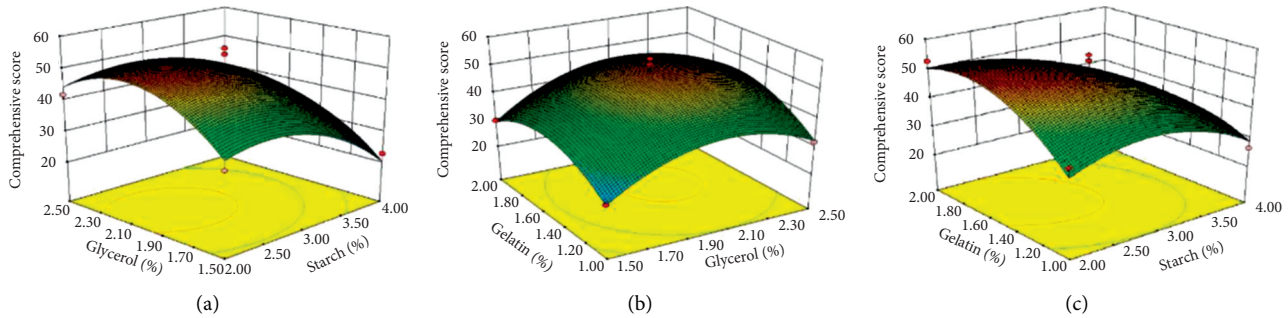


Figure 2: 3D responses surfaces.

Table 7: Predicted and experimental data for the responses at the optimum point.

Indexes	Predicted value	Experimental value	Percentage error (%)
T%	44.37	41.21 ± 0.13	7.12
Thickness (mm)	0.073	0.071 ± 0.02	2.73
E%	102.15	109.91 ± 2.31	7.59
TS (MPa)	4.52	4.47 ± 1.21	1.11
WS %	39.13	43.64 ± 0.03	11.53
Comprehensive score	27.48	28.68 ± 0.79	4.35

Rheology of film-forming solutions.

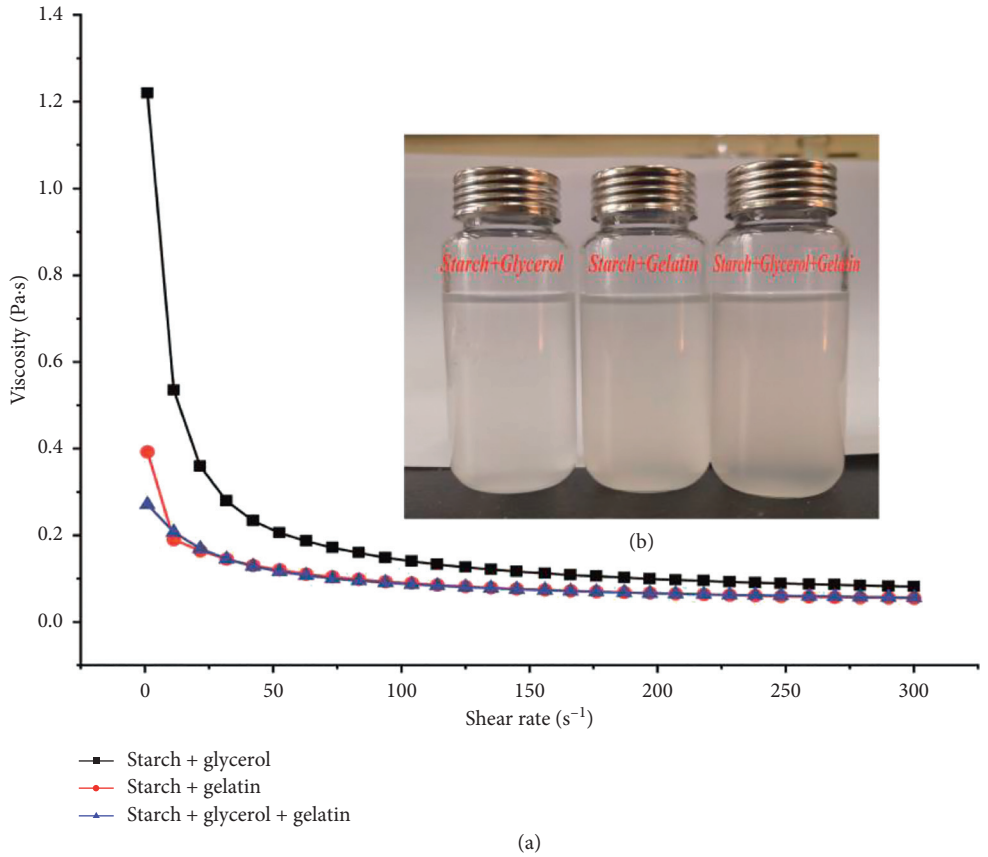


Figure 3: (a) Shear viscosity curves. (b) Pictures of film-forming solutions.

3.5. The Physical Properties of Films. The physical properties including thickness, TS, *E*%, *T*%, WS, and WVP of the three films were listed in Table 9. The optimized film (starch + glycerol + gelatin) showed high thickness due to the blend of three components. Meanwhile, lower WVP of the optimized three-component film was possibly attributed to

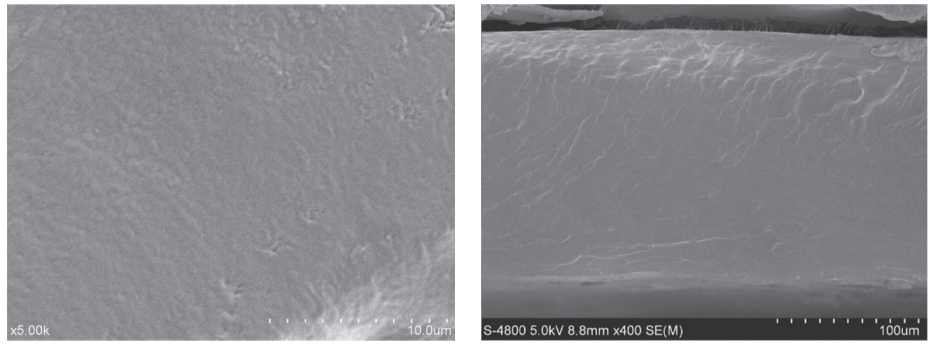
Table 8: The correlation coefficient fitted by the Cross model.

Sample	η_0 (Pa·s)	K (s)	p	R^2
Starch + glycerol + gelatin	0.28202 ± 0.00117	$0.03936 \pm 4.68937E-4$	0.98729 ± 0.0147	0.99975
Starch + glycerol	1.46702 ± 0.01155	0.18258 ± 0.00453	0.91593 ± 0.01207	0.99991
Starch + gelatin	0.68782 ± 0.11894	0.55409 ± 0.38086	0.47097 ± 0.06955	0.99778

Table 9: The physical performance of the optimized film.

Sample	Thickness (mm)	TS (MPa)	E%	T%	WS (%)	WVP ($\times 10^{-10}$ gs^{-1}m^{-1} Pa^{-1})
Starch + glycerol + gelatin	0.071 ± 0.02^a	4.47 ± 1.21^b	109.91 ± 2.31^b	41.21 ± 0.13^a	43.64 ± 0.03^a	1.50 ± 0.02^c
Starch + glycerol	0.064 ± 0.01^b	4.83 ± 0.5^a	138.32 ± 11.94^a	41.12 ± 0.34^a	44.83 ± 0.31^c	1.56 ± 0.04^b
Starch + gelatin	0.068 ± 0.03^b	4.15 ± 0.07^c	89.62 ± 3.64^c	41.09 ± 0.18^a	43.77 ± 0.53^b	1.67 ± 0.02^a

Different letters indicated significant ($p < 0.05$).



(a) (b)

Figure 4: The micrographs of optimized complex film (a) Surface. (b) Cross section.

the forming of intermolecular hydrogen bonds between glycerol and starch with the matrix, resulting in strong interfacial adhesion [16] and lower WS indicated water resistance enhanced. Meanwhile, the lower TS and E% of the three-component film may probably be due to the agglomerates formed by the incorporation of gelatin, which disrupted the compactness of the hydrogen bonds [40]. Moreover, the T% showed no significance, which may be explained by the great miscibility of three components.

3.6. SEM Analysis. As shown in Figure 4, SEM was applied to observe the microstructures of the surface and cross section of the optimized film. The smoothness and homogeneity of the surfaces presented in Figure 4(a) indicated that the components dispersed well within each other. The appearance of surface wrinkles did not mean that the film was uneven and discontinuous. Such wrinkles might be caused by adhesion between the film and the plastic plate when the film was peeled off. Additionally, as shown in Figure 4(b), distinctive ridged structures were presented in the cross-sections of the composite biodegradable film. The high uniformity of the biodegradable film was attributed to the plasticizing capability of glycerol and the thickening capability of gelatin. Furthermore, the numerous intermolecular hydrogen bonds that formed between hydroxyl groups in glycerol with the hydroxyl groups present in starch promoted close bonding in polymers [41] and further enhanced miscibility among starch, gelatin, and glycerol.

3.7. FTIR Analysis. The spectra of the single starch film and optimized biocomposite films are shown in Figure 5. Typically, the broadening of the O–H and the decreasing of wavenumber could be possible due to the characteristic of hydrogen bonding interaction in the film [42]. For the control film (single starch film), the characteristic band around 3267 cm^{-1} was originally from the O–H stretching vibration, and the band absorption intensity was broadened with incorporation with gelatin and glycerol. Also, the characteristic peak at 1644 cm^{-1} slightly shifts to 1636 cm^{-1}. This phenomenon indicated the formation of hydrogen bonds.

The similar characteristic peaks at approximately 2940 and 2875 cm^{-1} in the three biocomposite film spectra were attributed to C–H vibration and C=C vibration, respectively [43]. The peaks at 1636 and 1023 cm^{-1} represented the C=O and C–O stretching vibrations of glycoside bonds in the starch branch. The intensity of the peak at 1023 cm^{-1} increased with the incorporation of glycerol, and the enhancement in the –OH stretching vibration at approximately 3267 cm^{-1} suggested the occurrence of association [44]. Cross-linking between polymers tightened in the presence of intermolecular hydrogen and hydrogen-oxygen bonds (Figure 6). Such results illustrated the good

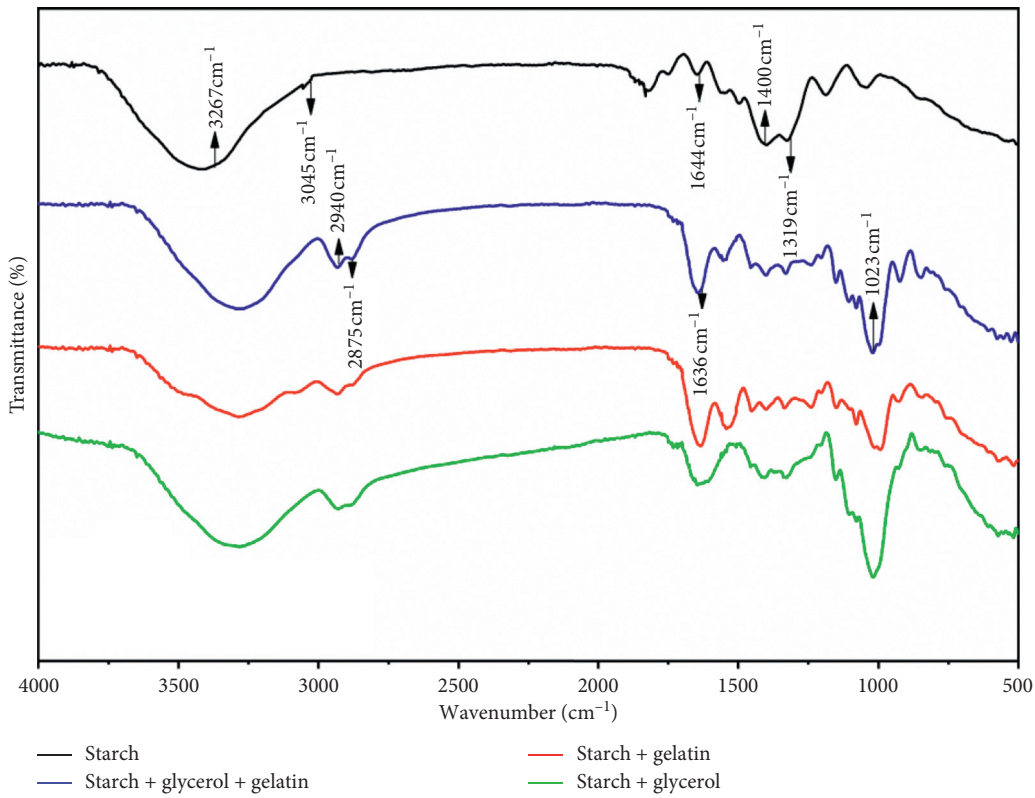


Figure 5: FTIR spectra of optimized films.

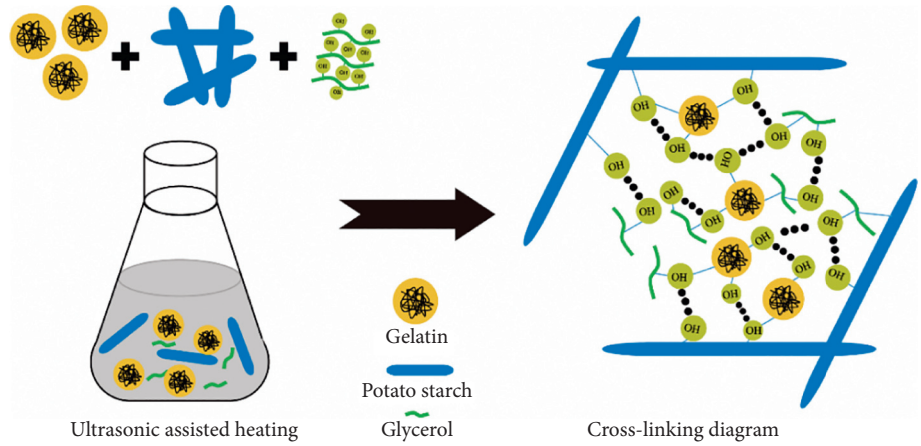


Figure 6: The schematic diagram of composite film.

compatibility between matrixes and were consistent with the SEM and rheology measurements.

4. Conclusions

A comprehensive performance film was successfully fabricated with biodegradable materials (starch, glycerol, and gelatin), and its formulation was optimized by using RSM. Responses, including mechanical, physical, and barrier properties, were subjected to a comprehensive multi-index evaluation. The RSM results verified the reliability of the model, and the optimized film exhibited excellent performance. In addition, the verification result indicated that the experimental values were highly consistent with the model-predicted values, and the optimal preparation conditions were 2.5% starch, 2% glycerol, and 1.5% gelatin. Rheological measurements proved that the film-forming solution exhibited non-Newtonian fluid behavior with low shear viscosity. Moreover, FTIR and SEM analysis demonstrated that the optimized films showed homogeneous with smooth microstructures, indicating good compatibility. In this work, the resulting biodegradable starch-based film with great comprehensive performance may be expected to become a green food packaging.

Data Availability

The data used to support the study are included within the article. Raw data can be acquired from the corresponding author upon reasonable request (Causunjf@hebau.edu.cn).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Emergency Food Product Packaging by Pectin-Based Antimicrobial Coatings Functionalized by Pomegranate Peel Extracts

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Emergency food products (EFPs) or energy bars are used in critical situations, such as natural disasters, to promote crisis management. EFPs require sophisticated packaging strategies. Edible coatings incorporated with natural antimicrobial agents could be considered as active packaging materials for increasing EFP safety. In this study, pectin-based coatings incorporated with pomegranate peel extracts were used to protect energy bars. Initially, total phenolic contents and antimicrobial and antioxidant properties of aqueous and ethanolic pomegranate peel extracts (PPEs) were determined. Also, PPEs were analyzed by HPLC. In the next step, the extracts were incorporated into the matrix of edible coatings as active substances. The sensory properties and microbial contamination of coated energy bars were investigated during 30 days of storage. Sixteen phenolic substances were detected in the extracts with gallic acid, ellagic acid, caffeic acid, coumaric acid, and quercetin as major ingredients. The ethanolic extract exhibited higher concentrations for all phenolic compounds. The results indicated that the ethanolic extract showed inhibitory effects on *S. aureus* and *E. coli* at concentrations of 30 and 50 mg/ml, and the aqueous extract's inhibitory effects were observed at concentrations of 50 and 80 mg/ml, respectively. The antioxidant and antibacterial effects could be attributed to high phenolic content and a combination of different substances. Microbial and sensory tests performed on coated energy bars showed that the active coatings were able to control and reduce the population of microorganisms during storage without adversely affecting sensory properties.

1. Introduction

Emergency food products (EFPs) or energy bars are known as processed food products specially designed for emergency conditions like natural disasters [1]. Energy bars are also often consumed as nutritious snacks by people who need a quick energy source, such as athletes with high physical activity. Individuals with nutritional problems or irregular meals can also use energy bars for nourishment. Energy bars boost the body's energy level and are often used as a substitute for a meal [2]. The EFP must be safe, nutritionally complete, palatable, easy to use, and easy to distribute [3]. In a crisis, due to nutritional deficiencies and unsuitable hygienic conditions, it is needed to ensure food products' safety. Packaging has an essential role in protecting food products from environmental contamination, extending shelf life, and minimizing food losses [4]. Novel packaging strategies such as the application of active packaging and edible coatings are gaining increasing attention. Currently, food packaging is mainly done using petroleum-derived materials. However, studies on alternative materials have been raised to reduce the harmful environmental effects of synthetic materials [5].

Edible coatings are defined as thin layers of edible materials on the surface of food products composed of natural biopolymers [6]. Biopolymers can be extracted from natural resources such as agricultural by-products [7]. Pectin is a colloidal carbohydrate in the middle lamellae and primary cell walls of many plants and fruits. Pectin has thickening and emulsifying properties. Pectin-based coatings are appropriate food packaging choices due to their good resistance to oxygen, oil, and aroma and also due to their good mechanical properties [8, 9].

Edible packaging may operate as a carrier of functional compounds that improve its functionality [7]. Considerable efforts have been made to enhance available coatings' performance, which increases the shelf life of the food product by delaying oxidation and controlling food-borne pathogens [4, 10]. The main functions of active coatings in food packaging are releasing active compounds into the surrounding medium or absorption of damaging components such as free radicals, moisture, and oxygen, negatively affecting the product quality [11]. As natural and inexpensive sources of bioactive compounds, plant extracts can be good choices for incorporation into active edible packaging [12]. The pomegranate peel as an agrowaste is an excellent source of bioactive compounds, and pomegranate peel extract (PPE) can be used as an active ingredient in matrix of bio-based coatings.

Pomegranate (*Punica granatum* L.) as a traditional plant is among the first plants cultivated by humans. Scientific confirmation of pomegranate health benefits has led to increased production and consumption in fresh and processed forms since the last century. During pomegranate processing, considerable amounts of pomegranate peels are produced as the major by-products. There are high levels of bioactive compounds in pomegranate peel and its extract, mainly phenolic acids, flavonoids, and hydrolysable tannins. PPE has therapeutic effects such as antioxidant, antimicrobial, anticancer, and anti-inflammatory activities [13, 14]. Pomegranate peel has higher antioxidant and antimicrobial activity than other parts of the pomegranate fruit [13, 15].

Previous studies have demonstrated the positive effects of PPE on food products' shelf life because of its antioxidant and antibacterial properties [16–18].

Qin et al. [12], evaluated the antimicrobial activity of active chitosan films containing PPE. The results demonstrated that 10 g/l incorporation of PPE has significant inhibitory effects on *S. aureus* [12]. Kanatt et al. [5] reported that active films from chitosan and polyvinyl-alcohol containing aqueous PPE increased protection against UV light. Active films containing pomegranate extract reduced the number of *S. aureus* by 2 log cycles and inhibited the growth of *B. cereus*. Tarkhasi [19] used active edible coating containing PPE for extending the shelf life of silver carp fish. The results confirmed that 5% PPE addition into the coating matrix considerably reduced the total viable count of bacteria and delayed lipid oxidation.

As a novel approach to extend the shelf life of food products, active edible coatings have gained remarkable attention in recent years because of their advantages compared to conventional packaging. A review of previous studies showed that no research had been done on the use of active coatings for emergency food product packaging, and the present study aims to open a window in this field. According to the nature and application of EFPs, active edible coatings can increase their shelf life and improve the health and physical function of consumers at the same time. Some compounds with high nutritional value are sensitive to various factors, including the process of production. Edible coating matrices have protective and carrier functions for these compounds. The present study introduces a new strategy for the enrichment of emergency food products with sensitive compounds with high nutritional value. The present research intended to study the potential of using active edible coating functionalized by pomegranate peel extract for safety improvement of EFPs. The main purpose of this study was to investigate the antimicrobial/antioxidant properties of aqueous and ethanolic PPEs as well as their effectiveness in active coating composition on microbial contamination of EFPs by considering the sensory properties.

2. Materials and Methods

2.1. Materials. Mature and healthy pomegranates (*Shirin Pust Ghermez* variety) with uniform size and appearance were collected from Saveh, Markazi Province, Iran. Fully ripened fruits with waxy (shiny/reddish) skin were selected (total soluble solids = 13.82 ± 1.59 °Brix/pH = 3.42/total acidity = 0.56 ± 0.12 g/100 g). The fruits were washed and sanitized with sodium hypochlorite in 20 ppm concentration for 15 minutes. Peels separated manually, dried at 60°C, and powdered by laboratory-scale mill (Moulinex, Type, Dap1, CMMF 8000 W, France). Microbial strains of *Escherichia coli* O157 : H7 (ATCC35218) and *Staphylococcus aureus* (PTCC1431) were prepared from the Department of Food Science and Technology, University of Tehran, Iran. Microbial culture media and chemicals including sulfuric acid (98%), 1,1-diphenyl-2-picrylhydrazine (97%), 2, 2-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS), dimethyl sulfoxide, ethanol (70%), methanol, Folin–Ciocalteu reagent, gallic acid, and sodium carbonate were procured from Merck Co. (Germany) and Sigma-Aldrich Co. (USA).

2.2. Pomegranate Peel Extracts. Solvents of deionized water and ethanolic solution (80% v/v) were used for extraction. The powdered pomegranate peels were homogenized with the solvents at the 1 : 20 ratio, and the mixture was kept away from the light and was allowed to stand for 48 h. The supernatant filtration was performed using Whatman No. 41 filter paper after centrifugation at 3500 rpm for 15 min. The filtrate was rota-vaporized at 50°C, and the concentrates were dried, powdered, and stored in amber bottles in a desiccator [20, 21].

2.3. Antioxidant Activity

2.3.1. DPPH Radical Scavenging Activity. DPPH radical scavenging activity of PPEs was determined following the method reported by Parseh and Shahablavasani [22]. 1 mg of PPE was added to 4 ml of DPPH ethanol solution (0.1 mM),

and the solution was shaken vigorously. The mixture was left to stand for 30 min in the dark medium at room temperature. Then, the absorbance was recorded at 517 nm to determine the remaining DPPH in the solution. The radical scavenging activity was calculated by the following equation:

$$\text{DPPH radical scavenging (\%)} = \frac{(A\text{control} - A\text{sample})}{A\text{control}} \times 100. \quad (1)$$

2.3.2. ABTS Radical Scavenging Assay. ABTS radical scavenging activity of PPEs was performed according to Malviya et al. [23]. The ABTS radical was generated by mixing 5 ml of ABTS (7 mM) with 88 μl of ammonium persulphate (140 mM) and incubating in the dark at 25°C for 16 h (stock solution). The working solution prepared by mixing stock solution and buffered saline (pH 7.2 PBS) until the absorbance value at 734 nm was equal to 0.70 ± 0.02. Then, 100 μl of PPE was mixed with 3 ml of the ABTS working solution and kept in a dark place for 10 min. The ABTS radical scavenging capacity of the sample was calculated by the following equation:

$$\text{ABTS radical scavenging (\%)} = \frac{(A\text{control} - A\text{sample})}{A\text{control}} \times 100. \quad (2)$$

2.4. Determination of Total Phenolic Content. The total phenolic contents of PPEs were measured using the Folin–Ciocalteu method with gallic acid as the standard. 0.1 ml of the diluted extract (10-fold diluted with distilled water) was mixed with distilled water (6 ml) and Folin–Ciocalteu reagents (0.5 ml). After 4 minutes, 20% sodium carbonate (1.5 ml) was added to the solution, and the final volume was adjusted to 10 ml with distilled water. The final mixture was kept in total darkness for 2 h, and the absorbance was measured at 765 nm using a spectrophotometer (CEILE- 2, UK). Total phenolic contents were calculated using the calibration curve of gallic acid concentration, and the results were expressed as milligrams of gallic acid equivalent (GAE)/g of dry extract. The standard curve of gallic acid solution (50 to 250 mg/ml) ($r^2 = 0.99$) was prepared using a similar procedure [22].

2.5. Analysis of Phenolic Compounds Using HPLC. High-pressure liquid chromatography (HPLC) equipped with UV detector was employed to determine the phenolic components in the PPEs. A combination of acetonitrile, water, and acetic acid (2%) was selected as the mobile phase with a flow rate of 0.5 ml/min. The reverse phase C18 column was the main system to separate the phenolic compounds. The length of the column and the packed particle sizes were 25 cm and 5 μm, respectively. Every injection was accomplished with a volume of 20 μl. To prepare the samples for analysis, 5 ml of the ethanol-HPLC grade was mixed with 5 ml water. Then, 8 ml of the prepared mixture was mixed with 2 ml of HCl (1.2 M), and 0.5 g of dried extract was incorporated. After heating, 10 ml of methanol was added to the obtained mixture and centrifuged for 5 min at the speed of 4000 rpm. The filtered transparent fluid was applied for HPLC phenolic determination. For quantification, initially, 0.1 mg of each standard was solved in 1 ml of ethanol-HPLC grade, and the concentrations of 0.005, 0.02, 0.04, 0.06, and 0.1 mg/l were prepared. The obtained solutions were injected into the HPLC apparatus to detect and determine the phenolic compositions [24].

2.6. Microbial Strain Activation. *S. aureus* (PTCC1431) and *E. coli* O157:H7 (ATCC35218) were refreshed in tripticase soy broth (Merck, Germany) at 37°C incubation and inoculated on tripticase soy agar (Merck, Germany) plates for checking the purity.

2.7. Antimicrobial Activity of Extracts. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined to assess the antimicrobial properties of extracts. Stock solutions of each PPE (500 mg/ml) were prepared using dimethyl sulfoxide as the solvent. A volume of 0.5 ml from each stock solution and 0.5 ml of tripticase soy broth (TSB) were added to the first tube and serially diluted in the next 10 to 15 tubes containing 0.5 ml of TSB medium. Then, 0.5 ml of 1/100 dilution of 0.5 McFarland bacterial cultures was added to each tube. Controls for culture medium, solvent, testing bacteria, and PPEs were considered. The peel extract concentration showing no bacterial growth and turbidity after 24 h incubation at 37°C was considered as MIC. Aliquots of 100 μl from each transparent tube with no turbidity were separately cultured on Mueller Hinton agar plates, and after 24 h of incubation at 37°C, the concentration that showed no bacterial growth was recorded as MBC [25].

2.8. Energy Bar Production. Energy bars were produced according to the following procedure in the form of 50 g solid bars: shortening (8 g) was melted in an oven at 112°C and other ingredients including wheat flour (25 g), milk powder (5 g), sugar (7 g), lecithin (0.5 g), vanillin (0.5 g), cocoa powder (0.5 g), coconut powder (0.75 g), vitamins/minerals premix (3.5 g), and salt (0.2 g) were added to melted shortening and mixed completely and 5 ml of water was added to the mixture. The semisolid mixture was molded in 4.4 cm × 7.6 cm dimensions using aluminium foil. Finally, the baking process was done in the oven at 150°C for 20 min [26].

2.9. Energy Bar Coating. Pectin (2% w/w) was dissolved in distilled water at 75°C under gentle stirring for 15 min, and glycerol (0.2% w/w), as a plasticizer, was added to the solution. The mixture was sterilized and cooled to 40°C, and then PPEs were added at the tested concentrations (0, 1, 1.5, and 2 × MIC). Energy bars were coated by immersing them into the coating solution (for 3 min) and drying with airflow (1 h). Under the same conditions, sterile distilled water was used as a coating solution for control samples. All the samples were stored at ambient temperature for 30 days [27].

2.10. Microbial Analysis of Coated Energy Bars during Storage Time. 10 g of samples was homogenized with 90 ml of sterile peptone water (0.1%) using a Stomacher lab blender. Appropriate dilutions were spread on sterile petri plates containing plate count agar and incubated at 35 ± 1°C, for 24 h, for the enumeration of total count of bacteria. Sabouraud dextrose agar medium was used to enumerate total molds and yeasts with 7 days incubation at 25°C. MacConkey agar was used for coliforms counting, and petri plates were kept at 37°C for 24 h [26, 28–30].

2.11. Sensory Evaluation. Hedonic sensory evaluation of energy bars was conducted with 10 trained tasters. Appearance, aroma, taste, texture, and overall acceptance of coated samples were determined and scored on a scale varying from "5 = like extremely" to "1 = dislike extremely." Samples were coded with three-digit random numbers and presented in white dishes [31].

2.12. Statistical Analysis. Experiments were carried out in triplicate and mean values with standard deviation were reported. Statistical analysis was performed using the SPSS software version 24 (SPSS Inc., Chicago, IL, USA). Homogeneity test was used for all data reported and then mean values were analyzed by one-way ANOVA, followed by Tukey comparison test. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Total Phenolic Contents of Pomegranate Peel Extracts. Total phenolic content of PPE samples is shown in Figure 1. Ethanolic extract, in comparison with aqueous extract, exhibited a higher phenolic content. Our results regarding total phenolic contents are compatible with that reported by Rosas-Burgas et al. [32] and lower than that mentioned by Derakhshan et al. [33] and Sumaiya et al. [34]. There is a direct relationship between phenolic compounds and the antioxidant activity as well as antimicrobial properties [35]. Iranian pomegranate cultivars were investigated for antioxidant activity by Tehranifar et al. [36], and the level of total phenolics was reported from 295.79 to 985.37 mg GAE/100 g. The total phenolic content of PPE has been reported equal to 342 mg GAE/g by [37]. It has been reported that differences between the concentrations of phenolic compounds and their antioxidant activities could depend on several factors, such as the part of the fruit, extraction methods, cultivar, stage, and climatic conditions during fruit maturation [38]. The presence and amount of various antioxidant compounds in plant materials, which have different chemical properties and solubility in different solvents, lead to changes in the yield and type of extracted antioxidants [39].

3.2. Phenolic Compound Detection and Quantitation by HPLC. The HPLC diagrams obtained from the analysis of ethanolic and aqueous extracts of pomegranate peel are shown in Figures 2 and 3, respectively, and diagram analysis data are presented in Table 1. As shown, gallic acid, ellagic acid, and caffeic acid are the most abundant compounds in pomegranate peel extract. The pomegranate peel has various tannins, flavonoids, alkaloids, and organic acids. Various flavonoids like flavan-3-ol, naringin, luteolin, luteolin 7-O-glucoside, pelargonidin, prodelphinidin, catechin, epicatechin, epigallocatechin-3-gallate, kaempferol, kaempferol-3-O-glucoside, kaempferol-3-O-rhamnoglucoside, quercetin, and rutin are found in PPEs [23]. Hmid et al. [40] studied phenolic compounds of eighteen pomegranate cultivars in Morocco. Phenolic compounds were identified as chlorogenic, caffeic, ferulic, gallic, and ellagic acids, catechin, phloridzin, quercetin, epicatechin, and rutin. Mabrouk et al. [37] determined phenolic compounds in PPE. Predominant phenolic compounds were gallic acid (6041.1 μg/g dw) and caffeic acid (1220.37 μg/g dw).

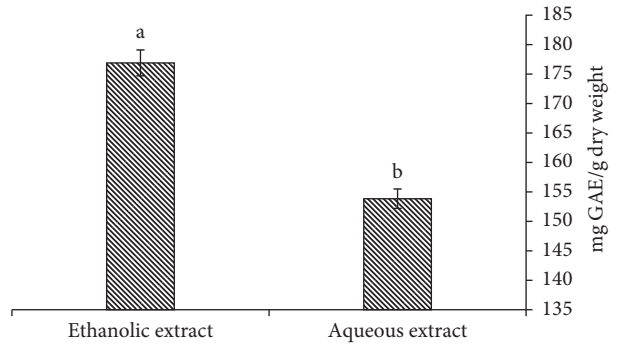

Figure 1: Phenolic contents of pomegranate peel extracts. Different letters on the bars indicate significant differences ($p < 0.05$).

According to our results, measured phenolic compounds in PPEs had higher values in the ethanolic extract except galangin. The chemical properties of the solvent and method of extraction lead to differences in the extraction of phenols [41]. The cultivar type, growing region, climate, maturity, and cultural practice strongly influence the chemical composition of pomegranate fruits. Also, significant variations were observed in organic acids, sugars, and phenolic compounds during the years [36].

3.3. Antimicrobial Activity of Extracts. Minimum inhibitory concentration (MIC) is introduced as the lowest concentration of antimicrobial agent with an inhibitory effect on the growth of a certain microorganism, meaning that the microorganism is present in the environment but cannot reproduce. Minimum bactericidal concentration (MBC) is the lowest concentration of the antimicrobial agent, leading to the death of the microorganism, and no living microorganism can survive in a medium containing MBC [22].

Table 2 shows the MIC and MBC of pomegranate peel extracts on *E. coli* and *S. aureus* microorganisms. According to the results, at the concentration of 80 mg/ml of aqueous PPE, 8 colonies of *E. coli* were grown, showing the inhibitory effect against the tested bacteria, and this concentration is introduced as MIC. Also, at the concentrations from 90 to 100 mg/ml, no growth was observed, and the concentration of 90 mg/ml was

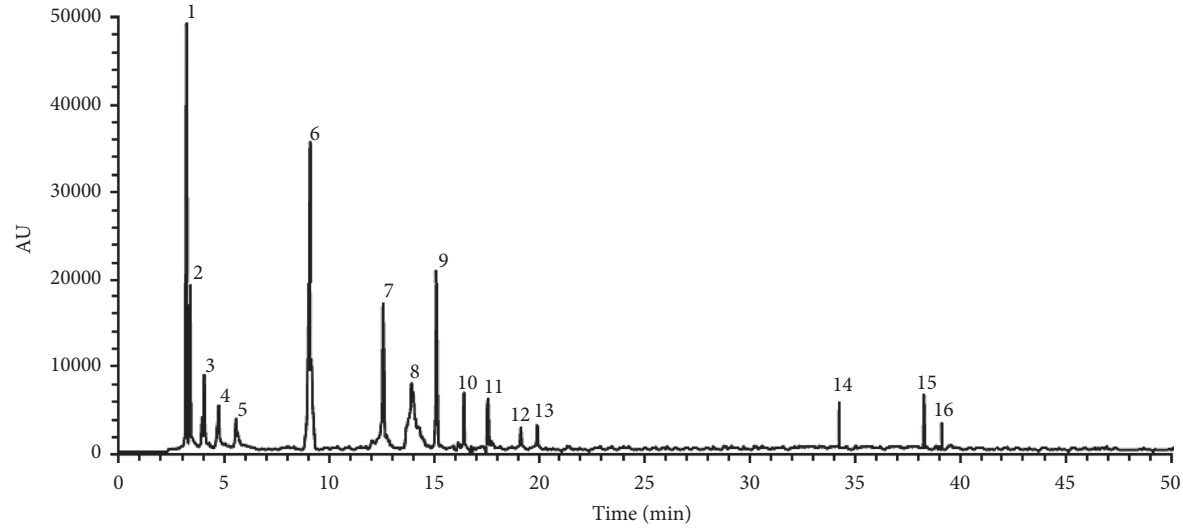

Figure 2: HPLC diagram of phenolic compounds of ethanolic extract.

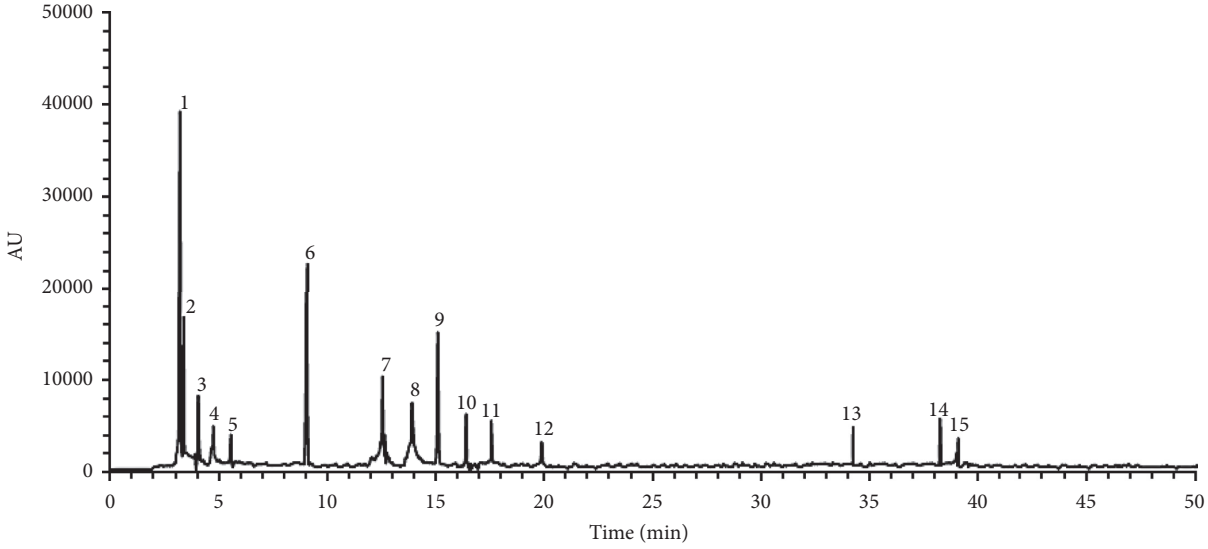

Figure 3: HPLC diagram of phenolic compounds of aqueous extract.

introduced as MBC. Concerning *S. aureus*, MIC was 50 mg/ml, and MBC was 60 mg/ml. Microbial growth in the presence of ethanolic extract showed that MIC and MBC on *E. coli* microorganism were at 50 and 60 mg/ml, and regarding *S. aureus*, they were at 30 and 40 mg/ml, respectively.

Gram-positive bacteria are more susceptible than Gram-negative ones. In agreement with the present study, other studies confirmed that *S. aureus* is more sensitive than *E. coli* to pomegranate extract [42, 43]. Al-Zoreky et al. [44] confirmed that *S. aureus* was more sensitive than *E. coli* to PPEs. The outer peptidoglycan layer in Gram-positive bacteria is an ineffective barrier. The phospholipid membrane's impermeability to lipophilic solutes in Gram-negative bacteria leads to resistance to antibacterial substances [45].

Aqueous and ethanolic pomegranate peel extracts were found to have antibacterial activity against different strains of *Escherichia coli* [46]. Also, the inhibitory effect of pomegranate peel extract on the growth of *Staphylococcus aureus* has been demonstrated [23]. As the results show, the ethanolic extract has more strong bactericidal and inhibitory effects on tested microorganisms. This finding is in agreement with Ahmad et al. [47] who demonstrated that alcohol has better performance in the extraction of antimicrobial substances compared to water.

The amount of bioactive compounds in different extracts, which depended on regional and variety changes, causes differences in the inhibitory activity of peel extracts [45].

3.4. Antibacterial Effects of Active Coating on Energy Bars during Storage. Antibacterial effects of active coatings incorporated by ethanolic and aqueous PPEs on energy bars are presented in Tables 3 and 4, respectively. Coated energy

Table 1: Phenolic compound concentration in pomegranate peel extracts.

No.	Compound	Retention time (min)	Concentration (μg/ml)	
			Aqueous extract	Ethanolic extract
1	Gallic acid	3.2	529.7740	674.9871
2	Caffeic acid	3.4	264.8438	299.1797
3	Catechin	4.05	28.01822	30.75171
4	Epicatechin	4.75	16.24309	26.51934
5	Ferulic acid	5.57	14.54545	22.36364
6	Ellagic acid	9.05	437.5254	628.6004
7	Quercetin	12.58	154.5000	255.2530
8	Quercetin-3-methyl-ether	13.9	113.5135	148.6486
9	Coumaric acid	15.1	172.9885	356.3218
10	Naringenin	16.4	25.54945	28.84615
11	Apigenin	17.55	25.31818	28.18182
12	Kaempferol	19.1	ND	15.15000
13	Luteolin	19.88	15.49180	15.98361
14	Chrysin	34.2	19.83471	24.29752
15	Pinocembrin	38.11	24.42857	28.92857
16	Galangin	39.05	14.67123	14.38356

*ND: not detected.

Table 2: Antimicrobial effects of pomegranate peel extracts on *S. aureus* and *E. coli*.

Type of extract	Type of microorganism	Extract concentration (mg/ml)											
		5	10	15	20	30	40	50	60	70	80	90	100
Aqueous	*S. aureus*	++	++	++	++	++	+	8 colonies	–	–	–	–	–
	E. coli	++	++	++	++	++	++	++	++	+	8 colonies	–	–
Ethanolic	*S. aureus*	++	++	++	+	5 colonies	–	–	–	–	–	–	–
	E. coli	++	++	++	++	++	+	6 colonies	–	–	–	–	–

++ shows the high growth, + indicates low growth, and – indicates lack of growth of microorganism.

Table 3: Antibacterial effects of different concentrations of pomegranate peel ethanolic extract incorporated in coatings of emergency food product during 30 days of storage.

Storage time (days)	Extract concentration (×MIC = 50 mg/ml)	Total count	Molds and yeasts	Total coliforms
1	0	212.00 ± 25.09^{dA}	28.33 ± 18.33^{aA}	6.33 ± 4.16^{aA}
	1	120.66 ± 8.02^{aB}	2.33 ± 0.57^{aB}	0.0 ± 0.0
	1.5	96.33 ± 8.50^{aB}	0.0 ± 0.0	0.0 ± 0.0
	2	58.66 ± 4.16^{aC}	0.0 ± 0.0	0.0 ± 0.0
10	0	310.66 ± 29.00^{cA}	15.33 ± 8.73^{aA}	6.33 ± 2.51^{aA}
	1	91.66 ± 9.60^{bB}	0.0 ± 0.0	0.0 ± 0.0
	1.5	45.00 ± 8.88^{bC}	0.0 ± 0.0	0.0 ± 0.0
	2	21.00 ± 5.29^{bC}	0.0 ± 0.0	0.0 ± 0.0
20	0	360.66 ± 27.61^{bA}	25.00 ± 8.71^{aA}	8.33 ± 2.51^{aA}
	1	36.66 ± 11.93^{cB}	0.0 ± 0.0	0.0 ± 0.0
	1.5	11.66 ± 3.05^{cBC}	0.0 ± 0.0	0.0 ± 0.0
	2	1.66 ± 1.52^{cC}	0.0 ± 0.0	0.0 ± 0.0
30	0	435.66 ± 12.66^{aA}	43.00 ± 19.46^{aA}	9.66 ± 5.03^{aA}
	1	11.33 ± 7.09^{dB}	0.0 ± 0.0	0.0 ± 0.0
	1.5	2.33 ± 0.57^{cC}	0.0 ± 0.0	0.0 ± 0.0
	2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

*Different small letters show significant differences ($p < 0.05$) at certain extract dosage during storage time. *Different capital letters show significant differences ($p < 0.05$) at certain storage time and different extract dosage.

bars had microbial contamination from the first date of storage time. The control coating was not able to reduce bacterial contamination of coliform bacteria, molds, and yeasts. Also, the total count of bacteria was increased during storage time in samples with control coating. While pectin-based active coatings exhibited pronounced antibacterial activity against tested pathogenic strains. However, coated samples with incorporated PPE have shown a reduction in the count of tested bacteria during storage time. So, it can be concluded that active coatings with PPE have inhibitory and

Table 4: Antibacterial effects of different concentrations of pomegranate peel aqueous extract incorporated in coatings of emergency food product during 30 days of storage.

Storage time (days)	Extract concentration (×MIC = 80 mg/ml)	Total count	Molds and yeasts	Total coliforms
1	0	212.00 ± 25.06^{aA}	28.33 ± 18.33^{aA}	6.33 ± 4.16^{aA}
	1	184.33 ± 48.95^{aA}	9.00 ± 8.71^{aA}	0.33 ± 0.57^{aB}
	1.5	180.67 ± 21.94^{aA}	0.0 ± 0.0	0.0 ± 0.0
	2	171.33 ± 28.57^{aA}	0.0 ± 0.0	0.0 ± 0.0
10	0	310.67 ± 29.01^{bA}	15.33 ± 8.73^{aA}	6.33 ± 2.51^{aA}
	1	146.67 ± 17.95^{aB}	1.66 ± 2.88^{aB}	0.0 ± 0.0
	1.5	86.67 ± 27.74^{bC}	0.0 ± 0.0	0.0 ± 0.0
	2	13.00 ± 9.54^{bD}	0.0 ± 0.0	0.0 ± 0.0
20	0	360.67 ± 27.61^{cA}	25.00 ± 8.71^{aA}	8.33 ± 2.51^{aA}
	1	145.33 ± 18.23^{aB}	0.0 ± 0.0	0.0 ± 0.0
	1.5	15.00 ± 5.00^{cC}	0.0 ± 0.0	0.0 ± 0.0
	2	3.67 ± 6.35^{bD}	0.0 ± 0.0	0.0 ± 0.0
30	0	435.66 ± 12.66^{dA}	43.00 ± 19.46^{aA}	9.66 ± 5.03^{aA}
	1	64.67 ± 30.24^{bB}	0.0 ± 0.0	0.0 ± 0.0
	1.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

*Different small letters show significant differences ($p < 0.05$) at certain extract dosage during storage time. *Different capital letters show significant differences ($p < 0.05$) at certain storage time and different extract dosage.

bactericidal effects, in a way that using 100 mg/ml ethanolic extract or 160 mg/ml aqueous extract incorporated in coating solution caused to complete inactivation of bacteria in energy bars during 30 days of storage. Edible coatings can prevent microbial growth by limiting their growth requirements, including oxygen, carbon dioxide, and moisture permeability. Also, active coatings suppress the growth of microorganisms by the gradual release of embedded antimicrobial agents onto the food surface, maintaining their critical concentrations over time [48]. Antimicrobial agents increase the shelf life of food products by increasing the lag phase and slowing the growth phase of microorganisms [49]. Due to the thermal processes in the production of energy bars, it can be assumed that the contamination is of the surface contamination type. The presence of active compounds, their gradual release, and the resulting limitations of the coating on the surface of energy bars can limit the growth of surface microorganisms.

Earlier studies have demonstrated the antibacterial activity of pomegranate peels [44, 50, 51]. The presence of broad spectrum antimicrobial compounds in pomegranate peel extract leads to inhibitory effects against Gram-positive and Gram-negative bacteria [23]. Antibacterial action of PPE is attributed to the phenolic toxicity that interacts with sulfhydryl groups of proteins in microorganisms [52]. The antimicrobial activity of phenolic compounds depends on their ability to damage and disrupt cell membrane integrity [53]. It has been concluded that pomegranate peel can be used as a natural preservative for food products [37].

3.5. Antioxidant Activity of Extracts. A single method alone cannot accurately measure the antioxidant potential of food systems, and therefore, several methods are used for this purpose. In this study, DPPH and ABTS scavenging activity assays were used for antioxidant activity determination.

The antioxidant activity characteristics of PPEs are shown in Figure 4. The DPPH scavenging activity of PPEs was reported in the range of 77.02% to 86.36% by Orak et al. [54]. Malviya et al. [23] reported that the highest DPPH inhibition activity of PPE was equal to $79.5 \pm 6.5\%$.

ABTS scavenging activity of ethanolic PPEs in previous studies was equal to $78.92 \pm 1.13\%$ [55] and $94.6 \pm 6.10\%$ [23].

The results suggest that the ethanolic extract has more antioxidant activity that can be explained by more total phenolic contents and their action as reducing agents, hydrogen donors, and singlet oxygen quenchers [56]. The nature of the solvent has a significant impact on the extraction capacity of phenolic compounds from plant materials [57]. Ethanolic solvent was found more efficient than water for phenolic compound extraction [58]. Organic solvents have better efficiency as compared to water for antioxidant extraction. Also, the extracts obtained from the mixture of solvents exhibited higher antioxidant activity compared to individual solvents [23].

3.6. Sensory Evaluation. Tables 5 and 6 present the scores given by the panelists to the energy bars, for sensorial preference. No significant differences ($p < 0.05$) were observed among the formulations for any of the attributes evaluated. Samples with active coating received the higher averages for appearance and texture, whereas other sensory parameters, including taste, aroma, and overall acceptance, were negatively affected. According to results, PPE incorporation in the coating solution for emergency food bar coating can negatively change sensory attributes, but there are no significant differences ($p < 0.05$) between samples and control.

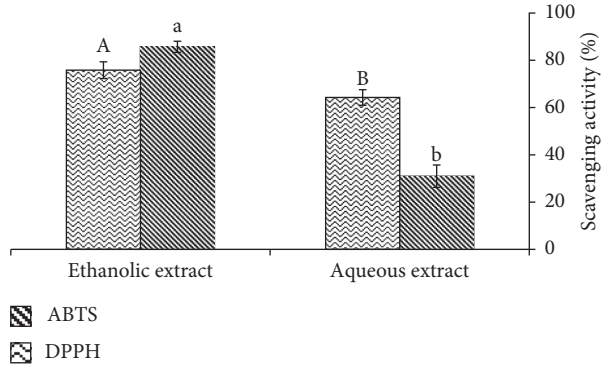

Figure 4: Radical scavenging activity of pomegranate peel extracts. Different small letters on the bars indicate significant differences ($p < 0.05$) between two extracts. Different capital letters on the bars indicate significant differences ($p < 0.05$) between two extracts.

Table 5: Sensory evaluation of the emergency food products coated with different concentrations of ethanolic pomegranate peel extract incorporation.

Extract concentration (×MIC = 50 mg/ml)	Appearance	Aroma	Taste	Texture	Overall acceptance
0	4.4 ± 0.54	5.0 ± 0.00	4.4 ± 0.54	5.0 ± 0.00	4.0 ± 0.70
1	4.8 ± 0.44	4.6 ± 0.54	4.6 ± 0.54	5.0 ± 0.00	4.6 ± 0.54
1.5	5.0 ± 0.00	4.2 ± 0.44	4.2 ± 0.44	5.0 ± 0.00	3.8 ± 0.83
2	5.0 ± 0.00	4.4 ± 0.54	3.8 ± 0.44	5.0 ± 0.00	3.6 ± 0.54

*No observed significant differences between the averages ($p < 0.05$).

Table 6: Sensory evaluation of the emergency food products coated with different concentrations of aqueous pomegranate peel extract incorporation.

Extract concentration (×MIC = 80 mg/ml)	Appearance	Aroma	Taste	Texture	Overall acceptance
0	4.4 ± 0.54	5.0 ± 0.00	4.4 ± 0.54	4.8 ± 0.44	4.0 ± 0.70
1	4.4 ± 0.54	4.4 ± 0.54	3.6 ± 0.54	4.8 ± 0.44	4.2 ± 0.83
1.5	4.8 ± 0.44	4.6 ± 0.54	4.6 ± 0.54	4.8 ± 0.44	4.4 ± 0.54
2	5.0 ± 0.00	4.8 ± 0.44	4.6 ± 0.54	5.0 ± 0.00	3.6 ± 0.54

*No observed significant differences between the averages ($p < 0.05$).

4. Conclusions

Overall, the results showed that the ethanolic extract of pomegranate peel had higher amounts of bioactive compounds as well as higher antioxidant and antibacterial activity. Gallic acid, ellagic acid, and caffeic acid were the most abundant phenolic compounds in pomegranate peel extracts. Pomegranate peel extracts exhibited inhibitory and bactericidal effects against Gram-positive and Gram-negative bacteria. Pectin-based active coating functionalized by PPEs showed good antimicrobial properties and extended the shelf life of energy bars. PPE incorporation in the coating solution slightly affected the sensory properties of samples, but there were no significant differences ($p < 0.05$) compared to the control sample. In summary, the application of active coatings containing pomegranate peel extract at a concentration of 2 × MIC could eliminate pathogenic microorganisms in energy bars within 30 days of storage.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Review Article

Reducing Meat Perishability through Pullulan Active Packaging

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The provision of safe products from the meat industry has been considered as the major source of protein for maintaining human health. Meat-borne outbreaks are mainly due to *Salmonella typhimurium* (*S. typhimurium*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *Clostridium perfringens* (*C. perfringens*), reducing the shelf life and consumer demands. A variety of vulnerable substances, including cholesterol oxidation products (COPs), are generated by the oxidation of meat induced by the microbial infestations. The use of certain biodegradable active packaging, including pullulan active packaging, is being focused by the meat industry due to their safety, stability, and negligible health risks. The potential of pullulan active packaging, incorporated with silver nanoparticles and essential oils, against *E. coli*, *S. typhimurium*, Mycoplasma, and other bacterial species is exclusive. Similarly, maintenance of organoleptic properties of meat with nominal oxidative rancidity and limited human health issues can be acquired by pullulan active packaging.

1. Introduction

The meat industry is facing several issues, and wastage of meat and meat products due to improper processing is the leading issue. According to an alarming report by the Food and Agriculture Organization (FAO) of the United Nations, approximately one-third or over 1.3 billion metric tons of all edible food products, including meat, is wasted annually due to poor practices in harvesting, storage, and transport [1]. Edible food items, especially meat, are also prone to chemical deterioration and microbial infestation causing the stunning threat to consumer health. These deteriorations represent huge economic losses to the food and meat industry every year. Due to the damaged quality of food items, the consumer confidence is being shaken. As a result, economic losses as well as litigation costs borne by food and meat industries are increasing day by day [2].

The losses may be greater in those areas and countries having less processing and sanitary control facilities. The foodborne pathogens in these countries cause a regular health issue for the tourists and consumers in the export regions [3]. The broiler meat industry is facing huge wastage and losses due to improper processing and preservation. Every year, the spoilage of raw meat could be as high as 40% along with the food supply chain (production, retailing, and consumers) representing a remarkable loss [4]. Alternative packaging strategies, such as designing various pack sizes according to consumer demands and manipulative processing and packaging to maintain food quality and shelf life, have been proposed to reduce these food losses [5]. This reduction in food wastage would enable the nations for sustainable food production and enhance the market development. Globally, more emphasis is done on food safety and it is one of the major objectives of the recent food legislation. The microbiological risks of food products, especially broiler meat, are the major sources of foodborne outbreaks which resulted in a high causalities rate of 13% around the world [6]. In addition to microbiological safety,

the colour and lipid stability of meat are the salient parameters of quality influenced by consumer acceptance [7, 8]. For extending the shelf life of meat and meat products, antimicrobially active, commercially based, economical, and smart preservation solution is "nanotechnology" [9]. Moreover, nanotechnology enables meat preservation with certain biological and chemical procedures by maintaining the compositional integrity, moisture, and gaseous exchange from the meat surface [10]. This advanced technology has a prominent impact on production, storage, processing, transportation, and safety of meat and meat products [11].

For the preservation of meat and meat products, many synthetic and natural antimicrobials/antioxidants have, continuously, been used to reduce meat spoilage with easier commercialization of meat [12]. These products were used either onto the meat surface, in the diet of animals, or along with edible films and coatings (EFCs) mostly referred as "active packaging" [12]. EFCs directly produced or obtained by biomass (polysaccharides and protein), synthesized chemically (i.e., polylactic acid), or obtained from microorganisms (i.e., pullulan) are most preferably utilized as active or biodegradable packaging [13]. The efficacy of EFCs has been investigated by many researchers in previous decades focusing on improvement of their effectiveness when incorporated with natural or metal nanoparticles [13, 14].

Pullulan is one of the best used biodegradable polysaccharides capable of forming better, colourless, and tasteless edible active packaging with better oxygen and gas barrier capacities [15]. The incorporation of various antimicrobial agents (including organic acids, essential oils, plant extracts, proteins, and metal nanoparticles) into "pullulan active packaging" can reduce the major microorganism species load in meat products [14].

Based on these facts, this review is aiming on the application of pullulan active packaging for efficient and prolonged meat preservation. In our review, we have provided comprehensive details regarding meat-borne outbreaks, meat oxidation, cholesterol oxidation products (COPs), use of active packaging, and pullulan active packaging for meat preservation.

1.1. Meat-Borne Outbreaks. Meat-borne outbreaks are mostly due to *S. typhimurium*, *S. aureus*, *E. coli,* and *C. perfringens* [6, 16]. The red meat-related outbreaks are mainly due to the infestation by *Salmonella* species, *Listeria* species, *Staphylococcus* species, and *Clostridium* species, and most of the outbreaks were due to *S. typhimurium* [5]. In the case of broiler meat-related outbreaks, most of the food poisoning incidences were due to *S. typhimurium*, *C. perfringens,* and *Campylobacter* species [5]. Most of the countries are prone to the attack of the bacterial strains in food items due to favourable environmental conditions [17–19]. This problem may be induced by lack of basic principles for food processing, unhygienic food packaging, preservation, and inappropriate microbiological safety measures during the processing of food items especially the meat [20, 21].

As preventive measures, the processing, preservation, packaging, storage, transportation, and distribution of meat and meat products should be in a hygienic environment [22]. When meat and poultry sectors do not compromise to compel minimum criteria to ensure the safety and quality of their products, the foodborne outbreaks will not be subsidized [20]. Meat industry must monitor the microbiological load and other biological hazards, causing potential hazards for consumer health, during the production and processing of food items [23]. The meat-related outbreaks occur during cutting and processing, cooking, packaging, transportation, storage, and preservation [24]. These meat-related outbreaks are not only causing serious threats to human beings in the last decade but also deteriorate meat quality in the form of "lipid and protein oxidation" [25].

Implementation of regulatory requirements, good manufacturing, and hygiene practices may be considered as a good methodology to achieve food safety all over the globe [23].

1.2. Meat Oxidation. The pathogenic species of bacteria can deteriorate the quality and shelf life of meat and meat products. Meat deterioration may be experienced as discoloration, off flavour, reduced shelf life, toxic compound formation in meat and meat products, and drip losses of the nutrients [3, 25, 26]. These negative changes in meat and meat products are, mainly, due to oxidation of lipids and protein. This process is affected by the temperature and duration of their storage [27]. The amount of COPs in meat is a reckonable parameter to detect lipid oxidation or "oxidative rancidity" [28]. Similarly, the adaptability of certain microbial strains against new weather and environmental stress is triggered by oxidative rancidity leading towards the production of highly vulnerable substances [29]. The customer demands may be declined by this pessimistic transformation in meat and meat products influenced by environment or storage conditions. These qualitative changes mainly happen due to "oxidation" of lipids resulting in the formation of COPs or protein oxidation [30]. When the packed fresh meat, in a transparent packaging material, is exposed to sunlight/oxygen or improperly stored, the oxidation is initiated, leading towards the reduction in the organoleptic or sensory properties with a higher rejection by the valued customers [31].

Protein oxidation is mainly induced by the bacterial species or due to the oxidative stress [32]. Covalent modification of protein molecules during the oxidation process not only harms the organoleptic properties of the meat and meat products but also influences human health [32].

COPs are formed when the animal-derived food products are heated, cooked dehydrated, stored, and exposed to radiations [33], reducing their demands especially in the persons having high blood cholesterol. Various food products reflect the changing amount of COP formation during processing and storage (Table 1) due to the lipid contents [30].

COP generation is, mainly, conjugated with the amount of fatty acids (unsaturated) and cholesterol in meat,

Table 1: Lipid contents of various food products and the amount of COPs [30].

Food products (stored/processed)	Lipid percentages (%)	COPs (g/ 100 g lipids)
Raw beef	13.8	0.50
Cooked beef	10.6–12.2	0.40–0.50
Whole egg powder	44	0.80–1.50
Whole milk powder	22.7	0.30–0.40
Grated cheese (Grana type)	18.4–28.4	0.30–0.40
Snacks prepared with eggs	12.7–30.9	0.4–2.60
Biscuits prepared with eggs	3.7–27.6	0.20–3.70

radiations (ultraviolet and gamma), moisture contents, and packaging materials [33, 34]. Addition of naturally available phenolic compounds (i.e., tannic acid and catechin) to the food products and raw meat may be beneficial to reduce the microbial growth and lipid oxidation [34]. The amount of COPs will be increased when the raw meat is packed and stored while they may be reduced when the processing/packaging of meat is carried out after cooking [35]. Similarly, vacuum packaging and a low amount of fatty acids in meat can also be considered as "tools" to reduce the COPs in the meat during processing [35]. The maintenance of the quality of meat is still a question mark for the food scientists. These vital issues of the commercial meat industry require appropriate attention by the researchers and government agencies to decrease the losses in near future.

2. Meat Active Packaging

The shelf life of meat has been prolonged by the use of synthetic antioxidants including butylated hydroxyl-toluene (BHT), butylated hydroxyl-anisole (BHA), and propyl gallate (PG) to reduce "oxidative rancidity" of fats [35]. However, human health safety is the major concern of the researchers regarding the extensive usage of BHT, BHA, and PG, switching the direction towards natural products [33, 35]. Deterioration of meat and meat products is also due to microbial infestation by native microflora in the gut of the animals, through environment, improper handling, transportation, and preservation methods [11]. To overcome these problems, antimicrobials are added to meat and meat products. The preservation of meat by using antimicrobial or antioxidant additives is considered hazardous for human health, and the development of green preservatives is highly required [12]. A study by Nithya and Mohankumar [36] showed that the pathogenic strains of bacteria, especially present in poultry meat, exhibit a maximum "multiple antibiotic resistance index" (MAR) especially *S. typhimurium*, *E. coli*, *S. aureus*, *Vibrio cholera* (*V. cholera*), *Campylobacter jejuni* (*C. jejuni*), and *Listeria monocytogenes* (*L. monocytogenes*). This issue is alarming as the ingestion of these pathogenic bacterial strains can cause huge outbreaks of foodborne problems due to their antibiotic resistance [37]. Moreover, physiochemical changes are continuously induced by these pathogens which may reduce the demands for meat and meat products [37]. EFCs are the best suited and green alternatives to maintain the quality and safety of meat products and to reduce microbial load [14]. The standardized procedures for the synthesis of green packaging products according to industrial requirements and incorporation with "green" antioxidants/antimicrobials are the major challenges to improve meat preservation [14].

In the recent few decades, food scientists have been working on green synthesized preservatives to enhance the quality and shelf life of meat (both fresh and frozen). Eco-friendly, safe, and biodegradable food and meat preservation may help to reduce the risk to the entire ecosystem caused by conventional and synthetic preservatives [38]. This practice may increase the consumer demands towards fresh food materials with extended shelf life and quality ensuring maximum distribution channels all over the globe [37, 38]. Additionally, the consumer acceptances pertaining to the "sensory characteristics" with better health safety and economical aspects in usage are the major objectives for the formation of such green synthesized packaging materials [38]. The meat-based items provide excellent media for bacterial growth which may modify the overall organoleptic, sensory, and chemical parameters, resulting in the decrease of shelf life and higher foodborne illnesses as mentioned by Kerry [39]. Nanotechnology can be a simple, green, and economical solution to lessen these issues. It is assumed that if nanotechnology paces at its current rate in the meat industry, the global hunger can be defeated in a very shorter period with an ample supply of processed meat [40–44]. This advanced technology has a prominent impact on the production, storage, processing, transportation, and safety of poultry products [11, 45–47].

The naturally available/green synthesized products can be used to preserve the various food items especially the meat (Table 2) due to their biodegradable qualities [16, 17].

Plant extracts and essential oils cannot be used without any active packaging for the preservation of food due to their variable characteristics and bioavailability. Active edible packaging incorporated with plant extracts and essential oils is a better choice for the long-term preservation of meat and meat products [12, 48–50].

The meat industry requires wide use of natural or green synthesized products to improve the quality of meat and meat products experienced by the consumers [11, 50, 51]. By this practice, excessive usage of synthetic meat preservatives can be reduced to increase the organoleptic and physicochemical properties of meat and meat products [14, 52]. Antimicrobial/antioxidant efficacy of the active substances in active packaging is, mainly, dependent on their uptake pathways, size, shape, composition, and surface modifications [53–62]. Moreover, the cellular and subcellular distribution of active compounds, from active packaging, to the

Table 2: Natural/green synthesized active packaging [16, 17].

Natural/green synthesized packaging products	Type of food	Active ingredient
Chitosan	Chicken breasts, cheese, and tilapia fillets	Organic acids
Starch/glycerol	Chicken breast meat, tilapia fillets, and cheese	Organic acids
Chitosan	Ground beef	Grapefruit extracts
Horseradish extracts	Beef	—
Clove, citronella, and cyprus	Food packaging in films	Clover oil
Seaweed extracts	Fruits, vegetables, and meat	—
Alginates	—	Polysaccharides and glycoprotein
Pullulan	Meat, eggs, and fruits	Polysaccharides

Table 3: Antimicrobial active packaging.

Active ingredients					
Edible films and coatings	Incorporated materials		Active against bacterial species	Techniques developed	References
	Nanoparticles	Others			
Chitosan	Silver	Ciprofloxacin	*P. aeruginosa*	Antimicrobial loaded films	[70]
Chitosan	Zinc oxide	Carboxymethyl cellulose	*S. aureus P. aeruginosa*, *B. cereus*	Bionanocomposite films	
—	—	Essential oils	*S. typhimurium, L. monocytogenes, E. coli*	Low-density polyethylene packaging (LDPE)	
—	—	Phenolic acid—gallic acid, benzoic acid, and flavonoids	*L. monocytogenes, C. jejuni*	Flexible bioactive packaging	
Whey proteins	—	Essential oils	*E. coli, L. monocytogenes, S. aureus, S. enteritidis*	Edible films from whey proteins	
Chitosan	—	Garlic oil	*E. coli, L. monocytogenes, S. aureus, B. cereus, S. typhimurium*	Edible films from chitosan	
Milk protein	—	Organic acids	*E. coli* and *Pseudomonas* spp.	Milk protein-based edible films	
—	—	Chitosonium acetate	*L. monocytogenes, S. aureus, Salmonella* spp.	Antimicrobial packaging	
Cellulose	Silver	—	*S. aureus, E. coli*	Active food packaging	[71]
Chitosan	Silver	Mandarin essential oil	*L. innocua*	Modified coating	[72]
Pullulan	Silver	—	*B. subtilis*, *S. aureus, S. marcescens*	Pullulan-silver nanopackaging	[73]
Alginate	Zinc oxide	Glycerol	*S. aureus, S. typhimurium*	Active packaging	[74]
Alginate	—	Cinnamon bark oil and soybean oil	*E. coli, L. monocytogenes,* and *Salmonella enterica*	Edible film packaging	[75]
Soybean	Zinc oxide	—	*S. aureus* and *E. coli*	Active packaging	[76]

food surface also governs their toxicity risks [61–67]. During the last few years, the development of suitable active packaging, with the lowest toxicity, gained the highest demands by meat and food industry with a better antimicrobial impact and customer safety [14, 67, 68]. Prolonged and microbial free meat preservation requires the application of better technology, such as EFCs, to ensure the quality of processed meat [52, 69]; Table 3. It will not only generate more revenue by the meat industry but also maintain customer demand and health.

For the long-term preservation of meat and meat products, emulsifiers and surface-active agents are also used as gas and moisture barriers in the form of "meat coatings" [14]. Pure lipids combined with hydrocolloids (such as protein, starch, cellulose, and their derivatives) may act as a component system able to be applied as "meat coatings" [69]. In fresh and processed meats, lipid incorporation into EFCs makes an excellent water barrier to improve the hydrophobicity, cohesiveness, and flexibility, leading to prolongation of freshness, colour, aroma, tenderness, and microbiological stability. Despite various advantages, protein films may be prone to proteolytic degradation in the presence of enzymes in meat products, or allergenic protein fractions can cause adverse effects on the susceptible people [77].

Polysaccharide-based films and coatings enhance the shelf life of meat and meat products due to the prevention of oxidative rancidity, dehydration, and surface browning [14]. These films dissolve and become integrated into the wrapped meat products exposed to smoke and steam. This parameter reduces the moisture loss from the stored meat products and improves the structure and texture, resulting in higher yields [69]. Moreover, polysaccharide-based films and coatings provide crispiness, hardness, viscosity, compactness, and gel-forming quality to the films.

3. Pullulan Active Packaging for Meat Preservation

Pullulan is an extracellular polysaccharide produced by a fungal species *Aureobasidium pullulans* [78] which is chemically constructed by repeated maltofructose units (Figure 1). This bioavailable polysaccharide is not exploited yet as "natural preservative and packaging material" except its application against rancidity [79].

Pullulan edible films are considered as more homogeneous and translucent with improved thermal stability and tensile strength when compared with other polysaccharide-based edible films, i.e., chitosan or seaweed edible films [80]. Further, this linear polymer exhibits the viscoelastic behaviour upon the drying and provides the entangled network of the fibers as compared to other bioavailable polymers, presenting less evaporation of water at complete drying [81, 82]. Studies conducted by Diab et al. [38] and Cheng et al. [78] revealed that active packaging obtained from pullulan showed colourless and tasteless texture that was resistant to oil and exhibited very low oxygen permeability with better barrier characteristics as compared to other edible films.

Pullulan active packaging, containing the sweet basil extract (SBE), reduces the chances of weight loss and colour changes in the stored food [82]. Chitosan active packaging (as compared to pullulan) may lead towards the intermolecular hydrogen bond formation instead of intramolecular hydrogen bonding, causing drastic changes in XRD (X-ray diffraction) measurements [82]. These results indicate that pullulan active packaging releases the silver ions in better quantity as compared to chitosan films [82]. Similarly, pullulan active packaging is considered as one of the emerging preservation technologies, to ensure the microbial safety and meat preservation, rarely influenced by external factors [83]. When the food materials are preserved with these packaging, the delicacy and originality of food taste are secured and preserved [14]. A study conducted by Karolina et al. [15] proved that pullulan active packaging preserves the quality of all food items (including fruits and meat) with safety against physical and mechanical damages. Incorporation of nanoparticles in pullulan film to control the foodborne pathogens of meat and meat products provided a new chapter in food and meat processing [84, 85].

3.1. Mechanism of Action. The incorporation of nanoparticles into pullulan provides tremendous antimicrobial activity against *E. coli* and *L. monocytogenes* by slow release of inorganic nanoparticles to targeted bacterial cells [86–88]. The delivery of specific "antimicrobial" substance directly to the surface of meat can reduce the population of various bacterial strains [89]. Antimicrobial mechanism of any antimicrobial substance incorporated into pullulan active packaging includes the following:

(a) Prolongation of the lag phase of growth of bacteria/fungi [55]

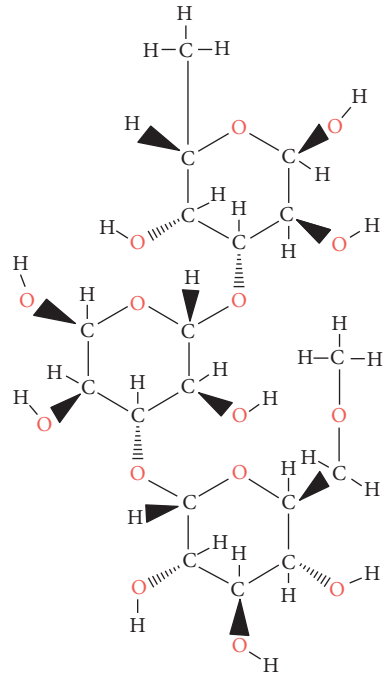

Figure 1: Pullulan chemical structure with repeated maltofructose units.

(b) Cell wall damage [62]

(c) Formation of holes and gaps in the outer membrane of bacterial/fungal cells [54]

(d) Destruction of the outer and inner membrane due to the penetration of metabolites into the bacterial/fungal cells resulting in the death of the target organism [54]

(e) Loss of integrity of cell membrane and cytoplasmic membrane which increase bacterial/fungal cell permeability [57, 58]

The antimicrobials or the active substances include silver nanoparticles, gold nanoparticles, TiO_2 nanoparticles, essential oils, and certain bacteriocins [90]. The bacteriocins are the peptides (proteins) synthesized by the ribosome of one strain of bacteria with an antimicrobial capacity against their allied strains [91, 92]. The delivery of nanoparticles, essential oils, and bacteriocins, incorporated in pullulan edible films, direct to the surface of meat can maintain its quality and prolongs the storage duration [91]. This is, directly, related to the fact that pullulan can adhere to the moist surface of meat and releases the "active agents" (nanoparticles, essential oils, and bacteriocins) right into the surface slowly [86]. It can surround nanoparticles in the form of a thin layer as well resulting in the reduction of the number of harmful bacterial strains [46].

Incorporation of nanoparticles/active substances from different origins, into pullulan, to formulate "active packaging" has been reported by many researchers in the last two decades, as a green preservative, to prolong the shelf life and quality of meat [85, 88, 91] (Table 4).

Table 4: Pullulan active packaging for meat preservation.

	Effective against bacterial strains	Type of food items	References
Pullulan with nanoparticles/essential oils	*S. aureus*, *L. monocytogenes*, *S. typhimurium,* and *E. coli* O157:H7	Poultry meat products	[84]
Pullulan with zinc nanoparticles	*S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, and *S. typhimurium*	Turkey meat	[86]
Pullulan with silver nanoparticles	*S. aureus* and *L. monocytogenes*.	Turkey meat	[86]
Pullulan with silver nanoparticles	*L. monocytogenes* and *S. aureus*	Turkey deli meat	[86]
Pullulan with LAE-nisin Z	*S. aureus*, *L. monocytogenes*, and *E. coli* O157:H7	Ham deli meat and raw beef	[87]
Pullulan with lauric arginate (LAE) and nisin Z	*S. typhimurium* and *S. enteritidis*	Turkey breast	[88]

Similarly, antimicrobial characteristics of silver nanoparticles (AgNPs) incorporated into active packaging, against various dangerous species of bacteria, cannot be ignored [79]. AgNPs synthesized from curcumin, pullulan, cellulose, and collagen sources can act as a strong antibacterial and antifungal agent [46, 91, 92]. For the customer health protection and product quality maintenance, the green synthesized AgNPs from pullulan (as stabilizing/reducing agent) can show tremendous results [14]. The use of "pullulan active packaging," incorporated with AgNPs is increasing day by day in the meat industry as a "green" preservative [85]. However, various factors may also affect the antimicrobial activity of "pullulan active packaging," incorporated with AgNPs including size, shape, and crystallographic structure of AgNPs [92]. Ganduri et al. [73] proved that the AgNPs, synthesized from the increasing amount of pullulan concentrations, showed an intense antimicrobial capacity for meat preservation [69]. Moreover, pullulan-mediated AgNPs can be utilized as an "active substance" for the preservation of meat and meat products, but the size, hydrophilic-hydrophobic character of pullulan-mediated AgNPs, and their concentration are the major factors affecting their efficacy [46, 93]. For the preservation of meat and meat products, the incorporation of silver nanoparticles into pullulan films to formulate "active packaging" is ever-increasing [85]. The conjugation of antimicrobial abilities of pullulan and AgNPs can improve the shelf life and quality of meat products [46, 92]. Khalaf et al. [86] reported that pullulan active films, containing silver nanoparticles, surprisingly inactivated the lethal impacts of *L. monocytogenes* and *S. aureus* causing a reduction in the spoilage of meat and meat products. The mentioned study was performed on turkey meat showed the antimicrobial activity of pullulan-incorporated silver nanoparticles for the safe processing of white meat. The stability of pullulan active packaging, incorporated with AgNPs, at a wide range of storage temperature, i.e., 4°C to 25°C, showed a novel route for safe, healthier, and biodegradable meat packaging [86, 94–97].

The superior biocompatibility, nonimmunogenic, nonmutagenic, noncarcinogenic, and easily degradable properties of pullulan-based active packaging can be considered as a better choice to improve the quality and shelf life of meat in preservation studies [98–100].

These advancements for meat processing can further reduce the microbial contamination in broiler meat with improved quality [101, 102].

4. Conclusion

The use of synthetic antioxidants (butylated hydroxyl-toluene, butylated hydroxyl-anisole, and propyl gallate) and antimicrobials, to overcome meat oxidation and perishability, is imposing serious health issues in human beings. The biodegradable pullulan active packaging, incorporated with any active substance (i.e., AgNPs and essential oils) may be adopted as the alternative technology due to their superior antimicrobial capacity against *S. typhimurium*, *E. coli*, *S. aureus*, *C. perfringens*, *V. cholera*, *C. jejuni*, *S. enteritidis*, and *L. monocytogenes*. Due to the effect of pullulan active packaging on microbial population reduction, the oxidative and compositional stability of meat is remained unchanged, resulting in higher customer safety. Furthermore, antimicrobial activity of AgNPs released from the active packaging is one of the complex phenomena influenced by their better composition, appropriate size, and shape. The green and reliable pullulan active meat packaging can not only reduce the chances of meat perishability but also lower down the oxidative rancidity, generation of COPs, and compositional losses.

Data Availability

No experimental data was utilized in this manuscript. The literature and referred journal research papers were considered.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Sazili AQ, Kumari S, and Khan MJ conceptualized the study; Khan MJ was involved in data curation and responsible for software development and wrote the original draft; Khan MJ, Sazili AQ, and Kumari S investigated the study; Khan MJ,

Kumari S, Selamat J, Shameli K, and Sazili AQ wrote, reviewed, and edited the manuscript.

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Research Article

Study on Biodegradable Chitosan-Whey Protein-Based Film Containing Bionanocomposite TiO_2 and *Zataria multiflora* Essential Oil

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In our research, a composite film of whey protein isolate (WPI)/chitosan incorporated with TiO_2 nanoparticles (NPs) and essential oil of *Zataria multiflora* (ZEO) was developed. The resulting composite films were evaluated by FTIR, SEM, and XRD, and also the physicochemical characteristics including color, mechanical properties, swelling ratio, and water vapor permeability (WVP) were studied. SEM graphs exhibited that the samples had a uniform and homogeneous structure where TiO_2 NPs and ZEO were well dispersed. FTIR and XRD findings also show that the hydrogen bonds and hydrophobic interactions are the main interactions between the composite WPI/chitosan and TiO_2. The crystalline nature of the composite samples increased with the increase of NP content. Nevertheless, ZEO had an insignificant effect on the functional groups and the crystallinity of composite samples. The film visual characterization revealed that, by adding and increasing the TiO_2 and TiO_2-ZEO, sample lightness and opacity significantly increased. Additions of TiO_2 remarkably ($p < 0.05$) improved the water vapor and mechanical properties of composite samples, although the loading of ZEO, regardless of TiO_2 incorporation, led to a considerable decrement of these properties. Furthermore, composite films containing ZEO combined with 2% of TiO_2 compared with 1% of NPs blended with ZEO had strong antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes*. Generally, the findings proposed that the addition of TiO_2 reinforces the properties of composite films with a synergistic effect of ZEO loading on the antibacterial ability, by which the resulting biodegradable composite samples can be used as a food active packaging material.

1. Introduction

Nowadays, the increase in the global concern of food quality and safety together with the environmental influences of nonbiodegradable plastic material wastes has led to the research and studies on the renewable and eco-friendly edible coating and films [1]. Biopolymers including proteins, lipids, polysaccharides, and their mixtures are considered as the main engaged materials for this purpose due to their benefits such as biodegradability, high availability, and renewability [2, 3]. Natural ingredients and essential oils extracted from plants are usually incorporated into the packaging ingredients to increase their mechanical and chemical properties and also prevent the packaged products from microbial activities and reduce the oxidation of lipids which can promote the quality of food products compared with conventional systems [4].

Chitosan as linear polysaccharides obtained from alkaline deacetylation of chitin is a promising good and applicable biopolymer [5]. Chitosan is insoluble in usual solvent, but chitosan because of their amino groups is soluble in some acid solutions with a pH value lower than 6. It has an excellent film-forming capability and high antimicrobial activity and can be employed as an active antimicrobial coating agent or packaging film [6]. Whey protein isolate (WPI) is another biopolymer with good film-making

capacity isolated from the by-products of cheese-making industries and has interesting mechanical properties [3]. In addition, films formed from whey protein have a transparent appearance and act as excellent gases barriers, but they do not exhibit any antimicrobial properties and do not have good moisture barrier properties unlike the films made of chitosan [7]. Therefore, composed films of whey proteins and chitosan have been developed to overcome these challenges and in order to combine the advantage of each component to film preparation with excellent characterization [8]. However, it was reported that the composite film from whey protein/chitosan had a poor efficiency in physical, mechanical, and permeability properties due to the incompatibility of both ingredients. A promising option to amend the compatibility problems between two polymers is to merge the nanoparticles (NPs) [9].

Titanium dioxide nanoparticles (TiO_2 NP_S) as metal oxides are usually used to improve the attributes of biopolymeric edible films [10]. TiO_2 is a cheap, inert, and safe compound which is extensively applied as an antiradiation and antimicrobial agent because of its photocatalytic properties in the edible films [11]. Furthermore, with respect to the proposed safe dosage, it is extensively applied in cosmetic and food industries to block light and create a white color [10]. Moreover, it was reported that, when a composite matrix was reinforced with TiO_2 NPs, the mechanical properties of the resulting biodegradable samples were significantly increased and the gas barrier and vapor permeability were also reduced [12]. In addition, the addition of TiO_2 NPs to the composite sample causes a decrease in the transmittance light and it is a suitable way to reduce the oxidation of lipid by light [13].

On the other hand, interest in using essential oils in the packaging films has been increased to prevent the chemical deterioration of the packaged foods due to the microbial contamination. Essential oils (EOs) are extracted from the aromatic herbal and are extensively used in food flavoring [14]. The active ingredients in EOs are terpenes, terpenoids, and aromatic ingredients having different antifungal, antiviral, antioxidant, and antibacterial properties. Among all essential oils, *Zataria multiflora* (ZEO) is well known due to antimicrobial properties. It has high-phenolic ingredients, such as carvacrol and thymol [15].

Therefore, it seems that the ZEO and TiO_2 NPs can be used as functional ingredients to enrich edible films based on whey proteins-chitosan owing to their different advantages such antimicrobial activity, nontoxicity, availability, biocompatibility, biodegradability, and good functional properties. The resulting enriched edible films also can be considered as excellent candidates for packaging of food products specially those which are prone to microbial growth to maintain their quality. The overall aim of the current research is to fabricate an edible film made of chitosan and whey proteins enriched with ZEO and TiO_2 NPs for producing biofunctional films with improved biological and functional properties. Subsequently, the antimicrobial, physical, structural, morphological, and mechanical properties of the resulting WPI/chitosan edible film incorporated with TiO_2 NPs and ZEO were studied.

2. Materials and Methods

2.1. Materials. Chitosan, WPI (higher than 91% protein), ZEO, TiO_2 NPs, and glycerol were from Bio Basic (Canada), Hilmar Canada, Barij-Essence Co. (Iran), Acros Co. (USA), and Merck Co. (Darmstadt, Germany), respectively. Also, some materials applied in our research were also from Merck with an analytical grade.

2.2. Composite Film Preparation. Whey protein suspension (3%, w/v) was made by dispersing WPI in DDW subsequently heated at 90°C for 30 min at a pH value of 8.0 and then cooled rapidly [5]. Chitosan solution (10 g/L) was made using dispersing chitosan in 2% (v/v) acetic acid solution by constant mixing for 3 h at 60°C [16]. Based on preliminary experiments, whey protein-chitosan suspension was made by blending two polymer suspensions at a constant ratio of WPI/chitosan (70 : 30) and mixing magnetically for 15 min at 25°C. In the next step, TiO_2 NPs (1 and 2% w/w) were incorporated, and after mixing for 15 min, glycerol (30% w/w) was incorporated to the composite suspension and again stirred for 30 min. Next, ZEO (1% v/v) was incorporated into the composites suspension and sonicated for 30 min with a power of 100 W. For degassing the film suspension, it was placed under vacuum for 20 min. The film suspension was cast onto the Petri dish and dried at 45°C for 24 h. The resulting dried samples were peeled and equilibrated for 2 days in a desiccator with saturated magnesium nitrate solution (RH: 53% at 25°C) until further tests [7].

2.3. Scanning Electron Microscopy (SEM). The morphology of composite WPI/chitosan samples was imaged by a SEM (VEGA II-550, TESCAN, Czech Republic) with an acceleration voltage of 10 kV. Before testing, samples were cut manually in liquid nitrogen. The specimens were stuck onto a stub by tape and sputter-coated by a thin layer of gold. Subsequently, the samples were placed into the SEM chamber and observed with a magnification of 1000x (cross section) and 5000× (surface area) [17].

2.4. X-Ray Diffraction (XRD). XRD patterns of composite sample, TiO_2 NPs, chitosan, and WPI powder samples were recorded with the X-ray diffractometer (Philips PW1730, PANalytical, Netherlands) using the Cu Kα radiation source (40 kV and 30 mA). The analysis was done in the 2θ range between 10° and 50° [18].

2.5. Fourier-Transform Infrared (FTIR) Spectroscopy. Composite WPI/chitosan sample FTIR spectroscopy was performed by a Bruker infrared spectrometer (Billerica, Massachusetts, USA) to evaluate the influence of addition of TiO_2 NPs and ZEO. The wavenumber region of samples mixed with potassium bromide was evaluated at 4000–600 cm^{-1} with 4 cm^{-1} interval [1].

2.6. Thickness of Films. Thickness was determined using a manual micrometer with a precision of 1 μm. Ten different locations of samples were measured, and the average was taken as the result [19].

2.7. Swelling Ratio. The composite film pieces of 20×20 mm in size were dried in an oven at 104°C, weighed, (W_i) and then immersed in 50 mL DDW at room temperature for 24 h. Excess liquid on the wet films were removed by the filter paper and weighed (W_f). The capacity of water absorption was determined according to the following equation:

$$\text{swelling ratio}\,(\%) = \frac{w_f - w_i}{w_i} \times 100. \quad (1)$$

2.8. Water Vapor Permeability. Water vapor permeability of samples was determined gravimetrically based on the technique of Jiang et al. [20]. A circular cup was filled with DDW to expose the lower film face to provide RH of 100%. Composite films were mounted with adhesives on the cups and then were maintained in a desiccator containing the saturation $MgNo_3$ solution ($53 \pm 2\%$ RH) at 25°C. The cup weight loss was determined every 1 h to 8 h to measure the line slope of the mass loss (g) against time (s). The WVP of the samples was calculated by

$$\text{WVP}\left(\text{g} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{kPa}^{-1}\right) = \frac{M \times T}{A \times \Delta P}, \quad (2)$$

where M is the linear portion (g/s), T is the sample thickness (m), A is the area of exposed film (m^2), and ΔP is the partial pressure difference of water vapor across the sample (kPa).

2.9. Mechanical Properties. Mechanical properties including tensile strength (TS) and elongation at break (EAB) of each composite sample were analyzed with a texture analyzer instrument (Testometric Co., Ltd., England). Rectangular piece samples ($1.6 \times 6\ cm^2$) were kept at an ambient temperature ($53 \pm 2\%$ RH) for 48 h. The sample specimens were mounted between the grips adjusted to 3 cm distance separation and then stretched with 83.33 mm/s. Mechanical properties were carried out with 6 replications [19].

2.10. Optical Characterization. The color indexes of samples evaluated by using a Minolta colorimeter (CR-300, Japan). L^* (lightness), a^* (green-red), and b^* (blue-yellow) were evaluated on the samples. In addition, the samples opacity were determined with placing directly the film specimens in the spectrophotometer, and their absorption was read at the 600 nm with an empty test cell as the reference using an UV-VIS spectrophotometer. Opacity of the composite samples was determined as follows:

$$\text{opacity} = \frac{A_{600}}{x}, \quad (3)$$

where x is the film thickness (mm) [21].

2.11. Antimicrobial Properties. Antimicrobial properties of composite films were measured by the disc diffusion technique. Three kinds of microorganisms including *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* were incubated in the nutrient broth media at 37°C overnight. A portion of 100 μL of each microorganism suspension containing 10^7-10^8 cfu/mL of the bacteria was spread in nutrient agar plate surfaces. Afterward, square pieces of the different composite films ($10 \times 10\ mm^2$) were placed on the inoculated agar plate surfaces and kept at 37°C for overnight. The inhibition zone surrounding samples was determined [22].

2.12. Statistical Analysis. SPSS v.16.0 (IBM Software, NY, USA) was applied to study the resulting data. Findings were first evaluated by using one-way analysis of variance (ANOVA), and then the Duncan post hoc test was used to indicate significant ($p < 0.05$) differences between mean film samples.

3. Results and Discussion

3.1. Film Morphology. The composite samples morphology is an important feature because it can ultimately determine the mechanical and physical characteristics of degradable packaging samples [21]. The morphology and microstructure of surface and cross section of the film samples are presented in Figure 1. In the control WPI/chitosan composite sample, some obvious agglomerates were observed in the sample surface due to the heterogeneous structure formation. Our finding was in agreement with the results of Zhang et al. [5] who evaluated the microstructure of chitosan/WPI films enriched by TiO_2 nanoparticles containing sodium laurate. They reported that the blend film made of chitosan and WPI had a ragged and rough surface attributing to the thermodynamic incompatibility and phase segregation of biopolymers. The SEM image shows that TiO_2 NPs and ZEO incorporation into the composite films had no significant influence on the surface morphology of WPI/chitosan samples. In agreement, Zolfi et al. [23] also showed that the surface morphology of kefiran-WPI sample had not been significantly changed by adding of TiO_2 nanoparticle at low concentrations indicating that good dispersion of nanoparticles can be obtained when the content of the particles is very low. However, in our study, some TiO2 particles were visualized on the composite film containing TiO_2 NPs with or without ZEO which can be due to the heterogeneous mixing resulting in agglomerate formation on the film sample surface. The cross-sectional images of all composite samples show smooth and continuous structure. The same observation has been stated for chitosan/TiO_2 nanocomposite films by the Siripatrawan and Kaewklin [24]. Also, the cross-sectional images showed that the composite films had some air bubbles in their cross sections which increased with the addition of ZEO to the composite films. These air bubbles can affect the barrier properties of the resulting films.

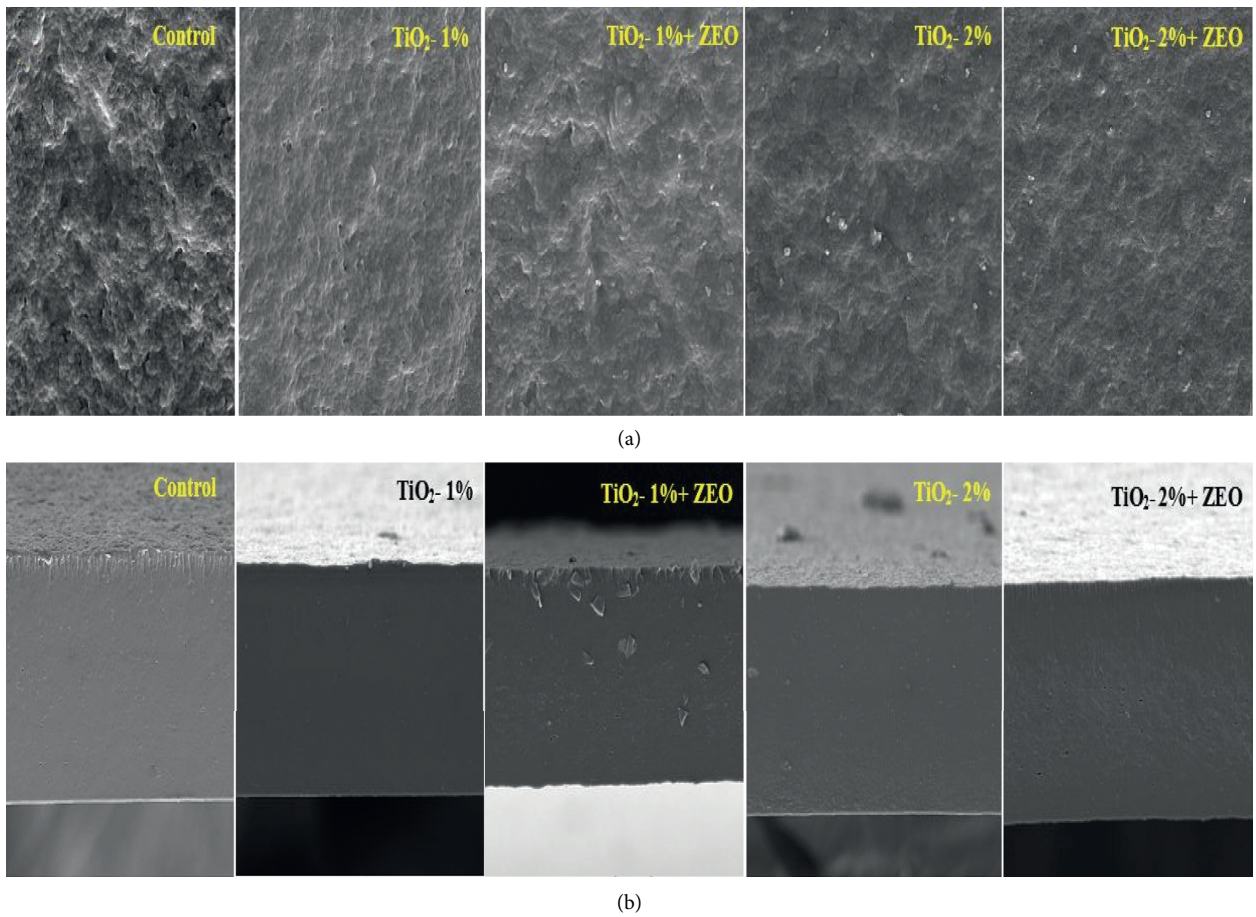

Figure 1: SEM graphs of surface (a) and cross-section (b) of WPI/chitosan samples (control) containing various amounts of TiO_2 (TiO_2-1% and TiO_2-2%) and combination of TiO_2 with ZEO (TiO_2-1% + ZEO and TiO_2-2% + ZEO).

3.2. XRD Pattern. In Figure 2, the XRD graphs of the films are shown. The XRD pattern of TiO_2 NPs showed the significant peaks at $2\theta = 25.69^\circ$, 27.84°, 36.44°, 37.29°, 38.24°, 39.04°, 48.49°, 54.34°, 55.54°, 63.09°, 69.34°, 70.69°, 75.44°, and 76.34° corresponding to the presence of different crystalline phases (anatase and rutile) in the TiO_2 NP structure. The characteristic peak of chitosan appears at 20.59°, which is the typical fingerprint for chitosan [25]. In contrast, WPI displayed an amorphous structure. Results of the formation of composite film between chitosan and WPI showed that the film has an amorphous pattern. This phenomenon can be explained with the modification of chitosan structure due to the amorphous complex formation because it has been reported that proteins are capable of changing the physical state of the chitosan film [2, 5]. When 1% of TiO_2 NP was incorporated in the composite film, two characteristic peaks appeared at 2θ of 21.89° and 25.64°. Addition of 1% ZEO into these composite films caused an inconsiderable shift at the displayed crystalline peak in the film containing 1% TiO_2 NPs at 20.59° and 25.59°, respectively. In accordance with this observation, Oleyaei et al. [11] stated that the appeared peak at around 20° could not be attributed to the TiO_2 NP crystalline phases (anatase or rutile), but it may rather be a good indicator of TiO_2 NP dispersion in the composite sample matrix. Raising TiO2 NP amount to 2%, the XRD pattern of composite films exhibited that the narrow peak intensity at around 25.64° increased and characteristic peak around 20° disappeared. Furthermore, two new signals appeared at the diffraction peak at 2θ of 38.14° and 48.54°. By incorporation of 1% ZEO at these composites film, no significant effect on peak angles at 25.64° and 48.54°, whereas after addition of ZEO in the composite film containing 2% TiO_2 NPs, peaks that appeared at 38.14° disappeared. The diffraction peaks that appeared at $2\theta = 25.64^\circ$, 25.59°, 25.69°, 38.14°, and 48.54° were ascribed to the anatase crystalline of TiO_2 NPs in the composite films with or without ZEO [5, 13]. The results were consistent with the findings of Li et al. [26] and Salarbashi et al. [12] who studied that the crystallinity of the WPI and soluble soybean polysaccharide (SSPS) samples, respectively, increased by TiO_2 NP incorporation.

3.3. FTIR Results. The FTIR results of samples were analysed to identify the interactions between WPI/chitosan, TiO_2 NPs (1 and 2%), and ZEO. The resulting spectra are depicted in Figure 3. The peaks situated at the wavenumbers of 857, 1038, 1155, 1401, 1454, 1538, 1631, and 3273 cm^{-1} were found in all composite films. The characteristic absorption peaks at 857, 1038, and 1155 cm^{-1} attributed to the C-H shaking vibration, N-C bond amide groups, and C-O-C stretching vibration bonds,

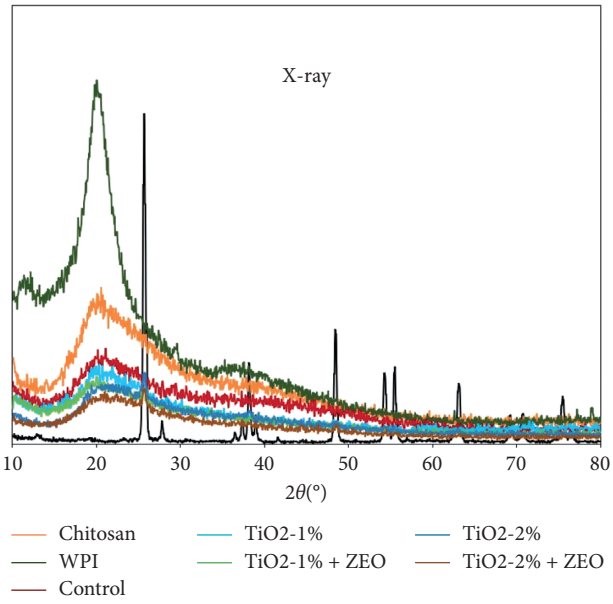

Figure 2: X-ray patterns of WPI/chitosan samples (control) containing various amounts of TiO_2 (TiO_2-1% and TiO_2-2%) and combination of TiO_2 with ZEO (TiO_2-1% + ZEO and TiO_2-2% + ZEO).

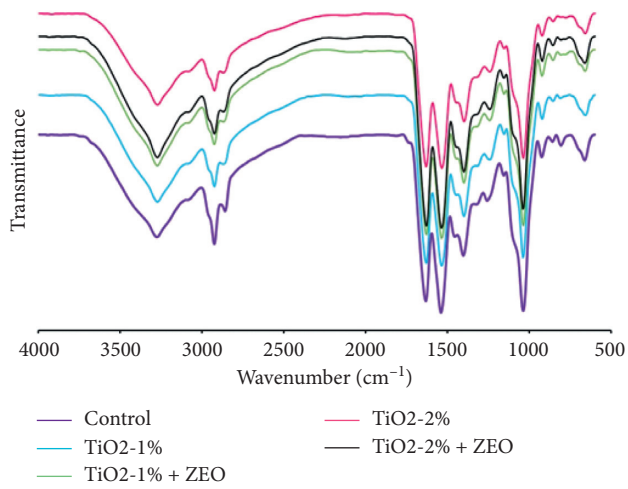

Figure 3: FTIR spectra of WPI/chitosan samples (control) containing various amounts of TiO_2 (TiO_2-1% and TiO_2-2%) and combination of TiO_2 with ZEO (TiO_2-1% + ZEO and TiO_2-2% + ZEO).

respectively [1]. The peaks around 1401 cm^{-1} and 1454 cm^{-1} could be related to the bending vibrations of C-H groups [12], but the main absorption broad peaks at 1631 cm^{-1} and 1538 cm^{-1} refer to the C = O and N-H bending with C-N stretching vibrations at the amide groups of composite film components, respectively, consistent with the existing literature [27]. Additionally, the other peak that appeared at 3273 cm^{-1} was assigned to the hydroxyl (-OH) group vibrations of WPI/chitosan, ZEO, and TiO_2 NPs. After the incorporation of TiO_2 NPs, some observable changes were observed in the composite films. The peaks observed at 804 cm^{-1} in control film disappeared after addition of TiO_2 NPs. Moreover, the band at around 666 cm^{-1} regions with incorporation of 1% and 2% TiO_2 NPs into composite films matrix shifted to ~660 cm^{-1}, indicating the bending vibration of Ti-O-Ti. It is because of hydrogen bond formation between -OH groups of WPI/chitosan and Ti from TiO_2 NPs [24]. Similar results were obtained for TiO_2 NPs with the addition of these NPs in the sample by Kaewklin et al. [28]. The peak situated at 1256 cm^{-1} is due to the vibrations in the plane of C-N and N-H groups of bound amides (amide III) or vibrations of CH_2 groups of glycerol [29]. The blue shift to the wavenumber of 1244 cm^{-1} occurred when TiO_2 NPs (1 and 2%) were added; this might be because of hydrogen bonding formation between functional N-H groups of WPI, chitosan, and -OH groups on the surface of the TiO_2 NPs [1]. Similarly, Arfat et al. [30] observed that the incorporation of ZnO NPs into the fish protein isolate/fish skin gelatin-based composite films caused the blue shift at amide bands to a lower wavelength. Furthermore, observed peaks at a wavenumber around 2923 cm^{-1} and 2857 cm^{-1} in the WPI/chitosan film sample mainly stemmed from the asymmetrical -CH_3 and symmetrical -CH_2 stretching vibrations, respectively [17]. Incorporation of 1 and 2% TiO_2 NPs at the composite films led to small red shift to 2925 cm^{-1} at the methylene groups and also considerably changed to 2871 cm^{-1} at -CH_2 groups, which belongs to the strong hydrophobic interactions in composite films. The major chemical groups in the essential oils are aldehyde, ketone, esters, and phenolic groups [31]. The findings showed that the addition of ZEO had no significant influence on the functional groups related to these compounds in the composite films structure containing 1 and 2% TiO_2 NPs. This can be due to the physical covering and entrapment of the functional groups of ZEO by the functional groups of WPI/chitosan and TiO_2 NPs, and some special interactions were able to remain in the network of the composite films. In fact, it seems that the entrapment of ZEO in the film matrix limited its stretching and bending vibrations; therefore, no significant effect was observed in the FTIR spectra when ZEO was added to the film samples. However, our results were not in line with the findings of Alizadeh-Sani et al. [1] and Ghadetaj et al. [32] who reported that the addition of rosemary and *grammosciadium ptrocarpum* essential oils, respectively, into the matrix of WPI/cellulose nanofibers and WPI films caused a significant change in the functional groups related to hydrophobic interactions and hydrogen bonds. This observed inconsistency in the results can be due to the differences in the biopolymers used to prepare the film samples as well as differences in the composition of EOs.

3.4. Color and Opacity Properties. The visual appearance of biopolymeric films is an important factor in overall acceptance by consumers [3]. The values of opacity, lightness (L^*), green-red (a^*), and blue-yellow (b^*) parameters of composite films are listed in Table 1. The films containing

Table 1: Thickness, swelling ratio, water vapor permeability (WVP), tensile strength (TS), elongation at break (EAB), and optical properties of WPI/chitosan samples containing TiO_2 and TiO_2-ZEO.

Films	Thickness (μmm)	Swelling ratio (%)	WVP (10^{-10} g·m MPa^{-1}s^{-1}·m^{-2})	TS (MPa)	EAB (%)	Color			Opacity
						L^*	a^*	b^*	
Control	66.93 ± 1.23^{b}	65.08 ± 1.62^{e}	3.47 ± 0.07^{b}	6.03 ± 0.22^{e}	22.37 ± 1.19^{a}	75.1 ± 0.67^{c}	-5.94 ± 0.1^{a}	11.25 ± 0.76^{a}	4.22 ± 0.01^{a}
TiO_2-1%	64.37 ± 1.14^{a}	46.56 ± 0.94^{d}	3.34 ± 0.059^{a}	8.80 ± 0.83^{b}	17.39 ± 0.99^{b}	90.45 ± 0.75^{b}	-5.79 ± 0.47^{ab}	13.63 ± 0.24^{b}	12.50 ± 0.04^{b}
TiO_2-1% + ZEO	71.31 ± 1.13^{c}	17.59 ± 1.30^{b}	3.70 ± 0.058^{c}	8.14 ± 0.45^{d}	13.62 ± 1.19^{c}	86.51 ± 0.48^{d}	-5.13 ± 0.16^{ab}	13.94 ± 0.36^{b}	14.24 ± 0.01^{c}
TiO_2-2%	66.81 ± 1.22^{b}	35.79 ± 1.29^{c}	3.47 ± 0.063^{b}	9.44 ± 0.48^{a}	12.88 ± 1.34^{d}	92.02 ± 0.71^{a}	-4.78 ± 0.61^{b}	13.55 ± 0.62^{b}	21.40 ± 0.05^{d}
TiO_2-2% + ZEO	73.50 ± 1.21^{d}	12.12 ± 1.00^{a}	3.81 ± 0.084^{d}	8.71 ± 0.56^{c}	11.28 ± 1.70^{d}	88.75 ± 0.79^{c}	-4.87 ± 0.78^{ab}	16.02 ± 0.63^{c}	22.15 ± 0.10^{e}

Data are shown as average ± standard deviation. Different letters in the same column show significant differences ($p < 0.05$).

TiO_2 NPs and a combination of TiO_2 NPs and ZEO presented significant differences in the case of all color parameters and values of opacity in comparison with the control sample. The composite films containing 1% and 2% TiO_2 NPs showed a considerable increase in b^* and a slight decrease in a^*. The lightness or whiteness of samples with the addition of TiO_2 NPs went up significantly ($p < 0.05$) from 75.1 to 92.02. The ZEO incorporation in the TiO_2-containing composite films caused a slight reduction in the lightness and an increase in the yellowness and the tendency of the film redness. On the other hand, the transmittance results at 600 nm presented that the control film had the highest transparency, whereas the transparency of composite films containing TiO_2 NPs considerably decreased and also there was an increase in the TiO_2 NP amount from 1% to 2%. The transparency of composite biopolymer films significantly decreased. Also, the ZEO combination had a significant influence on the TiO_2-composite sample opacity causing a decrease in the transparency of the films. In agreement with these findings, Hosseini et al. [33] showed that the reduction of film transparency with the incorporation of nanoparticles could be because of their aggregation which can block the light transmission. These results were in harmony with the findings reported on the incorporation of TiO_2 NPs to WPI/chitosan [5] and gelatin/agar films [13]. Similarly, it has been reported that the hake protein film transparency reduced after compounding with thyme EO [34].

3.5. Swelling Ratio. The swelling ratio is an important factor in composite films and represents its water absorption resistance property and type of film use [35]. Table 1 exhibits the swelling ratio of different composite films. As seen, the control presented the highest swelling ratio (65.08%). A significant decrease in the swelling ratio was exhibited for composite samples with the addition of 1 and 2% TiO_2 NPs and reached to 46.56% and 35.79%, respectively. A similar trend obtained by Achachlouei and Zahedi [9] showed that the water absorption of CMC films considerably reduced after combination with TiO_2 NPs. This result can be due to the hydrogen bond formation among WPI/chitosan, glycerol, and TiO_2 NPs which decreased the hydrophilic groups on the sample matrix and free space of the network to water molecule diffusion inside the films [13]. In addition, it has been suggested that the reduction in water uptake can be because of the hydrophobic nature of TiO_2 NPs [11]. In contrast, Ren et al. [36] presented that the water-binding capacity of PVA/xylan samples went up by TiO_2 NPs incorporation, and the film containing the highest amount of rutile TiO_2 NPs showed the highest swelling ratio. On the other hand, the results of the addition of the ZEO into the composite film formulation containing 1 and 2% TiO_2 NPs demonstrated that the swelling ratio of films significantly reduced to 17.59% and 12.12%, respectively. This phenomenon is due to the ZEO hydrophobic nature that caused an increase in the hydrophobicity of the composite-TiO_2 NPs films, which caused a lower affinity of samples to water [37]. It was also reported that the sample swelling ratio was altered by adding rosemary and mint essential oils and then decreased [35]. Thereby, it can be concluded that that the incorporation of TiO_2 NPs and essential oil into the composite films can improve the usability of films for high-humidity food packaging.

3.6. Composite Film Thickness and WVP. The thickness can be changed by the inclusion of ingredients to the blend film. The water vapor transfer is one the most important characteristics of food packaging polymer films. Low vapor permeability that reduces the transfer of moisture between the outer atmosphere and the food environment is a determining factor of the application of film produced in food packaging [35]. In general, similar findings were shown for both the thickness and WVP of composite samples by incorporation of TiO_2 NPs and ZEO. The findings showed that the average thickness and WVP of composite films were between 0.064 and 0.073 mm and 3.34 and 3.81×10^{-10} g·s^{-1}·m^{-1}·kPa, respectively (Table 1). The thickness and WVP of control composite film were 0.066 mm and 3.47×10^{-10} g·s^{-1}·m^{-1}·kPa, respectively, and inclusion of 1% TiO_2 into composite film matrix caused a remarkable ($p < 0.05$) effect on this parameter of composite films and reduced to a lower amount. Nevertheless, the increase in TiO_2 NP concentration from 1 to 2% had no effects on the thickness and WVP of composite samples in comparison with the control film. This can be because of the proper dispersion of the nanoparticles at lower concentrations, which, in addition to forming a compact structure and reducing the thickness, also blocks the water vapor entry [38]. It has been shown that the thickness of the WPI film significantly increased after loading 1.5% TiO_2 NPs into the WPI matrix which was inconsistent with our findings [1]. However, Vejdan et al. [13] about WVP decrease in the samples containing NPs theoretically stated that this could be attributed mostly to the tortuous path introduced by impermeable TiO2 NPs distributed in the matrix, in which water molecules permeate forcing to the increase of the transmission length. The positive effects of TiO_2 NPs on WVP of some polymers such as wheat gluten/nanocellulose [39], kefiran/WPI [23], and pectin [40] have been revealed in different investigations.

Furthermore, the incorporation of ZEO into the composite films containing 1 and 2% TiO_2 NPs caused a significant increase in the average thickness to 0.071 and 0.073 mm and WVP of composite films to 3.70 and 3.81×10^{-10} g·s^{-1}·m^{-1}·kPa, respectively. These results are in accordance with results of summer savory essential oil (SSEO) into the blend carboxymethyl cellulose-agar film [41]. Increase in the WVP of samples followed by loading ZEO in fact can be because of the negative effect of the essential oils on the intermolecular bonding thus creating the free spaces between the chains and phase separation due to matrix incompatibility and presence of water molecules throughout the network of the composite film [32]. The findings of our research are in agreement with data reports on WVP of essential oil-incorporated films [32, 42]. Also, the data of this study are in contrast with those in the article by Shojaei-Aliabadi et al. [15] and Nisar et al. [43] which reported that the lower WVP was

Table 2: The antimicrobial activity of WPI/chitosan samples incorporated with TiO_2 and TiO_2-ZEO.

Films	Diameter of inhibition zone (mm)		
	L. monocytogenes (Gram +)	*S. aureus* (Gram +)	*E. coli* (Gram −)
Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
TiO_2-1%	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
TiO_2-1% + ZEO	7.90 ± 1.20^{b}	14.0 ± 2.00^{b}	8.2 ± 1.30^{b}
TiO_2-2%	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
TiO_2-2% + ZEO	8.50 ± 1.00^{a}	23.20 ± 3.20^{a}	9.50 ± 1.50^{a}

Data are shown as average ± standard deviation. Different letters in the same column show significant differences ($p < 0.05$).

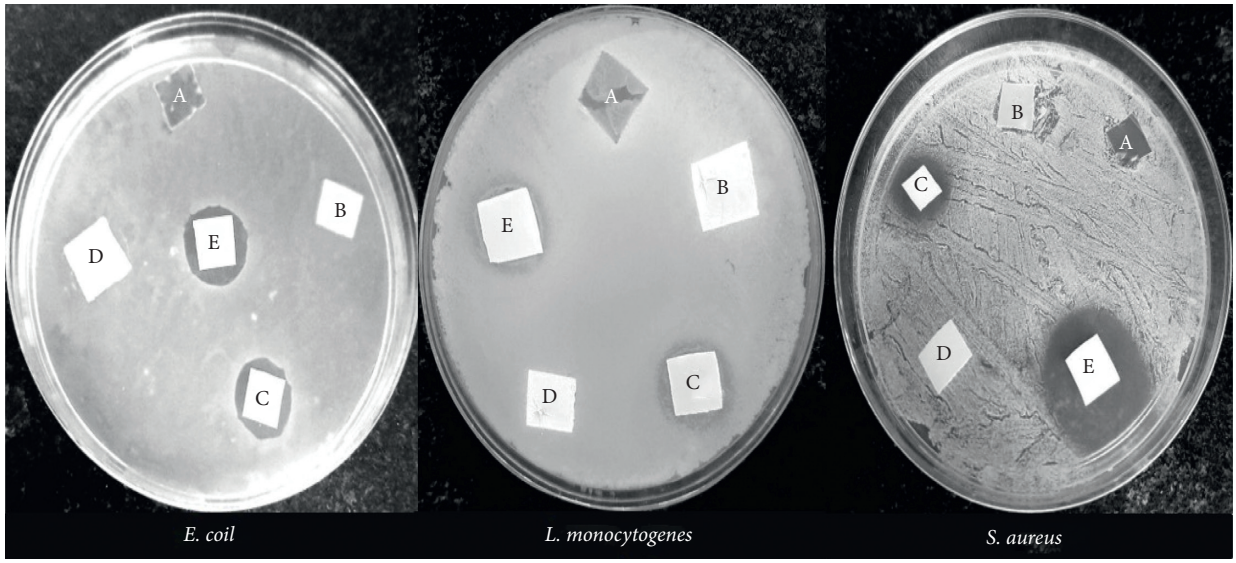

Figure 4: Antimicrobial activity of different composite films: control (A), containing 1% TiO_2 (B), 1% TiO_2 with ZEO (C), 2% TiO_2 (D), and 2% TiO_2 with ZEO (E) against three types of study bacteria.

obtained with formulation of ZEO oil into the kappa-carrageenan/nanoclay composite film and clove bud essential oil at the pectin film, respectively.

3.7. Mechanical Properties of Composite Films. The influences of TiO2 NPs and ZEO on the mechanical characterization of samples including TS and EAB are shown in Table 1. Compared with the control film, the incorporation of TiO2 NPs caused a significant ($p < 0.05$) increase in the TS values of composite films. Results indicated that the control film had a TS of 6.06 MPa with the loading of 1 and 2% TiO_2 NPs; the TS values considerably increased to 8.80 and 9.44 MPa, respectively. However, the addition of ZEO into the matrix of blends film containing 1 and 2% TiO_2 NPs led to a significant decrease at the resistance of the composite film to 8.14 and 8.71 MPa, respectively. In contrary to the TS, a drastic decrease from 22.37% to 17.32% and 12.88%, respectively, occurred because of the flexibility of the composite films with inclusions of 1 and 2% TiO_2 NPs. Hence, the addition of ZEO to the composite film containing 1% TiO_2 NPs remarkably reduced the EAB of film to 13.62%, while the incorporation of ZEO to the film matrix at a concentration of 2% TiO_2 NPs had an insignificant influence on the elongation capacity of sample (11.28%). The increase in strength and decrease in the flexibility of the composite films by TiO2 NPs addition can be because of the uniform distribution of TiO2 NPs through interfacial interactions such as electrostatic interactions, hydrogen bonds, and O-Ti-O bonds with other compounds in the film, consequently increasing the cohesion force of the film causes a restriction of the motion of polymeric network [29, 39]. These results coincide with the incorporation of TiO_2 NPs into soluble soybean polysaccharide [44] and starch/pectin film [40]; it was found that the sample TS was increased by the gradual increase of TiO_2 nanoparticles. Further, in contrast with our finding, Siripatrawan and Kaewklin [24] obtained that the tensile resistance of chitosan and TiO_2 nanocomposite films increases when low amounts were incorporated, but decrease in higher TiO_2 NP amount is probably because of the agglomeration of TiO_2 NPs in effect of inhomogeneous dispersion at a certain concentration. But, decrease in the tensile resistance and EAB after addition of ZEO may be because of the weakening of intermolecular connection of the sample by reducing the main interactions and increasing the breakup of film network due to heterogeneous with matrix of composite films, which caused a decrease in the film rigidity and resistance [1]. This result matched with that reported by the previous research about adding summer savory EO to CMC-agar edible composite film [41] or

incorporating cinnamon and ginger essential oils to CMC-chitosan blend samples [45].

3.8. Antimicrobial Properties. Antibacterial properties of films against *L. monocytogenes*, *S. aureus* (Gram-positive), and *E. coli* (Gram-negative) food pathogenic bacteria are illustrated in Table 2 and depicted in Figure 4. Results indicated that control films and films containing 1 and 2% TiO_2 NPs had no antimicrobial activity, whereas TiO_2-containing (1 and 2%) composite films loaded with ZEO had an antimicrobial effect against all pathogenic bacteria. This may be related to the non-UV treatment of the NPs at this study because it has been reported that UV modification significantly increases the functionality of the NPs such as antimicrobial activity. Same findings were previously shown by Ahmadi et al. [46]. But, TiO2 NPs have a synergistic influence on the antimicrobial properties of ZEO; in that way, the film containing the essential oil showed a higher antimicrobial activity when combined with 2% TiO_2 NPs than with 1%, which was evident in the results of the growth inactivation ofall three microbes, especially *S. aureus*. Also, overall, composite films showed a lower microbial inhibition zone against Gram-negative than against the Gram-positive bacteria. This is due to the structural difference in the bacterial cell wall. Gram-negative bacteria have complex cell wall compared with Gram-positive bacteria [47]. In agreement with these findings, some researches showed a higher antimicrobial activity for essential oil and NPs against Gram-positive bacteria in comparison with the Gram-negative ones [14, 15, 48]. It has been reported in various studies that chitosan due to the presence of positive-charge amino groups has the ability to interact with the negatively charged cell wall of microorganisms, which leads to the breakage of the proteinaceous substrate [16, 49]. Hence, the lack of antibacterial activity in chitosan/WPI-based films in this study, regardless of the addition of essential oils, NPs, and a type of chitosan, could be because of the less concentration of chitosan in comparison with WPI in the film matrix, which could not show antimicrobial effects. Generally, the antimicrobial effect of TiO_2 NPs and ZEO was associated with its crystalline structure, shape, and size of the NPs [50] and the presence lipophilic and hydrophobic functional group with phenolic components such as carvacrol and thymol with a stronger antimicrobial activity in ZEO [15, 51, 52]. They can bind to the bacterial membrane by different mechanisms, damaging the microbial membrane and cell wall and finally damaging proteins and DNA, causing the cell death [53].

4. Conclusion

WPI/chitosan films loaded with different concentrations of TiO_2 nanoparticles and ZEO showed excellent potential to be applied for packaging. The nanoparticles were well distributed in the film structure, and the resulting composite films were homogeneous without any phase separation as observed by SEM. The XRD showed that the incorporation of nanoparticles gradually increased the crystalline structure of samples, which may be because of new bond formation between WPI/chitosan matrix and TiO_2 NPs as investigated by FTIR spectroscopy. However, the findings showed that the incorporation of ZEO had a negligible influence on the crystalline nature of the samples containing TiO_2 nanoparticles. The results of the water vapor permeability test showed that the WVP of WPI/chitosan samples decreased when enriched with TiO_2 nanoparticles, whereas the combination of TiO_2 nanoparticles with ZEO caused a significant increase in the WVP of composite samples. The opacity and color characterization of the samples also changed through the enrichment of films with TiO_2 nanoparticles and ZEO. The addition of TiO_2 nanoparticles and particularly TiO_2-ZEO into the film samples resulted in a significant reduce in their swelling ratio. The mechanical attributes (TS and EAB) of WPI/chitosan films were also modified by the addition of TiO_2 nanoparticles and ZEO. The control WPI/chitosan binary sample and samples containing 1 and 2% TiO_2 did not show any antibacterial activity, while composite films containing TiO_2-ZEO had high antimicrobial activity on both types of bacteria. Generally, the results of our research proposed that WPI/chitosan edible films functionalized by TiO_2 particles and ZEO can be considered as promising candidates for use as active packaging materials for food products owing to their useful antimicrobial properties as well as the suitable physicomechanical characteristics.

Data Availability

The data used to support the study are included within the article. Raw data can be acquired from the corresponding author upon reasonable request (hlashkari@gmail.com).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Shelf Life Extension of Veal Meat by Edible Coating Incorporated with *Zataria multiflora* Essential Oil

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The present research aimed to investigate the preservative effects of a sodium caseinate (SC) coating enriched with *Zataria multiflora* Boiss. essential oil (ZMEO) at 0.5, 1, or 1.5% on the product life of meat during storage at 4°C. Over a 15-day period, the meat samples were refrigerated and analyzed every five days. The treated samples had markedly less psychrotrophic bacteria, lactic acid bacteria, Enterobacteriaceae, and total viable counts relative to the control throughout storage. In terms of the sensory, chemical (PV, TBARS, and pH), and microbial characterization, undesirable results were attained in the control sample after 10 days of refrigerated storage, whereas samples coated with SC/ZMEO, especially at higher essential oil concentrations (1 and 1.5%), proved to be significantly more stable ($P < 0.05$). However, high concentration of ZMEO (1.5%) gave an unpleasant effect on sensory attributes of meat samples. Notably, the SC/1% ZMEO coating led to good overall acceptability of the veal specimens even after 15 days of refrigeration. Hence, this coating is recommended as a replacement for synthetic preservatives and flavorings for meat products given that it preserved the quality of refrigerated veal samples for over two weeks.

1. Introduction

High moisture and nutrient levels make veal meat highly susceptible to microbial spoilage, with aerobic conditions facilitating lipid and protein oxidation. The quality of stored meat can be improved if measures are taken to avert such processes [1]. Numerous factors can influence microbial spoilage of meat and meat products. Later than slaughtering, meat can be contaminated with bacteria from various sources including washing water, air, and soil as well as human resources and the meat processing equipment [1].

Over the past few years, many researchers have attempted to prolong the shelf life of foods with a wide range of methods, among which the use of films and coatings prepared from natural products seems highly promising given the health-related problems of synthesized preservatives and the deteriorative effects of thermal [2–4]. Various organic substances such as carbohydrates, lipids, and proteins can be used to develop edible films and coatings [5]. Among these substances, polysaccharides are highly regarded for such applications due to possessing appropriate film-forming characteristics.

Consumers are highly interested in the incorporation of essential oils into food products given the benefits of such natural additives. *Zataria multiflora* Boiss., which grows in Iran, Pakistan, and Afghanistan [6–8], has antiseptic, analgesic, and carminative properties [9]. Due to containing a large amount of phenolic oxygenated monoterpenes (e.g., menthol and carvone), *Z. multiflora* essential oil (ZMEO) possesses potent activity against both microbes and oxidants [10, 11]. A novel research issue that is yet to be explored is the supplementation of sodium caseinate-based films with ZMEO and the evaluation of the related applications in the packaging of real foods (e.g., veal meat). Hence, the current study aimed to examine caseinate coatings supplemented with ZMEO in terms of their effects on the chemical, microbiological, and sensorial properties of veal stored for a period of 15 days at 4°C.

2. Materials and Methods

2.1. Extraction of the Essential Oil. The *Z. multiflora* Boiss. plant was obtained locally in Shiraz, Iran (30.060 N, 52.560 E). To extract the ZMEO, we followed the technique of Moosavi-Nasab et al. [12]. In brief, the aerial segments of *Z. multiflora* were dried and then placed in a Clevenger type (Jal Tajhiz, Iran) apparatus for 3 h to allow hydrodistillation. The distilled ZMEO was dried over anhydrous sodium sulphate (Merck Co., Germany). Ahead of experimentation, the samples were sealed within dark vials and kept at a temperature of −18°C.

2.2. GC-FID Characterization. To determine the chemical composition of the ZMEO, a gas chromatograph device (Agilent 7890A) containing a flame ionization detector (FID) was used. The experimentation was done on a fused silica capillary HP-5 column (30 m, 0.32 mm i.d.; film thickness 0.25 mm) and 250°C and 280°C were used as the injector and detector temperatures, respectively. The carrier gas used was nitrogen, with a flow rate of 1 mL/min being employed; the temperature of the oven was boosted at a constant rate of 4°C per minute from 60 to 210°C, before being augmented to a final temperature of 240°C at a rate of 20°C per minute. The final temperature was maintained for 8.5 min. A split ratio of 1 : 50 was used.

2.3. Preparation of Coating Solutions. To prepare the coating solutions, 50 g of sodium caseinate (Merck Co., Germany) was gradually added under constant stirring at 550 rpm to a solution (at 60–65°C) composed of 1 L of distilled water and 15 g of glycerol (Merck Co., Germany). Stirring and heating of the mixture took place for an hour using a heater stirrer (IKA® RCT basic, Staufen, Germany) set at 80 ± 3°C in an effort to enhance the mechanical properties of films by establishing disulphide bounds in casein structure. To eliminate any undissolved particles, the solution was then passed through Whatman filter paper (No. 3). The prepared solution was then distributed equally across four different groups. Subsequently, the ZMEO (0.5, 1, or 1.5% v/v) was incorporated into three of the prepared film solutions, with the fourth solution containing only sodium caseinate. The immersion technique was used to coat the meat samples. To remove excess biopolymer solution from coated samples, they were allowed for around 10 minutes at ambient temperature. Storage of the veal occurred over a period of 15 days at 4°C, with experimentations being performed every five days.

2.4. Chemical Characterization. To obtain meat quality over the storage, the pH variations were evaluated using the technique presented by Moosavi-Nasab et al. [12]. Briefly, meat specimens (10 g) were homogenized for five min in a stomacher blender (Jal Tajhiz, Iran) with 10 volumes of deionized water. Then, the electrode of a Suntex TS-1 pH-meter (Taiwan) was directly immersed into the sample to evaluate the pH. Peroxide values were evaluated following the technique of Shahamirian et al. [13]; the results were reported as the mmol of O_2 per kg of the veal. To evaluate the extent of lipid oxidation, the thiobarbituric acid reactive substance (TBARS) values were determined and reported in terms of the milligrams of malondialdehyde (MDA) per kilogram of the veal. In this procedure, a mixture of veal specimens and trichloroacetic acid (Merck Co, Germany) was centrifuged before the filtrate was vortexed together with thiobarbituric acid. After homogenization, the resulting samples were incubated for 20 min within a water bath set at 97°C. Finally, absorbance was evaluated at a wavelength of 532 nm. The calibration curve was prepared using a precursor of MDA called 1,1,3,3-tetramethoxypropane (99%). To convert the final results related to 1M of 1,1,3,3-tetramethoxypropane equivalent per gram of the veal specimens, the values were multiplied by the MDA's molecular weight.

2.5. Microbiological Analysis. The microbiological population of meat samples over the storage was analyzed using the technique described by Moosavi-Nasab et al. [12]. First, a Stomacher blender was used to mix 10 g veal samples with 90 ml of saline solution (as the diluent). Subsequently, two further dilutions were made and 1 mL samples were added to the culture media 15 mL situated within Petri dishes. The bacterial counts were made in duplicate and were reported in terms of log CFU per gram. The culture media (all supplied by Merck Co.) and incubation conditions varied for the different investigations. Plate count agar was used for the psychrotrophic bacteria and total viable counts, while the Enterobacteriaceae and lactic acid bacteria were enumerated after growth on violet red bile agar and MRS agar, respectively. The incubation duration and temperature prior to each of the four counts in the order mentioned were 10 days at 4 ± 2°C, two days at 37 ± 2°C, one day at 37 ± 2°C, and two to three days at 37 ± 2°C, respectively.

2.6. Sensory Characterization. To assess the veal samples in terms of changes in organoleptic characteristics during storage, ten panelists who were trained for such experimentations were recruited. The panel was blind to the nature of each sample. Each panelist was asked to score the samples from one (dislike extremely) to nine (like extremely) on a hedonic scale in terms of texture, odor, color, and overall acceptability. Sample acceptability was defined as a mean sensory score of five or above [3].

2.7. Color Variables and Visual Scores. Color variables of meat samples were evaluated at 1, 5, 10, and 15 days according to the procedure of Hosseini et al. [14]. For each sample, the L^* (brightness), a^* (red-green nature), and b^* (yellow-blue nature) measurements were made using Adobe Photoshop® CS6 at six different points that were selected at random from the entire surface of the veal samples. The photos were taken using a Canon PowerShot A540 (resolution: six megapixels) within a wooden box ($50 \times 50 \times 60\,cm^3$) under natural daylight (6500 K) [14].

2.8. Statistical Analysis. The experimentation was done in triplicate. Significant differences between means were determined using the SAS 9.1 program (SAS Inc., USA) at a significance level of 0.05; the tests used were Duncan's test and one-way analysis of variance (ANOVA) [15].

3. Results and Discussion

3.1. Chemical Composition of ZMEO. A 1.19% extraction efficiency was achieved for ZMEO. The composition of this essential oil is summarized in Table 1. Through the GC/FID study, a total of 14 different components (98.49%) were identified in the ZMEO, with carvacrol (42.22%) and thymol (26.93%) being the chief constituents. These findings are in agreement with those of Ziaee et al. [15], who used the same technique and identified 14 components of ZMEO, among which carvacrol (39.29%) and thymol (25.24%) were the chief components. Furthermore, Moradi et al. [16] stated that the content of ZMEO in the aerial portion of *Z. multiflora* Boiss. was approximately 1.2% v/w, with carvacrol (41.2%) and thymol (27.4%) again being the major components. Other results show that the proportion of the components of ZMEO varies according to the origin of the *Z. multiflo*ra Boiss. plant, although the chief components of this essential oil are monoterpene hydrocarbons, oxygenated monoterpenes, thymol, and carvacrol.

3.2. Chemical Analysis. Table 2 summarizes the chemical properties of the veal specimens during refrigeration (4°C) for 15 days. The initial pH values of coated meat samples with sodium caseinate (control) and sodium caseinate incorporated with 0.5, 1, and 1.5% ZMEO were 5.50, 5.39, 5.38, and 5.28, respectively. These results are in line with those of a different study [17]. However, different species, diet, season, and stress level before and during slaughtering can lead to different pH values among meats. The pH differences among meat samples can be derived from coating solutions pH and higher concentration of ZMEO. All meat samples experienced considerable rises in pH values during storage. In the control sample, this could be explained by the activity of enzymes (e.g., protease and lipase) present in the meat and/or microbes, which leads to an increased concentration of volatile bases like ammonia and trimethylamine [18]. Generally, the veal specimens coated with the sodium caseinate/ZMEO coatings maintained lower pH values compared with the control. This could be explained by the antimicrobial activity of the ZMEO given the strong presence of constituents like thymol and carvacrol.

The primary oxidation products were measured by peroxide valued index. Meat products possess high susceptibility to both microbial spoilage and chemical deterioration [19]. Table 2 summarizes the impact of the coatings on the alteration of the PV in the veal samples. While all samples (control/treatments) experienced significant increments in PVs during storage ($P < 0.05$), the samples coated with sodium caseinate/ZNEO had significantly lower PVs ($P < 0.05$) than the control on day 15. Hence, the treatments were able to impede the process of peroxidation in veal during refrigerated storage. Sharififar et al. [20] found ZMEO to effectively inhibit the oxidation of linoleic acid and proposed this to be the result of the free radical scavenging activity of the phenolic compounds present in ZMEO. In our study, the samples coated with sodium caseinate alone showed the highest PV values, which reached 7.82 on day 15. Significantly lower PV values were obtained for treated samples, especially for those coated with higher ZMEO concentration. Table 2 summarizes the findings related to the TBARS analyses for veal specimens stored at 4°C. The lipid oxidation of all meat samples was initially low (below 0.5 mg MDA/kg). In general, the TBARS values gradually increased until the tenth day of storage, after which they fell until the closure of the experimentation (day 15). A similar trend has been reported by Chouliara et al. [21] in regard to chicken breasts. These results are explained by the production of MDA during the early days of storage ahead of its breakdown, which occurred toward the end of the experimentation. It should be noted, however, that, relative to the control sample, the MDA values of the veal fell significantly ($P < 0.05$) with the addition of different ZMEO concentrations (0.5, 1, or 1.5%). The vast amounts of phenolic components in ZMEO give rise to excellent antioxidant characteristics. These findings are in line with those reported in the literature [1, 22]. Furthermore, we found that the sodium caseinate coating significantly promoted the activity of ZMEO (1 or 1.5%) against lipid oxidation, which can perhaps be ascribed to the protective impact of sodium caseinate on the phenolic compounds of ZMEO [23].

3.3. Microbiological Analysis. Table 3 summarizes the microbiological parameters of meat samples coated with sodium caseinate with and without different concentrations of ZMEO during 15 days of refrigerated (4°C) storage. The initial total plate count (TPC) of control and control plus 0.5, 1, and 1.5% ZMEO meat samples was 4.16, 4.22, 3.88, and 3.22 log CFU/g, respectively. In line with the results of similar studies, the TPC progressively increased in the control sample [12, 24–26]. After 10 days, the TPC of the control sample and control plus 0.5% ZMEO passed the suggested limit for raw meat (7 log CFU/g), reaching 7.64 and 7.42 log CFU/g, respectively [27]. However, the TPCs of veal specimens coated with sodium caseinate and 1–1.5% ZMEO stayed under 6 log CFU/g throughout the 15 days of storage. A number of researchers have stated that the utilization of coatings enriched with antimicrobial agents can prolong fresh quality of meat and meat products [12, 17, 27].

Table 3 also outlines the alterations in the PBC of the veal samples during the storage time. The initial count for control and control plus 0.5, 1, and 1.5% ZMEO was 2.34, 2.4, 2.01, and 1.68 log CFU/g, respectively. During storage, meat samples coated by sodium caseinate plus 1.5% ZMEO had the minimum PBC. After the 15-day storage period had come to an end, the PBC of the control and control plus 0.5, 1, and 1.5% ZMEO coated samples was 6.6, 5.8, 3.8, and 2.5 log CFU/g, respectively. Hence, ZMEO had a remarkable effect against the psychrophilic bacteria present in refrigerated meat samples.

Table 1: Chemical compositions of essential oil obtained by hydrodistillation from *Zataria multiflora* using GC/FID.

No	Compound	Retention index	Retention time (min)	Relative peak area (%)
1	α-Thujene	924	1.533	0.1532
2	α-Pinene	932	4.232	3.933
3	3-Octanone	984	5.620	3.203
4	Myrcene	988	6.527	1.202
5	α-Terpinene	1014	6.849	10.87
6	p-Cymene	1020	7.931	2.239
7	γ-Terpinene	1054	13.060	0.4015
8	Linalool	1095	15.422	0.5058
9	Carvacrol methyl ether	1241	17.863	0.9719
10	**Thymol**	**1289**	**18.363**	**26.93**
11	**Carvacrol**	**1298**	**18.776**	**42.22**
12	Eugenol	1361	23.088	1.268
13	Carvacrol acetate	1370	29.882	2.346
14	β-Caryophyllene	1417	40.757	2.253

Entries in bold are the main components of *Zataria multiflora* essential oil.

Table 2: Changes in chemical properties of SC and SC + ZMEO coated meat samples during 15 days' storage at refrigerated temperatures[a].

		Storage days at 4°C			
		1	5	10	15
pH	SC	5.50 ± 0.01^{Da}	5.92 ± 0.02^{Ca}	6.47 ± 0.03^{Ba}	7.82 ± 0.04^{Aa}
	SC + 0.5% ZMEO	5.45 ± 0.04^{Da}	5.82 ± 0.04^{Ca}	6.58 ± 0.03^{Ba}	7.42 ± 0.07^{Ab}
	SC + 1% ZMEO	5.38 ± 0.06^{Da}	5.65 ± 0.03^{Cb}	5.91 ± 0.07^{Bb}	6.32 ± 0.07^{Ac}
	SC + 1.5% ZMEO	5.38 ± 0.11^{Ca}	5.33 ± 0.04^{Cc}	5.69 ± 0.02^{Bc}	5.89 ± 0.05^{Ad}
PV	SC	1.38 ± 0.07^{Da}	3.78 ± 0.11^{Ca}	5.94 ± 0.07^{Ba}	7.82 ± 0.05^{Aa}
	SC + 0.5% ZMEO	1.45 ± 0.08^{Da}	3.11 ± 0.09^{Cb}	3.98 ± 0.06^{Bb}	5.66 ± 0.04^{Ab}
	SC + 1% ZMEO	1.28 ± 0.06^{Da}	2.61 ± 0.13^{Cc}	3.36 ± 0.05^{Bc}	4.21 ± 0.05^{Ac}
	SC + 1.5% ZMEO	1.33 ± 0.9^{Da}	1.87 ± 0.08^{Cd}	2.06 ± 0.04^{Bd}	2.67 ± 0.05^{Ad}
TBARS	SC	0.28 ± 0.12^{Ca}	0.49 ± 0.08^{Ca}	1.29 ± 0.07^{Aa}	1.08 ± 0.04^{Ba}
	SC + 0.5% ZMEO	0.31 ± 0.11^{Ca}	0.43 ± 0.04^{Ca}	1.12 ± 0.08^{Ab}	0.88 ± 0.05^{Bb}
	SC + 1% ZMEO	0.27 ± 0.14^{Ca}	0.33 ± 0.06^{Cb}	0.86 ± 0.02^{Ac}	0.71 ± 0.09^{Bc}
	SC + 1.5% ZMEO	0.31 ± 0.12^{Ba}	0.33 ± 0.08^{Bb}	0.62 ± 0.07^{Ad}	0.51 ± 0.04^{Ad}

SC, sodium caseinate; ZMEO; *Zataria multiflora* essential oil. Data represent the mean value of three replicates ± SD. Means within each row with different uppercase letters are significantly different ($P < 0.05$), and means within each column with different lowercase letters are significantly different ($P < 0.05$).

Meat spoilage is mostly the result of the activity of lactic acid bacteria (LAB), with certain species (*Lactobacillus* spp., *Carnobacterium* spp., and *Leuconostoc* spp.) being more involved in this process [28]. In our study, the initial LAB counts of control and control plus 0.5, 1, and 1.5% ZMEO coating in meat samples were 1.28, 1.33, 1.28, and 1.16 log CFU/g, respectively. The minimum LAB counts were found in the veal samples that had the sodium caseinate + 1.5% ZMEO coating, amounting to 1.16 and 2.88 log CFU/g on days 1 and 15, respectively. The LAB count was significantly different between the various samples ($P < 0.05$), though a progressive rise in the number of LAB was generally apparent. On day 15, the LAB counts of control and control plus 0.5, 1, and 1.5% ZMEO coated samples increased to levels of 5.22, 4.6, 3.84, and 2.88 log CFU/g, respectively. It should be noted that, among the various types of Gram-positive bacteria, LAB reportedly possess the maximum resistance against essential oils [29]. According to our results, LAB growth was markedly stunted in specimens coated with sodium caseinate and elevated concentrations of ZMEO, which could be related to the presence of phenolic compounds in the essential oil. Frangos et al. [30] proposed that such antimicrobial resistance of LAB is a result of the ability of these species to deal with both the osmotic stress and the efflux of potassium ions induced by the essential oils. In another research, Khorsandi et al. (2018) examined 5 different essential oils against LAB causing spoilage in vacuum packed curd sausage. They reported that EOs have antimicrobial activity against LAB and their activity depended on their main components. The minimum growth of LAB was seen in the veal specimen coated with sodium caseinate/1.5% ZMEO, confirming the favorable antimicrobial properties of ZMEO [31].

Table 3 also depicts the results related to Enterobacteriaceae, for which the initial counts of control and control plus 0.5, 1, and 1.5% ZMEO coating were 3.12, 3.22, 3.08, and 2.96 log CFU/g, respectively. At the end of storage, the control specimen had an Enterobacteriaceae count of 7.8 log CFU/g. However, meat specimens coated with sodium caseinate incorporated with 1 and 1.5% ZMEO had 2 and 3.3 log CFU/g less counts than the control, respectively (Table 3). This could be explained by the activity of ZMEO against such spoilage bacteria, which has also been described by other researchers [4, 15, 16]. The meat sample coated with

Table 3: Changes in microbial counts of SC and SC + ZMEO coated meat samples during 15 days of storage at refrigerated temperatures[a].

		Storage days at 4°C			
		1	5	10	15
APC	SC	4.16 ± 0.11^{Da}	5.11 ± 0.16^{Ca}	7.64 ± 0.32^{Ba}	8.35 ± 0.08^{Aa}
	SC + 0.5% ZMEO	4.22 ± 0.08^{Da}	5.33 ± 0.12^{Ca}	7.42 ± 0.08^{Ba}	8.21 ± 0.12^{Aa}
	SC + 1% ZMEO	3.88 ± 0.06^{Db}	4.22 ± 0.22^{Cb}	4.82 ± 0.14^{Bb}	5.54 ± 0.07^{Ab}
	SC + 1.5% ZMEO	3.22 ± 0.12^{Dc}	3.36 ± 0.08^{Cc}	3.88 ± 0.11^{Bc}	4.42 ± 0.21^{Ac}
PBC	SC	2.34 ± 0.08^{Da}	3.82 ± 0.15^{Ca}	4.9 ± 0.12^{Ba}	6.6 ± 0.09^{Aa}
	SC + 0.5% ZMEO	2.4 ± 0.12^{Da}	2.62 ± 0.11^{Cb}	3.2 ± 0.18^{Bb}	5.8 ± 0.08^{Ab}
	SC + 1% ZMEO	2.01 ± 0.11^{Db}	2.45 ± 0.14^{Cb}	3.3 ± 0.12^{Bc}	3.8 ± 0.12^{Ac}
	SC + 1.5% ZMEO	1.68 ± 0.06^{Cc}	1.99 ± 0.09^{Bc}	2.2 ± 0.11^{Bd}	2.5 ± 0.06^{Ad}
LAB	SC	1.28 ± 0.12^{Da}	2.67 ± 0.12^{Ca}	4.22 ± 0.15^{Ba}	5.22 ± 0.09^{Aa}
	SC + 0.5% ZMEO	1.33 ± 0.08^{Da}	2.43 ± 0.17^{Ca}	3.8 ± 0.13^{Bb}	4.6 ± 0.12^{Ab}
	SC + 1% ZMEO	1.28 ± 0.08^{Da}	2.02 ± 0.19^{Cb}	2.92 ± 0.14^{Bc}	3.84 ± 0.16^{Ac}
	SC + 1.5% ZMEO	1.26 ± 0.15^{Da}	1.88 ± 0.12^{Bb}	2.12 ± 0.11^{Bd}	2.88 ± 0.15^{Ad}
EBC	SC	3.12 ± 0.06^{Da}	4.62 ± 0.11^{Ca}	6.8 ± 0.12^{Ba}	7.8 ± 0.22^{Aa}
	SC + 0.5% ZMEO	3.22 ± 0.09^{Da}	4.5 ± 0.10^{Ca}	7.1 ± 0.17^{Ba}	7.5 ± 0.14^{Aa}
	SC + 1% ZMEO	3.18 ± 0.13^{Da}	3.88 ± 0.05^{Cb}	5.2 ± 0.15^{Bb}	5.8 ± 0.12^{Ab}
	SC + 1.5% ZMEO	2.96 ± 0.12^{Da}	3.12 ± 0.14^{Cc}	3.9 ± 0.19^{Bc}	4.5 ± 0.18^{Ac}

SC, sodium caseinate; ZMEO, *Zataria multiflora* essential oil; TPC, total plate count; PBC, psychrotrophic bacterial count; LAB, lactic acid bacteria; EBC, Enterobacteriaceae counts. Data represent the mean value of three replicates ± SD. Means within each row with different uppercase letters are significantly different ($P < 0.05$), and means within each column with different lowercase letters are significantly different ($P < 0.05$).

sodium caseinate plus 1.5% ZMEO had the minimum count, highlighting the potent activity of ZMEO against the bacteria. Ziaee et al. [15] examined the mechanisms by which ZMEO exerts its antibacterial activity against *L. curvatus*. They reported that carvacrol and thymol, as the chief constituents of ZMEO, were the main antibacterial agents. Moreover, other researchers have also reported that these two compounds are mostly responsible for both the antibacterial and antioxidant activity of ZMEO [32–36]. Despite the fact that the bacterial counts for all veal specimens increased over time, the rate of this increment was significantly lower ($P < 0.05$) among the samples coated with SC/ZMEO relative to the control.

3.4. Color Variables. Table 4 shows the results related to the color parameters of the veal specimens during storage. Meat samples coated with sodium caseinate and sodium caseinate incorporated with 0.5% ZMEO after 10 days were sticky due to spoilage, meaning that the color analysis was probably inaccurate; data related to those samples are hence not shown.

Coating materials can change consumer acceptability of food, since optical properties of an edible coating depending on the material type and concentration can change the overall appearance of food. Furthermore, myoglobin is a protein that mostly determines the color of meat; this protein takes the form of deoxymyoglobin or oxymyoglobin depending on the availability of oxygen, thereby influencing consumer acceptance [37]. Hence, color evaluation of a meat product during its shelf life is essential. Table 4 summarizes the color variables (L^*, a^*, and b^*) during the refrigerated storage for all treatments. The L^*, or lightness values, showed a decreasing rate over the storage for all specimens. The control had the minimum L^* value after ten days of storage, probably due to alterations in meat color secondary to protein conformational changes that occurred due to oxidizing reactions and microbial growth [38]. In this regard, Soladoye et al. [39] reported that cross-linking between proteins and the carbonylation of protein molecules are related to decreases in muscle protein function and changes in the sensory characteristics of meat products. Over the 15-day period, the control sample underwent a significant decrease ($P < 0.05$) in terms of its a^* or redness value; this color loss was significantly less in the coated samples ($P < 0.05$). In fact, at the end of the storage period, the coated samples had a^* values higher than 10, representing a bright red color [40]. A similar trend was seen in the b^* values, with the drop in this parameter being more pronounced in the control sample ($P < 0.05$). Again, the coated samples had the maximum b^* values, though this was probably because of the yellowish color of the coating and ZMEO.

3.5. Sensory Analysis. In order to attain the desired antioxidative and antimicrobial performance, elevated concentrations of essential oils are required. However, this gives rise to concerns regarding the effects of such oils on the sensory attributes of food products; this is particularly important for the essential oils of plants such as oregano and *Z. multiflora* Boiss., which exert strong flavors and odors [3]. Table 5 summarizes the sensory scores related to the color, texture, odor, and overall acceptability of the veal samples, with a general decrease over time being apparent. During the initial part of the storage time, the introduction of elevated ZMEO concentrations resulted in decreased odor scores. The samples coated with sodium caseinate and 0.5% and 1% ZMEO achieved the maximum sensory scores during the study time. Due to unsuitable organoleptic characteristics, the taste and

Table 4: Changes in color variables of SC and SC + ZMEO coated meat samples during 15 days of storage at refrigerated temperatures[a].

		Storage days at 4°C			
		1	5	10	15
L*	SC	41.08 ± 0.43^{Aa}	38.95 ± 0.16^{Ba}	31.25 ± 0.33^{Cc}	—
	SC + 0.5% ZMEO	40.56 ± 0.26^{Aa}	38.12 ± 1.08^{Bab}	33.42 ± 0.27^{Cb}	—
	SC + 1% ZMEO	37.54 ± 018^{Ab}	37.95 ± 0.23^{Bbc}	36.05 ± 0.41^{Ca}	35.11 ± 0.09^{Da}
	SC + 1.5% ZMEO	36.06 ± 0.38^{Ab}	35.95 ± 0.13^{Ac}	34.28 ± 0.37^{Bab}	33.11 ± 0.12^{Cb}
a*	SC	12.54 ± 0.11^{Ac}	10.11 ± 0.14^{Bd}	7.92 ± 0.22^{Cc}	—
	SC + 0.5% ZMEO	12.45 ± 0.08^{Ac}	11.09 ± 0.11^{Bc}	7.65 ± 0.22^{Cc}	—
	SC + 1% ZMEO	13.87 ± 0.18^{Ab}	13.93 ± 0.09^{Ab}	12.26 ± 0.28^{Bb}	13.11 ± 0.08^{Ab}
	SC + 1.5% ZMEO	15.11 ± 0.22^{Aa}	15.45 ± 0.21^{Aa}	15.87 ± 0.08^{Aa}	15.33 ± 0.09^{Aa}
b*	SC	15.19 ± 0.17^{Ad}	13.23 ± 0.32^{Bd}	11.41 ± 0.19^{Cc}	—
	SC + 0.5% ZMEO	16.76 ± 0.21^{Ac}	14.76 ± 0.14^{Bc}	11.09 ± 0.06^{Cc}	—
	SC + 1% ZMEO	17.33 ± 0.35^{Ab}	17.45 ± 0.08^{Ab}	16.11 ± 0.08^{Bb}	17.09 ± 0.04^{Ab}
	SC + 1.5% ZMEO	19.05 ± 0.19^{Aa}	18.98 ± 0.17^{Aa}	19.27 ± 0.11^{Aa}	19.08 ± 0.13^{Aa}

SC, sodium caseinate; ZMEO, *Zataria multiflora* essential oil. Data represent the mean value of three replicates ± SD. Means within each row with different uppercase letters are significantly different ($P < 0.05$), and means within each column with different lowercase letters are significantly different ($P < 0.05$).

Table 5: Changes in sensorial properties of SC and SC + ZMEO coated meat samples during 15 days of storage at refrigerated temperatures[a].

		Storage days at 4°C			
		1	5	10	15
Color	SC	8.8 ± 0.42^{Aa}	6 ± 0.82^{Bb}	4.6 ± 0.84^{Cb}	1.5 ± 0.71^{Dc}
	SC + 0.5% ZMEO	8.9 ± 0.32^{Aa}	7.9 ± 0.74^{Aab}	5.9 ± 0.99^{Bab}	3.2 ± 0.42^{Cb}
	SC + 1% ZMEO	8.8 ± 0.42^{Aa}	8.7 ± 0.48^{Aa}	7.6 ± 0.70^{AB}	5.9 ± 0.99^{Ba}
	SC + 1.5% ZMEO	8.7 ± 0.48^{Aa}	8.7 ± 0.48^{Aa}	7.6 ± 0.70^{ABaa}	6.6 ± 0.70^{Ba}
Odor	SC	9 ± 0.00^{Aa}	6.9 ± 0.52^{Bab}	3.1 ± 0.74^{Cb}	1.5 ± 0.71^{Dc}
	SC + 0.5% ZMEO	8.9 ± 0.32^{Aa}	7.9 ± 0.74^{Aa}	4.6 ± 0.84^{Bab}	3.2 ± 0.42^{Bb}
	SC + 1% ZMEO	8.6 ± 0.52^{Aa}	8.2 ± 0.63^{Aa}	5.9 ± 0.99^{Ba}	6.7 ± 0.67^{Ba}
	SC + 1.5% ZMEO	6.9 ± 0.52^{Ab}	6 ± 0.82^{Ab}	5.9 ± 0.99^{Aa}	6.1 ± 0.57^{Aa}
Texture	SC	8.8 ± 0.42^{Aa}	6.6 ± 0.70^{Bb}	4.6 ± 0.84^{Cb}	3.6 ± 0.57^{Cc}
	SC + 0.5% ZMEO	8.8 ± 0.42^{Aa}	7.6 ± 0.70^{ABab}	5.9 ± 0.99^{BCb}	4.5 ± 0.85^{Cbc}
	SC + 1% ZMEO	8.7 ± 0.48^{Aa}	8.2 ± 0.63^{ABa}	6.6 ± 0.70^{Bb}	5.9 ± 0.99^{Bab}
	SC + 1.5% ZMEO	8.8 ± 0.42^{Aa}	8.8 ± 0.42^{Aa}	8.2 ± 0.63^{ABa}	7.6 ± 0.70^{Ba}
Overall	SC	8.8 ± 0.42^{Aa}	6 ± 0.82^{Bb}	4.6 ± 0.84^{Cb}	3.2 ± 0.42^{Cb}
	SC + 0.5% ZMEO	8.9 ± 0.32^{Aa}	7.6 ± 0.70^{ABab}	5.9 ± 0.99^{BCab}	4.4 ± 0.97^{Cab}
	SC + 1% ZMEO	8.8 ± 0.42^{Aa}	8.2 ± 0.63^{ABa}	6.4 ± 0.70^{Ca}	5.9 ± 0.99^{Ca}
	SC + 1.5% ZMEO	7.9 ± 0.74^{Aa}	7.6 ± 0.70^{ABab}	7.6 ± 0.70^{ABa}	6.2 ± 0.63^{Ba}

SC, sodium caseinate; ZMEO, *Zataria multiflora* essential oil. Data represent the mean value of three replicates ± SD. Means within each row with different uppercase letters are significantly different ($P < 0.05$), and means within each column with different lowercase letters are significantly different ($P < 0.05$).

odor of some specimens could not be evaluated on the 15th day of storage. Notably, the veal specimens coated with 1.5% ZMEO achieved the maximum scores for texture and overall acceptability. In line with similar studies, the sensory acceptability was confirmed with scores higher than five [3, 26, 41]. In terms of overall acceptability, the control sample fell below this limit prior to the tenth day of storage (below 5), whereas the samples coated with sodium caseinate/ZMEO (1 and 1.5%) maintained their overall acceptability until the completion of the 15-day study period. Ojagh et al. [4] and Jouki et al. [3] examined fish fillets and found that biopolymer-based antimicrobial coatings could provide significantly higher overall acceptability scores. Furthermore, Moosavi-Nasab et al. [12] described the beneficial effect of essential oils in terms of the overall quality and product life of fish fillets.

4. Conclusion

It can be concluded that packaging films comprised of sodium caseinate and ZMEO are able to extend the product life of veal and preserve its sensory traits by postponing both chemical and microbial alterations. Given the consumer demand for the use of natural alternatives to synthetic additives, we recommend the use of ZMEO-incorporated coatings for the preservation of meat products as they were found to possess considerable activity against both oxidants and microbes during the refrigerated storage of veal. Furthermore, the phenolic degradation of ZMEO was impeded

by the low temperature (4°C), meaning that the beneficial activity of ZMEO was sustained during the storage time. Ultimately, we found that while SC enriched with 1.5% ZMEO provided the maximum inhibition of microbes and highest prevention of chemical alterations, it gave rise to unpleasant sensory traits such as odor and probably taste. Therefore, SC plus 1% ZMEO is recommended to prolong shelf life of meat without any undesirable effect on sensory properties.

Data Availability

All data and their analysis are included within the manuscript (tables). Raw data are available from the corresponding author upon request (hlashkari@gmail.com).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Chitosan-Based Coatings Incorporated with Cinnamon and Tea Extracts to Extend the Fish Fillets Shelf Life: Validation by FTIR Spectroscopy Technique

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The aim of this study was to evaluate the effect of active coatings prepared from the chitosan on the quality parameters of fish fillets. Antimicrobial and antioxidant properties were improved by addition of tea and cinnamon extracts. Different quality parameters including free fatty acids (FFA), thiobarbituric acid value (TBA), trimethylamine (TMA), total volatile basic nitrogen (TVBN), whiteness, and pH of coated and noncoated samples were evaluated during storage for 20 d at 5 ± 1°C. Moreover, FTIR characterization (i.e., wavenumber and absorbance values) was used to investigate the oxidative stability. Extracts addition to chitosan coating had noticeable influence on reducing FFA and TBA. Moreover, modified chitosan coating decreased TVBN and TMA significantly. Based on FTIR finding, control sample showed the highest oxidation value, while the treated samples with chitosan\incorporated with tea and cinnamon extracts (CTCECS) had the lowest oxidation. The results showed that FTIR technique could be successfully applied to monitor the lipid oxidation of fish fillet. Therefore, FTIR provides a fast approach to study the compositional changes of food products rather than conventional chemical analysis. The findings of our research showed that chitosan coating modified with tea and cinnamon extracts could be used as a novel active packaging to prolong the shelf life quality of fish fillet.

1. Introduction

Fish fillet is one of the main highly perishable food products, which can be spoiled with different mechanisms such as microbiological, chemical, and enzymatically changes. These mechanisms reduced the shelf life of fish fillet and the nutritional quality parameters during storage time. Over the last few years, researchers used several methods to increase storage ability of fish fillets such as high-pressure treatment, cold storage and freezing, vacuum packaging, and modified atmosphere packaging. Among them, active edible films and coatings provide the promising opportunities to extend the fish shelf life [1, 2].

The main biopolymers which can be used as bio-based coatings are proteins, polysaccharides, lipids, or their combination. The biopolymer films can be controlled by the moisture and gas barriers properties. Incorporating natural preservatives, including extracts with good antimicrobial and antioxidant properties, flavours, spices, and colourants to film and coatings, can improve the functionality of edible packaging to shelf life extension of food products such as fish samples [3] by controlling the active components release into the surface. Chitosan shows great potential of applications in different food products [4]. Some useful technofunctional properties of chitosan are antioxidant activity, antimicrobial activity, and application as active coatings [4, 5]. Several recent studies have shown the effect of essential oils-incorporated edible films on shelf life extension of fish samples. Ojagh et al. [4] reported the effects of a chitosan coating loaded with cinnamon oil on the quality of rainbow trout. Jeon et al. [6] also confirmed the reduction of moisture loss and lipid oxidation for Atlantic cod and herring samples treated with chitosan coatings. Souza et al. [7] showed that chitosan edible films could be used as an alternative to improve the shelf life of salmon fillets due to its excellent antibacterial and antioxidant activities.

The main active components of cinnamon extract are phenolic ingredients including proanthocyanidins, which are a group of flavonoids created by flavan-3-ol polymers and oligomers [8]. The aqueous extracts extracted from different varieties of cinnamon are known to have some beneficial influence on health including antioxidant, antidiabetic, and antimicrobial properties [9]. Tea is an important source of polyphenol ingredients, such as catechins. Tea extract also has other ingredients, including phenolic acids and flavonoids but in lower proportion [10], which is recognized by its antimicrobial, antioxidant, anti-inflammatory, and anticarcinogenic properties [11].

The aim of our research was to evaluate the influence of chitosan coatings incorporated with tea and cinnamon extracts on the quality parameters of fish fillets during refrigerated storage. Moreover, the FTIR spectroscopy was used as a promising tool to assess the fish fillet oxidation behavior in a fast and cost-effective approach.

2. Materials and Methods

2.1. Materials. Tea and cinnamon plants were prepared from a local market (Kazerun, Iran). Chitosan was obtained from Sigma-Aldrich (St. Louis, USA). 1,3,3-tetraethoxypropane, thiobarbituric acid (TBA), FRAP, methanol, hydrochloric acid, tert-butylhydroquinone (TBHQ), sodium hydroxide, trichloroacetic acid (TCA), and ethanol were prepared from Merck Co. (Darmstadt, Germany). Also, double distilled water (DDW) was applied to prepare dispersions.

2.2. Extract Preparation. Plant powder (10 g) was completely mixed with hot distilled water (100 mL) for 30 min at 100°C followed by filtration (Whatman grade No. 1 filter papers) and concentrated in a rotary evaporator at 50°C and finally freeze-dried.

2.3. Antioxidant Properties of Coating Materials. TPC was determined by Folin–Ciocalteu based on the technique modified by Shahbazi et al. [12]. The method of the FRAP assay was modified by Justine et al. [13].

2.4. Fish Sample Preparation and Coating. To prepare coating solution, 0.2 g chitosan with 30–32 kD MW was dispersed in 100 mL distilled 1% w/w acetic acid; then, 2 g chitosan with 75 kD MW was added to it in 40°C during 3 h. Glycerol (0.75 mL for 1 g chitosan) was used as a food grade plasticizer in coating suspension. Then, fillets were immersed in the coating solutions. Then, fillets were placed to remove extra solution.

In our research, four treatment were used: (1) the untreated sample (control), (2) coated with tea extract (0.5%) (tea extract-coated sample, TECS), (3) coated with tea extract (0.25%) + cinnamon extract (0.25%) (tea and cinnamon extract-coated sample, TCECS), and (4) coated with chitosan (2%) + tea extract (0.25%) + cinnamon extract (0.25%) (chitosan, tea, and cinnamon extract-coated sample, CTCECS). The fish fillets were kept at 5°C, and evaluations were performed every five days for twenty days.

2.4.1. Free Fatty Acid (FFA) Measurement. FFA of samples was determined by a standard method introduced by AOAC. Fillets (15 g) were homogenized in a solvent (120 mL) of chloroform-methanol (1 : 1 V/V). After 24 h, 48 mL of water was added, and oil was collected. The extracted oil in presence of phenolphthalein was titrated by sodium hydroxide. FFA content are reported as an oleic acid percentage in the sample [14].

2.4.2. Thiobarbituric Acid Reactive (TBA) Measurement. The colorimetric technique modified by Joukar et al. [1] was used for measuring the secondary lipid oxidation products content of the fillets. Homogenized fillet (10 g) was added into 97.5 mL of DDW and 2.5 mL HCl 4 M. Mixture (5 mL) was mixed with fresh thiobarbituric reactive solution (4 mL). Sample was kept in an oil bath (90°C–95°C) for 0.5 h and then arrived to 25°C immediately. The sample absorbance (As) was determined against the blank (water) (A_b) at 532 nm. TBARS contents of sample were measured based on the following equation:

$$\text{TBARS}\left(\frac{\text{mg of malondialdehyde}}{\text{kg of sample}}\right) = 50 \times \left[\frac{As - A_b}{200}\right]. \quad (1)$$

2.4.3. Total Volatile Basic Nitrogen (TVBN) Measurement. Fish fillets and MgO were distilled by a Kjeldahl apparatus. Then, distillate vapor moved in a boric acid solution (2%) for titration. The indicators of this titration were methylene blue and methyl red solution (0.1%w/v in ethanol). Titration of suspension was performed with sulfuric acid (0.01°M). TVBN values (mg N_2/100 g of sample) were determined according to Yu et al. [15].

2.4.4. Trimethylamine (TMA) Measurement. TMA content was measured according to the technique modified by Yu et al. [15]. Fillets (10 g) were homogenized with 30 mL TCA solution (7.5% w/v) using a high speed homogenizer for 3 min with 15,000 rpm. The sample was centrifuged at $4000 \times g$ at 5°C for 10 min using a high-speed cryogenic centrifuge, and the supernatants were neutralized using NaOH (1M) for the analysis of TMA. The neutralized supernatant, formaldehyde (20%), anhydrous toluene, and saturated K_2CO_3 were combined in 2 : 1 : 5 : 3 ratio (v/v) in a tube. Then, incubated at 32°C for ten minutes, the toluene layer was combined with anhydrous Na_2SO_4 (0.2 g) and picric acid solution (5 mL, 0.02%, w/v). The absorbance of the sample was determined at 410 nm. TMA standard curve of was used at 0–2 mg/mL.

2.4.5. pH Measurement. Five gram of fillets was mixed in forty-five mL of DDW. Liquid phase was separated by a

Table 1: Total phenol content and antioxidant activity of coating materials.

	TPC (mg/g)	AA (mg/g)
Tea extract	4.97 ± 00.16C	1.79 ± 00.10D
Cinnamon-tea extract	80.61 ± 00.18A	34.83 ± 00.09A
Chitosan powder	0.65 ± 00.16D	3.69 ± 00.10C
Chitosan-cinnamon tea coating	7.71 ± 00.15B	18.66 ± 00.13B

Data represent mean ± standard deviation of three independent batches. Different uppercase letters in each column indicate significant differences ($p < 0.05$).

filter. pH of liquid phase was determined by a pH meter (Sartorius, PB-11, Japan).

2.4.6. Color Measurement. The color of fish fillets was determined by the modified technique introduced by Gahruie et al. [16]. A Canon camera (5 MP) and a box ($0.5 \times 0.5 \times 0.6\ m^3$) with a daylight source was applicated for evaluating color parameters. The lab parameters of samples were extracted by Adobe Photoshop, and whiteness index was calculated based on this parameters.

2.4.7. Fourier Transform Infrared Spectroscopy Measurement. Fish fillets were dried, powdered, and finally homogenized with potassium bromide. The functional groups of sample were evaluated by a FTIR spectrometer (BRUKER, TENSOR, Germany).

2.5. Statistical Analysis. The properties of treatment was analyzed at least triplicate. The findings were evaluated using variance one-way analysis at $p < 0.05$. The averages' significant differences were determined by Duncan's multiple range tests (SAS, V. 9.2, USA).

3. Results and Discussion

3.1. Antioxidant Properties of Coating Materials. Table 1 shows the total phenol content (TPC) and antioxidant activity (AA) of coating materials. The TPC of tea extract, cinnamon-tea extract, chitosan, and chitosan-tea-cinnamon were 4.97, 80.61, 0.65, and 7.71 mg/g, respectively. Also, AA of tea extract, cinnamon-tea extract, chitosan, and chitosan-tea-cinnamon were 1.79, 34.83, 3.69, and 18.66 mg/g, respectively. High antioxidant activity of cinnamon extract is related to phenolic components and reported previously [9, 17]. Also, tea extract is a good source of gallic acid and catechins: catechin, epicatechin, catechin gallate, epicatechin gallate, gallocatechin, epigallocatechin, gallocatechin gallate, and epigallocatechin gallate [11]. The antioxidant activity of chitosan is related to OH groups on the backbone [18].

3.2. Properties of Fillets

3.2.1. Changes in FFA Content. FFAs were produced by degradation of triglycerides, and this phenomenon was called hydrolysis. The content of FFAs can be used as a degree of lipolysis, and it is a parameter for evaluation freshness of fish fillets. Table 2 shows that FFAs were raised during shelf life due to lipolysis of lipids, and this phenomenon decreased the quality of fillets. The same results were reported by Andevari and Rezaei [19]. At the beginning of storage, the content of FFA in all of fish fillets was around 0.45. The FFA content significantly increased during first five days of storage. At the end of storage, CTCECS sample (chitosan coating incorporated with extracts) was the best sample in reducing the FFA production rate. Samples coated with tea (TECS) and tea-cinnamon (TCECS) had lower FFA production rate in comparison with control. Gómez-Estaca et al. [20] studied the influence of gelatin coating modified by oregano and rosemary extracts on the quality parameters of sardine. The same results on the FFA production was reported in this research.

3.2.2. Changes in TBARS Content. Lipid oxidation secondary products such as aldehydes and ketone were reported by TBA value. Malonaldehyde is one of the aldehyde which can produce red color with TBA reagent. There are some different aldehydes which can involve in this reaction. Therefore, the secondary products amounts (TBA value) were reported in mg MDA/kg fillets. Raeisi et al. [21] reported that TBA value under 5 mg MDA/kg of fillets is the standard level for fish quality. Based on Table 2, TBA value of uncoated sample significantly raised during storage time. In this reaction, hydroperoxides convert to secondary oxidation products such as aldehyde [22]. Based on the coating materials, various raising rates were shown in TBA value of samples. Usually, creating a coating layer had good effects on decreasing the lipid oxidation secondary products. The TBA value increase is related to the oxygen presence [23]. Contact of sample with oxygen can be reduced by polymer coatings, and thus, lipid oxidation can be controlled. So, production of aldehyde and ketone in the control was higher than that in coated samples. Phenolic compounds of extracts are potent antioxidants. At day 20, the highest TBA value was determined in control samples, and coated samples with tea and cinnamon in coating formulation with chitosan (CTCECS) had the lowest content. TBA values of untreated and treated fish fillet were under standard level during storage.

3.2.3. TMA Content. TMA was produced from trimethylamine oxide by microorganisms. Researcher reported that production of TMA is the main reason for bad odor in fish fillets, and it is called fishy. Table 3 shows that the TMA content at the beginning of storage was around 0.15 mg/100 g fillet. For chitosan-coated sample (CTCECS), a low increase was observed after 5 days of shelf life and it is arrived to 0.33 mg/100 g fillet. The TMA content of all samples significantly increased during storage. The TMA content of control sample was arrived to 5.17 mg/100 g sample at the end of storage. This sample had bad odor during last week of shelf life due to high content of TMA. The TMA content of tea (TECS), tea-cinnamon (TCECS), and chitosan-tea-cinnamon (CTCECS) samples at the end of storage was 3.13, 2.51, and 2.06 mg/100 g sample,

Table 2: Changes in FFA and TBA values of rainbow trout fillets during storage at 4 ± 1°C.

		Storage (day)			
	Sample	0	5	15	20
FFA (% oleic acid)	Control	0.41 ± 0.09Ad	1.87 ± 0.09Ac	3.28 ± 0.16Ab	4.14 ± 0.26Aa
	TECS	0.35 ± 0.06Ad	1.40 ± 0.11Bc	2.44 ± 0.05Bb	3.24 ± 0.14Ba
	TCECS	0.41 ± 0.03Ad	1.13 ± 0.07Cc	2.10 ± 0.17Bb	2.45 ± 0.05Ca
	CTCECS	0.40 ± 0.04Ad	0.79 ± 0.04Dc	1.38 ± 0.07Cb	2.05 ± 0.06Da
TBA (mg malonaldehyde per kg of sample)	Control	0.33 ± 0.04Ad	0.65 ± 0.03Ac	2.52 ± 0.06Ab	3.71 ± 0.04Aa
	TECS	0.25 ± 0.02Ad	0.39 ± 0.02Bc	2.20 ± 0.03Bb	2.54 ± 0.02Ba
	TCECS	0.26 ± 0.03Ad	0.35 ± 0.03Bc	2.07 ± 0.06Cb	2.22 ± 0.03Ca
	CTCECS	0.24 ± 0.02Ad	0.31 ± 0.02Cc	1.89 ± 0.05Db	2.03 ± 0.05Da

Data represent mean ± standard deviation of three independent batches. At a same time of storage, different uppercase letters indicate significant differences ($p < 0.05$). For same sample, different lowercase letters indicate significant differences ($p < 0.05$) over time. Control, sample without any coating; TECS, sample coated with tea extract (0.5%w/v); TCECS, sample coated with tea (0.25%w/v) + cinnamon (0.25%w/v) extract; and CTCECS, sample coated with chitosan (2% w/v) + tea extract (0.25%w/v) + cinnamon extract (0.25%w/v).

Table 3: Changes in TMA and TVB-N values of rainbow trout fillets during storage at 4 ± 1°C.

		Storage (day)			
	Sample	0	5	15	20
TMA (mg/100 g)	Control	0.20 ± 0.06Ad	0.75 ± 0.03Ac	3.21 ± 0.11Ab	5.17 ± 0.15Aa
	TECS	0.17 ± 0.09Ad	0.64 ± 0.08Bc	2.56 ± 0.15Bb	3.13 ± 0.08Ba
	TCECS	0.14 ± 0.08Ad	0.42 ± 0.08Cc	1.94 ± 0.04Cb	2.51 ± 0.05Ca
	CTCECS	0.13 ± 0.06Ad	0.33 ± 0.04Cc	1.63 ± 0.11Db	2.06 ± 0.11Da
TVB-N (mg N/100 g of sample)	Control	13.09 ± 2.11Ad	31.11 ± 3.10Ac	61.44 ± 4.12Ab	113.01 ± 6.05Aa
	TECS	11.23 ± 2.09Ad	21.10 ± 2.46Bc	43.39 ± 1.20Bb	85.29 ± 4.15Ba
	TCECS	12.34 ± 1.36Ad	17.20 ± 2.12Cc	32.62 ± 3.31Cb	53.87 ± 5.23Ca
	CTCECS	11.45 ± 2.45Ac	14.19 ± 1.09Cc	22.42 ± 1.23Bb	39.50 ± 3.36Ba

Data represent mean ± standard deviation of three independent batches. At same time of storage, different uppercase letters indicate significant differences ($p < 0.05$). For same sample, different lowercase letters indicate significant differences ($p < 0.05$) over time. Control, sample without any coating; TECS, sample coated with tea extract (0.5%w/v); TCECS, sample coated with tea (0.25%w/v) + cinnamon (0.25%w/v) extract; and CTCECS, sample coated with chitosan (2% w/v) + tea extract (0.25%w/v) + cinnamon extract (0.25%w/v).

respectively. Researchers reported that production of 5–10 mg TMA content in 100 g sample (sea bream and sardine) showed the starting of spoilage [24], but there are not any standard limitation for this parameter. We think, based on our results, production of 2.5 mg TMA in 100 g fillet for control sample is the starting of spoilage. This limitation was selected based on sensory attributes. Based on this standard limitation, TECS and TCECS samples were spoiled at 15~20 days of shelf life, but CTCECS sample was utilizable after 20 days of shelf life. Apparently, chitosan coating inhibited the TMA accumulation, and the inhibitory influence could be increased by addition of extracts. This finding was similar with the results by Souza et al. [7] in salmon samples. Gram and Dalgaard [25] showed that the increase of TMA content in fish was related to spoilage by microorganisms, including *Aeromonas spp* and *Shewanella putrefaciens*. The low TMA content in chitosan-coated fillets is due to the bacterial inhibition, which was confirmed by in another research [26].

3.2.4. Changes in TVBN Content. One of the most important measurement of fish fillets quality is TVBN index. Also, one of the common factors for evaluating the spoilage of fish fillet is TVBN. European Commission reported that 30–35 mg N_2/100 g of sample shows the beginning of spoilage. But, for rainbow trout, 25 mg N_2/100 g of sample was reported by researchers [27]. Table 3 reports that the TVBN value was increased from 13 to 113 mg N_2/100 g of sample during storage. Same findings were observed by Ojagh et al. [28] and Jouki et al. [29]. After 15 days, the TVBN value of the chitosan-coated sample was 22 mg N_2/100 g of sample, but the content of TVBN in the control sample and TECS was larger than the standard level. The raising trends of the TVBN value in coated fillets were lower than that determined in control samples. Our findings showed that chitosan modified with extracts (CTCECS) can improve the shelf life due to low barrier properties. TVBN content is related to the microbial count. The lowest content of the TVBN value was determined in the chitosan-coated fillet incorporated with tea and cinnamon (CTCECS), at the storage end. This influence can be due to extract and chitosan bactericidal properties.

3.2.5. Changes in Whiteness Index. Table 4 shows the whiteness index of fillets during 20 days of storage. Changes in color parameters can be due to the myofibrillar proteins destruction and myofibrils disarrangement. Jung et al. [30] reported that this reaction was placed by enzymatic process and nonenzymatic process. Whiteness value of samples raised during storage. Our findings are similar with findings of Dehghani et al. [2]. The fish meat color is affected by free water content, heme pigments, and muscle physical

Table 4: Changes in whiteness and pH values of rainbow trout fillets during storage at 4 ± 1°C.

		Storage (day)			
	Sample	0	5	15	20
Whiteness	Control	47.53 ± 0.29Ad	51.53 ± 0.40Ac	55.95 ± 0.33Ab	63.30 ± 0.46Aa
	TECS	44.23 ± 0.20Bc	46.43 ± 0.38Bc	49.07 ± 0.30Bb	56.26 ± 0.08Ba
	TCECS	45.31 ± 0.30Bc	45.58 ± 0.39Bc	48.50 ± 0.40Bb	52.10 ± 0.06Ca
	CTCECS	45.63 ± 0.32Bb	46.20 ± 0.46Bb	46.53 ± 0.40Cb	48.08 ± 0.47Da
pH	Control	5.59 ± 00.04Bc	5.70 ± 00.03Bc	6.17 ± 00.05Ab	6.54 ± 00.03Aa
	TECS	5.71 ± 00.02Ac	5.82 ± 00.04Ac	5.95 ± 00.02Bb	6.13 ± 00.04Ba
	TCECS	5.67 ± 00.03Ac	5.68 ± 00.03Bc	5.88 ± 00.01Cb	5.96 ± 00.02Ca
	CTCECS	5.51 ± 00.04Cc	5.57 ± 00.01Cc	5.70 ± 00.03Db	5.88 ± 00.02Da

Data represent mean ± standard deviation of three independent batches. At same time of storage, different uppercase letters indicate significant differences ($p < 0.05$). For same sample, different lowercase letters indicate significant differences ($p < 0.05$) over time. Control, sample without any coating; TECS, sample coated with tea extract (0.5%w/v); TCECS, sample coated with tea (0.25%w/v) + cinnamon (0.25%w/v) extract; and CTCECS, sample coated with chitosan (2% w/v) + tea extract (0.25%w/v) + cinnamon extract (0.25%w/v).

structure [31]. Whiteness increasing was due to the denaturation of different proteins and reducing the bond water [2]. Based on the results, CTCECS sample was the best sample in discoloration during shelf life. This sample was fortified with cinnamon and tea extracts. Changes in double bond of carotenoids and production of free radicals by lipid oxidation were the main reasons of discoloration of fillets.

3.2.6. Changes in pH. pH of coated and uncoated samples during storage are reported in Table 4. pH of fillets at the beginning of storage were 5.5–5.7. Different factors including fish species and size, water composition, catching season, geographical position, and stress level have effect on this parameter [23, 28]. The lowest (5.88) and highest pH (6.54) was measured in chitosan-tea cinnamon sample (CTCECS) and control at the end of storage, respectively. Different raising rate of pH was due to the antioxidant ingredients and its properties. However, in the 5th day of shelf life, because of lactic acid presence in the fillets, the pH of sample was similar. Proteolytic and autolytic enzymes produced by microorganism react with nitrogen ingredient and raise the pH of samples [32].

3.2.7. Fourier Transform Infrared Spectroscopy Analysis. There are a lot of research papers in which FTIR technique was used for evaluating the modification of structure. FTIR analysis was carried out during storage, and the spectra obtained are shown in Figure 1. The band around 1745 cm^{-1} was related to the triglycerides C=O stretching. Oxidation of fillets oil increased the intensity of this band (Figure 1 and Table 5). Researchers reported that production of secondary oxidative components such as aldehydes creates bands between 1728 cm^{-1} and 1780 cm^{-1} [33]. Figure 1 indicates an asymptotic response of the carboxylate absorption at 1542 cm^{-1} due to increasing % FFA [34]. Figure 1 shows that the changes that determined in the 600–1500 cm^{-1} are related to the oxidation of lipids. Bands at 1382 (-CH_3 aliphatic groups or C-H bonds of cis disubstituted alkenes) and 1461 (-CH2- aliphatic groups) are related to oxidation of lipids in samples. The saturated

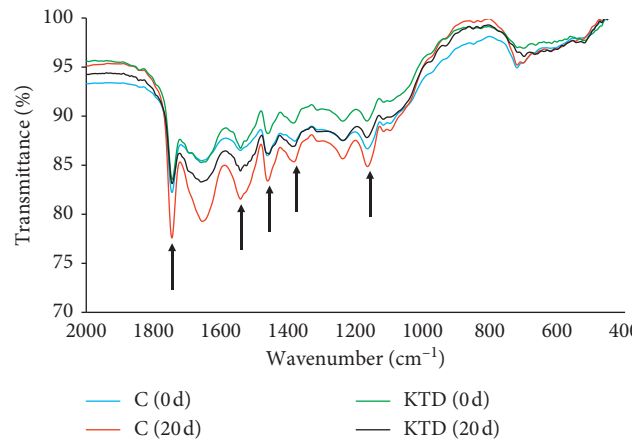

Figure 1: FTIR spectra of control (C) and CTCECS (KTD) samples during storage.

acyl groups contents in the fish oil can be calculated by evaluation bands at 1166 cm^{-1} [35]. Changing the location of bands to higher wavenumber after oxidation of lipids is due to the production low molecular weight saturated acyl components during lipolysis of triglycerides. Moomand and Lim [36] reported that after oxidation of lipids, the absorbance around 1099 and 966 cm^{-1} was increased. Based on our findings (Figure 1 and Table 5), the control sample had the highest oxidation, and the treated sample with chitosan, tea, and cinnamon extracts had the lowest oxidation (CTCECS). The results showed that oxidation of fish fillet can be evaluated by FTIR technique, and our results showed that the results of chemical analysis were similar to the results of FTIR.

4. Conclusion

The shelf life of samples improved by application of chitosan-based coating incorporated with tea and cinnamon extracts as a natural preservative. Main factors including FFA, pH, TBA, and TVBN of chitosan-coated fillets incorporated with extracts were in the standard range during storage. Our results showed that the results of chemical analysis were similar to the results of FTIR. The shelf life of

Table 5: FTIR peak assignments of different samples.

	Control		CTCECS	
	0 day	20 days	0 day	20 days
Saturated acyl groups	1145.10	1164.78	1166.01	1163.27
-CH_3 aliphatic groups	1377.23	1382.74	1382.74	1382.74
-CH_2- aliphatic groups	1461.96	1461.31	1460.97	1456.81
Asymptotic response of the carboxylate absorption	1539.11	1542.00	1541.70	1542.21
C=O stretching of the triglycerides	1745.52	1745.80	1745.00	1745.00

Control, sample without any coating; CTCECS, sample coated with chitosan (2% w/v) + tea extract (0.25%w/v) + cinnamon extract (0.25%w/v).

the control and chitosan-coated sample with high quality properties was 5 and 15 days, respectively. This result showed that this coating incorporated with extracts is a good case for improving the shelf life of meat products.

Data Availability

The data used to support this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

The Effects of Edible Coatings on the Postharvest Quality of Citrus Fruits as Affected by Granulation

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The world population is growing day-by-day, while the available natural resources for agricultural production, i.e., soil and water, are rapidly decreasing. Moreover, consumer preferences are highly affected by some quality characteristics of food products, which can be classified as external, internal, and hidden attributes. Among the internal quality attributes, granulation is a significant factor damaging the inner quality of citrus fruits and reducing the consumer attraction. The main symptoms of granulation (also known as section drying, crystallization, or scarification) in citrus are shrivelling in juice sacs due to gel formation, hardening, white colour, and low extractable juice content. A well-known result of the granulation is the reduction in soluble solid concentration (SSC), total sugar, and titratable acidity (TA). Granulation is known to affect the citrus fruit quality all over the world. Since citrus is one of the world's most popular fruit species, it is highly important to identify and manage this physiological problem to help sustainable production throughout the world. Consumer's preferences have also been moving towards the use of eco- and environmental-friendly alternative methods in postharvest fruit storage, including edible coatings. Edible coatings act as a barrier for the air and water transitions through the surface of fruits which results in retarding the deterioration of fruits, preventing and/or controlling the microbial decay, improving the fruit quality, and hence extending the storage duration. The mechanism of citrus fruit granulation is highly associated with the oxidative stress, and edible coatings have been reported to significantly reduce granulation and improve the quality of the fruits. In line with this information, this review article aimed to summarize the reasons, results, and managements of granulation of citrus fruits.

1. Introduction

Citrus is the leading fruit crop throughout the world and best suited to grow in Mediterranean countries, China, America, and Brazil. The total area devoted to citrus production is about 7.8 million hectare. The total production of oranges, mandarins, lemons, grapefruits, and others was about 73.3, 33.4, 17.2, 9.0, and 13.5 million tons, respectively, in 2017 [1]. Citrus fruits are rich in nutrients, antioxidants activities, ascorbic acid, and phenolic and other bioactive compounds and are traditionally and scientifically known to be very beneficial for human health [1, 2]. Citrus fruits are among the most important fruits in world trade (import/export), and the consumer's demand on the citrus fruits has been increasing throughout the world mainly because of the scientifically accepted health benefits and high antioxidant and high ascorbic acid contents. However, citrus fruits suffer from numerous production and postharvest problems, including pests, diseases, weeds, fruit cracking, yield problems, and postharvest quality loss. Granulation at the juice sac is

one of the most important problems causing quality loss and reducing the consumer attraction [3].

The granulation (also known as section drying, crystallization, or scarification) in citrus fruits mostly appear when the fruits are kept on-tree after ripening with an extended harvesting period which significantly affects the individual juice vesicles within the segments, and the most important symptom is the solid and dry nature of the fruit segments. This can also happen in postharvest storage for almost all citrus species [4–6]. The juice sacs of affected fruits shrivel because of gel formation become hardened and granular with low extractable juice content and white colour [3, 7–11]. However, the granulation can also develop after harvest during storage and significantly affects the marketability of the citrus fruits. The granulated fruit vesicles become solid, enlarged, and discoloured. The granulation is generally associated with the fast growth of the large size fruits which is common in young trees [4, 12]. The soluble solid concentration (SSC) and titratable acidity (TA) have also known to be reduced in the granulated fruit vesicles [4]. Further studies also showed that the granulation in citrus fruits causes secondary cell wall formation and thickening in the cell wall [13]. Previous studies have reported that the on- and off-tree storage, fruit size, and juice vesicle position have a significant influence on the granulation of grapefruits (*Citrus paradise* Macf.). It was found that the granulation is higher in the stylar juice vesicles than in others. Studies have also shown that the larger fruits (about 600 g) have higher granulation than smaller fruits. It was also noted that the on-tree storage (late harvesting) significantly increases the granulation in grapefruits. On the other hand, early harvest and storage at high temperatures (21°C) were found to increase the susceptibility of the fruits to granulation. Therefore, if the fruits are meant to be stored for later consumption, on-tree storage is more effective to reduce granulation at the large size grapefruits [5]. However, previous studies have also showed that the granulation in the late-season fruits mostly appears in storage after harvest [12, 13].

Biological activities continue in the harvested fruits and vegetables, whereas the water and gaseous exchange with surrounding environment happens through transpiration and respiration. Thus, these phenomena result in the water and solute loss which significantly reduces the postharvest quality and storability of fresh products. There are several chemical and nonchemical methods used in postharvest handling practices. However, numerous studies reported significant hazards on human and environmental health caused by mis- or excessive-use of agrochemicals and the acceptability of agrochemicals by consumers have been decreasing. One of the successful methods used in postharvest handling practices to maintain postharvest fruit quality is the application of edible coatings alone or incorporated with plant extract, essential oils, and active metabolites [14, 15]. Edible coatings act as a barrier for the movement of water and air on the fruits' surface which reduces the transpiration and respiration. This reduces water loss, delays fruit senescence, and increases antimicrobial properties of fruits [16, 17]. The commercial use of waxes as edible coatings began around the 1920s; however, the use of edible coatings in postharvest handling of fruits and vegetables is an old technique dating back to the 12th century in China. Edible coatings are composed of food-grade materials, and in addition to retard fruit deterioration, coatings may cause several physicochemical changes which delays or prevents fruit granulation [17].

There is a gap in the published literature about the use of edible coatings in the management of citrus granulation. Therefore, this review aims to summarize the main causes and mechanisms of the granulation in citrus, its effects on the postharvest storage quality, and its management by edible coatings.

2. Main Causes and Mechanism of Granulation

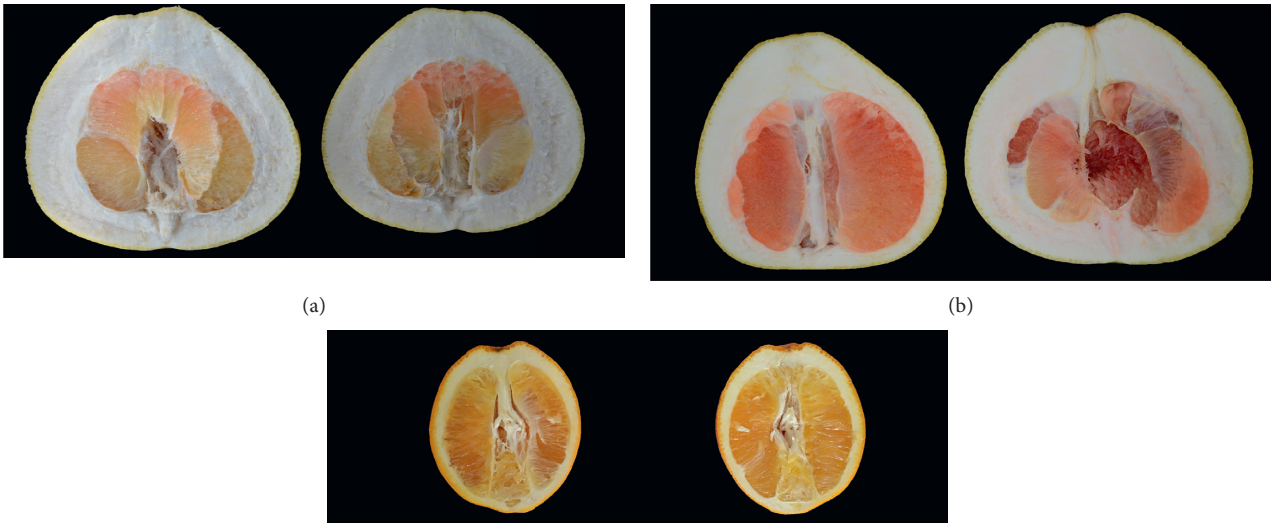
As described above, granulation can be described as a pre- and postharvest problem which resulted in shrivelling of the juice sacs due to gel formation and solid, enlarged, and discoloured fruit vesicles (Figure 1). It is common for large size fruits and generally associated with the fast growth of the fruits [4, 12]. The extractable juice content of the affected fruits decreases mainly because the moisture is binding to the cell walls or in gels [4]. These gels are the main causes of cell wall thickening at the fruits. In the granulated fruits, the pectin is incorporated with the juice to promote gelation formation [18]. Granulation generally starts from the stem end of the fruit pulp and extends towards the stylar end, and the main causes of it are the number of abnormal physiological activities (Table 1).

Water potential is of utmost importance to develop granulation in citrus fruits. Water can easily move within the plant organs from cell to cell due to their different (higher and lower) water potentials. When the SSC and acidity decrease at the fruit cells, these cells are generally turgid with high water potential and are susceptible to lose water to the other adjacent cells (which have lower water potential with higher SSC and low turgor). Researchers suggest that the granulation might be a result of the defensive system of the fruits to lose water [25]. For this purpose, cell wall thickening happens to prevent moisture loss [13]. Previous studies have also showed that polyphenol oxidase (PPO) activity and phenolic contents have a significant influence on granulation where the granulated fruits have lower PPO activity and phenolic content [27].

3. Effects of Granulation on the Postharvest Storage Quality

The granulation cause deterioration of fruit quality and hence decreased their marketable value [4, 12]. Granulation is known to reduce the concentrations of SSC and TA which results in developing insipid taste in the fruits [4]. The storage of the granulated fruits is more difficult as compared with the nongranulated normal fruits.

3.1. Effects of Granulation on Mineral Nutrients. Granulation has known to significantly influence the organic acid metabolism of the fruits and have a relationship with



(a) (b)

(c)

Figure 1: A view of granulation on the citrus fruits, pummelo fruits (a, b), "Newhall" navel orange (c).

Table 1: Main causes of granulation in citrus.

Causes	Reference
Delay in harvesting and/or late maturity	[4, 19, 20]
Vigorous and rapid tree growth	[12, 21]
Quick ripening	[21]
Adverse atmospheric condition: cold weather during the maturity period	[5, 20]
Sink competition among the different plant parts, i.e., fruits, roots, and leaves.	[9, 22, 23]
Rootstocks and cultivars type	[3, 24]
Higher water potential in juice cells	[25]
High temperatures during flowering and fruit set	[12]
Calcium deficiency	[20]
Enzymes activity: polyphenol oxidase (PPO), peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), pectin-methylesterase (PME), and cellulose	[26]

mineral nutrient contents in the "Guanximiyou" pummelo fruits. Similar to SSC and TA, the granulated juice sacs lead to a decrease in citrate and isocitrate concentrations while an increase in the malate concentration in the citrus fruits. Granulation was also found to enhance the accumulation of mineral nutrients (especially P, Mg, S, Zn, and Cu) in juice sacs [28]. Postharvest granulation of "Ponkan" fruits was reported to begin from the stem part of the fruits and extend towards the stylar end of the segment. Yao et al. [29] also conducted a study on "Ponkan" fruits and identified 768 genes which differentially expressed under granulation during the postharvest storage. Among these, researchers determined the genes responsible from enzyme encoding which are involved in the reduction of SSC and TA. In line with these results, Yao et al. [29] suggested that SSC and TA metabolisms are adjusted to the synthesis pathway of the cell wall. In another study, Zhang et al. [30] studied the role of miRNAs in the granulation of citrus fruits. They reported that the miRNAs have a regulating role in lignin processes which relate to citrus granulation during postharvest storage.

3.2. Effects of Granulation on Nutritional Ingredient. Granulation was reported to cause a significant reduction at the concentrations of SSC and TA [4]. Sharma et al. [27] noted that the granulated "Kinnow" mandarin fruits exhibit lower antioxidants activity and ascorbic acid content. Fruit granulation also causes a reduction in the juice content of the citrus fruits resulting in colour change (lighter) of the juice sac [12, 28, 31]. This causes the fruits to have an insipid taste because of the loose of SSC and TA contents. Both SSC and TA of citrus fruit were reported to decrease significantly at the granulated fruits where the sac colour became lighter and caused a decline in the internal fruit quality [27, 32–34].

3.3. Effects of Granulation on Reactive Oxide Species and Other Related Enzymes. Some of the fruit enzymes (i.e., polyphenol oxidase (PPO), peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT)) were reported to have a high correlation with the granulation [3, 24, 32, 35]. This means that the enzyme activities are higher in granulated fruits as compared with those in the healthy fruits. Wenqin

et al. [26] have also reported that the inbred pumelo fruits have higher granulation than hybridized fruits. Researchers also noted that no new isoenzymes appear when both inbred and hybridized pumelo fruits are granulated. The activities of isoenzymes of POD and SOD in granulation juicy sac were also reported to be higher than those of the nongranulated fruits. Similar results were also reported by Sharma et al. [24] who noted that the granulated fruits have lower PPO activity and phenolic content. Granulated "Kinnow" mandarin fruits were also reported to exhibit higher lipoxygenase (LOX) enzyme and higher rates of CO_2 and ethylene production [24]. On the other hand, recent research has also noted that ROS has some signalling roles on the biotic stress, including fruit granulation [36]. Thus, the evidence suggests that ROS plays an important role in the management of plant stress to different conditions [37].

3.4. Effects of Granulation on Hydrolase (Pectin Methylesterase, Cellulase, Etc.). Previous studies have also suggested an association of pectin-methylesterase (PME) with citrus granulation [3, 27, 32, 35]. Wu et al. [34] have also reported that the PME's activity is higher in the granulated citrus fruits. They also noted that the pectic acid concentration was also increased in the granulated fruits. In a different study, it was noted that the genes encoding PME catalyze the pectin degradation in citrus fruits [38]. In another study with Ponkan fruits, it was reported that the increase in the levels of those genes improves pectin biosynthesis and leads to an increase in the pectin level granulated juice sacs [29]. Similar results were also previously reported for granulated grapefruits [17].

4. Detection of Granulation

It is reported that the detection of the granulation in citrus fruits is almost impossible until cutting the fruits. However, a higher incidence of granulation was reported causing the fruits to feel softer at the last stage of maturity as compared with the healthy fruits. If harvest delays and the severity of the granulation increase, the fruit peel may turn greener, especially around the stem end, as compared with a nongranulated fruit [4]. It is very difficult to reliably detect the granulated fruits without cutting the fruits. Dael et al. [39] developed an algorithm to process fruit images for the detection of granulation. They reported that scanning orange fruits in an X-ray system (75 kV, 468 mA, 60-ms exposure) and processing the images at high-speed and robust algorithm could be used for the detection of the granulation. In another study, researchers aimed to develop a simple and noninvasive technique for the identification of the granulated "Sai Num Pung" tangerine fruits by near-infrared (NIR) spectroscopy and multivariate chemometric analysis. They have analyzed the moisture content, SSC, and TA of the granulated and nongranulated fruits to investigate any relationship among the characteristics. Researchers noted that the idea of using spectroscopic measurement for the identification of the granulation could be successful [40].

5. Development and Management of Citrus Granulation

As understood from the mechanism and causes of granulation, there are some important characteristics which significantly affect the fruit granulation. It was reported that granulation can be significantly affected by different characteristics, i.e., rootstock type, yield performance, tree age, and climatic conditions [4, 32]. Researchers previously reported that the rootstocks which are supporting earliness, on the other hand, promote granulation in fruits. The same phenomena appear on vigorous rootstocks [32, 41]. Granulation in citrus fruits is also reported to be strongly linked with high temperatures during flowering and fruit set [12]. Rootstocks are also known to have an impact on the plant water relationship which might influence the mechanism of granulation [42]. The tree vigour on grafted plants could be associated with the ease movement of water through the rootstocks and scion [43], and this affects the water potential of the plants. Thus, high water potential is known to significantly increase the granulation. There are some previous studies supporting this information where the tree vigour and SSC were reported to be inversely related [44]. Similarly, Hofman [25] reported that the rootstocks have significant influence on the granulation.

Not only the rootstocks and crop load but also the soil type is known to significantly affect tree vigour and fruit development, which indirectly affect the granulation in citrus fruits. Linked with the mechanism, the availability of water is crucial for the granulation and sandy soils have high availability which may increase the fruit granulation [25, 45]. Fruit size is highly related to the number of fruits per tree and is reported to influence granulation in citrus fruits. A low number of fruits is known to have an increase in fruit size, and granulation is more prevalent in these fruit trees [3–5, 12, 25, 32]. The main reason for this relationship is that the SSC and TA concentrations are low in the large fruit which promotes the mechanism of granulation in citrus fruits. The fruit position is also reported by Hofman [25] to significantly influence the granulation. The fruits located inside the canopy are reported to be susceptible to granulation as compared with the outer fruits. This can be associated with the SSC content of the fruits where the fruits inside the canopy are far away to the carbohydrate sources (fewer leaves are found inside the canopy as compared with outside), and it is reported that the leaves generally export their sugar primarily to the nearest fruits [46]. The SSC of the inner fruits is lower than the fruits located outside the canopy.

It was noted that the alcohol-insoluble solids (AISs) (composed of pectin and some cell wall materials) are higher at the granulated fruits [5]. Several studies suggest that the large fruits are more sensitive to granulation [4, 5]. It was also reported that the harvest time significantly affects the granulation of the Guanxi pomelo (*Citrus grandis*). Previous studies showed that fruits at low altitudes with timely harvest have high SSC and TA contents and light granulation, but fruits at higher altitudes have delayed maturity than those at

the lower altitudes; those fruits are reported to have less SSC and TA contents with higher granulation [19]. The above listed factors which influence the development of granulation in citrus made it possible to do some recommendations for its management. The selection of the rootstocks for citrus trees is highly important for successful control of granulation. It could be recommended to select some moderate vigorous rootstocks as compared with high vigorous rootstocks. It is also of utmost importance to regularly irrigate and fertigate plants to promote flowering and ensure early fruit ripening. Adequate application of nitrogen and phosphorus is highly important during the early stages of flowering and fruit set. Regarding the fruit location and SSC contents, tree pruning is also important to ensure adequate light penetration inside the canopy. This would increase the number of leaves inside the canopy, thus increasing the SSC content of the fruits. Fruit thinning can also increase the SSC content of the fruits and may promote earliness, which can reduce the granulation.

Researchers also suggested that the white colour shading net (20%) could increase the SSC and reduce the granulation at "Ponkan" fruits [47, 48]. Granulation is reported to be strongly related to the high temperatures during flowering and fruit set [12], and shading net could reduce the incidence of granulation. It is also known that plant nutrition has a significant influence on the granulation development [28, 49] and foliar application of macro and micronutrient, i.e., potassium, zinc, calcium, and boron could prevent the development of granulation [11, 50, 51]. Shade netting could also stimulate the development of longer roots [52] which can indirectly improve nutrient uptake and help to reduce the granulation. Among those plant nutrients, calcium was reported to be highly effective in the prevention of granulation in citrus. On the other hand, the selection of cold tolerant and early maturing varieties is suggested [20].

In a different study, Xiong et al. [53] noted that the exogenous application of spermidine (Spd) significantly inhibits the ROS accumulation in "Huangguogan" citrus fruits, when applied to seedlings, and affects antioxidant enzyme activities. Furthermore, researchers suggested that the exogenous Spd application may protect fruits from granulation by improving metabolic defence systems. According to the researchers' notes, exogenous Spd application decreases the permeability of cell membrane (which reduces the water movement) and malondialdehyde (MDA) content of the fruits [53].

6. Effects of Edible Coatings on Citrus Granulation and Maintaining the Postharvest Quality of Citrus Fruits

Edible coatings reduce the movement of water (transpiration) and gaseous (indirectly reduces respiration) through the fruits' surface which results in a reduction in the water and solute loss, an increase in antimicrobial activities and hence the improvement in fruit storability [16, 17]. Edible coatings and films are very important which are developed from edible, environmentally friendly ingredients and have no damage to human health [54]. Because of the consumers' preferences, recent studies have mainly condensed on the development and use of edible coatings in postharvest applications. Edible coatings are reported to be applied to fruits in several forms, including spraying, dipping, and brushing [55]. Edible coatings cover the fresh fruits, seal the stomata, and fill any pores and mechanical cracks [16, 17]. The main mechanism of the edible coatings for maintaining postharvest quality and storability is because of its characteristics for reducing the gaseous exchange and respiration. However, because of the anaerobic respiration, coatings should not completely inhibit but control gaseous exchange [56]. Therefore, some characteristics of edible coatings, i.e., waxiness, adhesion, thickness, transparency, and plasticity, play a critical role in the success of edible coatings. The characteristics of the fruits are also important for obtaining the desired condition [16].

Edible coatings are generally composed of lipids, proteins, polysaccharides, or from their combinations [57]. These have different advantages and disadvantages in commercial use. For example, lipids provide a good barrier for moisture due to their hydrophobic in nature [16] but have poor characteristic for gas barrier [58]. On the other hand, contrary to lipids, proteins have a good ability to act as a barrier for gaseous exchange, but they are brittle and may have allergenic risks [58, 59]. Polysaccharides have better characteristics as compared with lipids and proteins and have widely used, such as *Aloe vera* [60], alginate [61], chitosan [62], and Arabic gum [17]. Combination of multiple coating materials improves functionality of the materials [58]. In general, edible coatings reduce respiration rate [63, 64], prevent weight loss [63–65], control ethylene biosynthesis [63, 64, 66], maintain fruit firmness [63–65], prevent colour changes (by controlling anthocyanin and carotenoid synthesis [67]), delay the break-down of soluble solids [64, 65], minimise loss in TA [63–65], reduce the loss of phenolic compounds and ascorbic acid and maintain antioxidant activity [62], delay or control chilling injury [64], and reduce microbial decay [68]. In a previous study, Kharchoufi et al. [68] reported that the edible coatings enriched with pomegranate peel extracts have antifungal effectiveness and reduce *Penicillium digitatum* decay in orange fruits. Edible coatings with *Ficus hirta* + 1.5% sodium alginate treatment was reported to enhance the antioxidant activities of Nanfeng mandarins which also stimulated the accumulation of phenolic compounds and some defence-related enzymes (i.e., SOD, CAT, POD, chitinase (CHI), and phenylalanine ammonialyase (PAL)) [61]. The senescence of the citrus fruits is generally known as the cause of granulation during postharvest storage, and uncontrolled metabolism reactive oxygen species (ROS) increases the senescence and granulation of the fruits [69, 70]. Thus, the prevention of the ROS formation inhibits the formation of the granulation. In a previous study, chitosan treatment was reported to reduce the post-harvest oxidative damage, inhibit the $O_2^{\cdot-}$ production, and MDA accumulation, which reduces the granulation of the "Wuye" lithci fruits. It was also noted that edible coatings enhance the activity of some enzymes, i.e., superoxide dismutase (SOD), ascorbate

peroxidase (APX), and catalase (CAT) [71]. Edible coatings enhance or induce some enzymatic activities in grapefruits [72] and "Murcott tangor" fruits [73]. Similar results were also reported for "Newhall" navel orange and "Xinyu" tangerines where it was noted that that the chitosan coating forms a protective barrier on the surface and inhibits the oxidation process [74]. This result is in accordance with the previous knowledge about the positive relationship between granulation and oxidative stress [75]. Thus, it can be concluded that the prevention of oxidative stress prevents the granulation of the citrus fruits.

7. Conclusion and Further Suggestions

Although there are numerous studies about the mechanism and management of granulation in citrus, it is still an important problem throughout the world and there is not a simple practice for prevention of granulation. Studies suggest that high temperature during flowering, higher water potential of the tree, vigorous tree growth, imbalanced nutrition, cold weather during maturity period, and delay in harvesting are the main reasons for granulation. At this point, another important problem arises which is the drastic change in the world climatic data due to climate change. In line with this information, farmers have to reconsider climatic data when renewing their plantations or establishing new orchards. It is also crucial to pay attention to the rootstock selection, irrigation, and fertilization. Granulation is known to highly affect the postharvest storage life of the citrus fruits, but very few studies have found about the effects of granulation on the postharvest storage duration. It is important to study this subject for the prevention and/or reducing the postharvest granulation. Edible coatings have a great potential in reducing the quality loss during storage and extending the storage duration of citrus fruits. Besides the quality retention, edible coatings provide favourable conditions for reducing oxidative damage and inhibiting $O_2^{-\cdot}$ production and MDA accumulation, which reduces the citrus fruit granulation. A review of the existing literature also suggests that the edible coatings have high potential in the prevention of the postharvest granulation; however, further studies need to clarify the mode of action and those studies should focus on technological readiness of edible coatings. Commercial performances of edible coatings should be tested by comparing with different applications and by considering its cost and sustainability. This would also make it possible to develop edible coatings industrially, and they will be used for the prevention of citrus fruit granulation during storage.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

C. W. was responsible for the conceptualization of the study; İ. K., C. C., Z. G., and C. W. investigated the study; İ. K. and C. C. reviewed the study; İ. K. and C. W. were involved in the discussion; İ. K. and C. W. were responsible for writing, reviewing, and editing the original draft; C. W. was involved in the project administration and funding acquisition.

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