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COMPARISON OF QUALITY PARAMETERS OF EDIBLE FILMS WITH RHUBARB ADDITION PRODUCED BY TWO DIFFERENT METHODS

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ABSTRACT. In edible films with added plant extracts, the effects of the polymer matrix used and the production method on film performance have not yet been sufficiently clarified. Therefore, in this study, the quality parameters of starch-gelatin and chitosan-based edible films with added rhubarb (*Rheum ribes* L.), produced by two different methods, were comparatively examined. The films were characterized in terms of thickness, water solubility, mechanical properties, water vapor permeability, optical properties, color parameters, antioxidant capacity, biodegradability, and structural properties. The results showed that the film properties changed significantly depending on both the polymer matrix used and the rhubarb extract concentration. Starch-gelatin-based films exhibited higher water solubility and flexibility, while chitosan-based films showed higher mechanical strength and better water vapor barrier properties. Rhubarb addition significantly increased the antioxidant potential of the film-forming mixtures in both methods. Color analyses revealed that the films darkened with increasing rhubarb concentration. SEM and FT-IR analyses showed that rhubarb addition caused physical changes in the film structure but did not alter the chemical structure. Chitosan-based films with rhubarb addition may be considered as a potential alternative for active and sustainable food packaging applications.

Keywords: *Edible film, rhubarb (Rheum ribes L.), chitosan, starch-gelatin, antioxidant activity, biodegradability*

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INTRODUCTION

Increased packaging use stands out as one of the most significant problems causing environmental pollution today. One of the main reasons for this is that consumers cannot properly classify and dispose of packaging waste for recycling. It was reported that approximately 40% of the packaging put on the market in Türkiye cannot be recycled [1]. The use of traditional food packaging materials such as plastic, paper, glass, metal and composite in edible packaging applications is limited. Petroleum-based plastics such as polyethylene and polypropylene pose serious ecological threats due to their non-environmentally friendly structure [2, 3]. Most of them are single-use plastics and are released directly into nature, especially oceans or soil, after use. In addition, burning these materials causes greenhouse gas emissions, triggering global warming [3-5].

To mitigate these environmental threats, the use of recyclable or biodegradable packaging materials is encouraged, and public awareness campaigns are being conducted. In this context, edible films and coatings, offering environmentally sustainable and functional properties, have attracted significant attention, particularly in recent years [3, 6-9]. Edible packaging extends the shelf life of food products, prevents microbial spoilage, and reduces waste. Thanks to developing technologies, these types of biopolymers can be transformed into edible films and coatings, and they can be applied directly to food as packaging or bags in film form, or as coatings. One of the most important advantages of edible packaging is that it is consumed as an integral part of the product, eliminating the need to open the packaging [3, 10]. Furthermore, the biodegradability of edible packaging provides environmental benefits in terms of reducing plastic waste and decreasing dependence on fossil fuels [3, 11-14]. Edible films and coatings can be produced based on lipids, polysaccharides, and proteins, or their combinations [15, 16]. Today, the most preferred edible film polymers are polysaccharides. Edible films and coatings of polysaccharide structure are hydrophilic, and have low moisture barriers and high gas barriers. They are produced from polysaccharide-structured substances such as starch, chitin, chitosan, cellulose, gums and pectin [17].

Chitosan is the polymer material most commonly preferred in the production of edible packaging. It has non-toxic, biocompatible, biodegradable, and biologically functional properties. Thanks to these properties, it is widely used in the production of edible films [6]. Among various biopolymers, chitosan is a good candidate for food packaging material due to its film-forming ability and biodegradability. However, pure chitosan film does not have the ideal antioxidant capacity for antioxidant-active food packaging materials. This situation creates a disadvantage in terms of long-term storage

of foods with high fat content [18]. To overcome these shortcomings, it is necessary to improve the antioxidant capacity of pure chitosan film to expand its application in food packaging [19]. In addition, improvements are made in the formulation of these films by adding functionality-enhancing additives such as plasticizers, antimicrobials, colorants, flavorings, probiotics, and live microorganisms for biocontrol purposes [3, 12-14, 20-24]. In these improvements, the use of purees, pulps and extracts of fruit and vegetable origin is carried out in order to improve the sensory and nutritional properties and suitable mechanical performance of these films [11].

Starch, another preferred material in the production of edible packaging, is a natural storage carbohydrate produced in plants. Starch is composed of two types of structures called amylose and amylopectin, which are classes of α-D-glucose [25]. Not all starch is digested by amylase enzymes and is absorbed in the small intestine. Due to this structure, it controls blood sugar levels, has positive effects on cholesterol, diabetes, colon cancer prevention, and gastrointestinal health [26]. Also, starch, which contains a high amount of dietary fiber, can be used as a fiber source in healthy eating [27].

Starch granules are insoluble in water, and they swell by absorbing water. The ability of starch to absorb water stems from the numerous hydroxyl groups it contains. When starch is added to water, hydrogen bonds weaken above 56 °C, and the granule size increases several times its original size in a phenomenon called gelatinization. As the granules absorb moisture, the solution becomes clear and the viscosity increases [28]. Starch is widely used in many sectors such as textiles, paper, pharmaceuticals, paints, cosmetics, and the food industry. Its widespread use is due to its tasteless and odorless nature, natural origin, minimally altering of the product's structure, and viscosity, flow properties, gel resistance, and yield properties [27, 28]. In this study, we aimed to compare the characterization parameters of chitosan and starch-based films by adding rhubarb to them.

Rhubarb (*Rheum ribes* L.), a perennial herbaceous plant of the Polygonaceae family, grows in Iran, Iraq, Lebanon, Palestine, and Turkey [29]. Rhubarb is also known in the region by names such as wild rhubarb and mountain banana [30]. Reaching up to 150 cm in height, rhubarb is a green, leafless plant. It has a sour taste due to its low pH of 3.56 [31]. It was reported that 100 g of rhubarb contains 20.4 mg ascorbic acid, 2.25 g protein, 0.24 g fat, 1.15 g ash, 25.1 mg phosphorus, 114.4 mg potassium, 60.3 mg calcium, and 24.6 mg sodium [32, 33]. However, research has shown that rhubarb contains chrysophanol, physcion, and emodol anthraquinones, as well as quercetin, 5-deoxyquercetin, quercetin 3-O-rhamnoside, quercetin 3-O-galactoside, and quercetin 3-O-rutinoside flavonoids [34]. The results of the studies indicated that rhubarb has a high amount of phenolic compounds [31, 35]. It was also reported to have an antibacterial effect [36, 37].

The present study aimed to determine the extent to which the addition of rhubarb (*Rheum ribes* L.) improves the antioxidant capacity, and functional physicochemical properties (mechanical strength, barrier properties, color, and thickness) of chitosan and starch-based edible film matrices, and to identify the relationship between this improvement and the film matrix.

The present study primarily focuses on evaluating the effect of different polymer matrices on the physicochemical, mechanical, and functional properties of rhubarb-enriched edible films. Although two different preparation methods were employed, these methods were selected based on the specific processing requirements of each polymer system rather than as independent variables for comparison. Starch–gelatin and chitosan matrices differ significantly in terms of solubility, film-forming mechanisms, and structural behavior, which necessitates the use of matrix-specific preparation conditions.

In addition, different forms of rhubarb (puree and lyophilized powder) were used to ensure compatibility with each polymer system. The use of rhubarb puree in starch–gelatin films is suitable due to its aqueous and gelatinization-based structure, whereas lyophilized rhubarb powder provides better dispersion and stability in chitosan-based systems. Therefore, the primary comparison in this study is based on the polymer matrix type, while preparation methods and rhubarb forms were adapted to achieve optimal film formation within each system.

RESULTS AND DISCUSSION

A total of 12 edible films were produced using two different methods (Figure 1). Characterization studies were performed on all film samples.

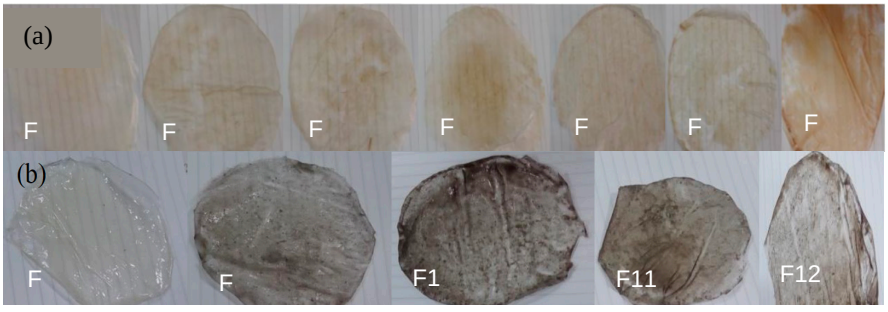

Figure 1. Film samples produced by both methods

The statistically analyzed film thickness (FT) values of the film samples revealed significant differences among the samples ($p \leq 0.05$) (Table 1). F1, F2, F3, F4, and F5 samples were classified within the same statistical group ("a"), and no significant difference was determined among the thickness values of these samples. In contrast, when the F6 and F7 films were examined, the effect of increasing gelatin concentration on film thickness in the presence of lecithin was found to be statistically significant ($p \leq 0.05$). When the film thickness values of the F8–F12 samples were statistically evaluated, the addition of rhubarb extract was found to have a significant effect on film thickness ($p \leq 0.05$) (Table 1).The fact that the control group F8, which does not contain rhubarb, showed the lowest thickness value revealed that the addition of the extract volumetrically strengthened the polymer matrix. The continuously increasing thickness in formulations F9, F10, F11, and F12 where the rhubarb ratio was gradually increased, showed that the extract interacts with the chitosan structure, expanding the net structure of the film and increasing its water-holding capacity. These results revealed that rhubarb extract concentration is one of the determining factors of film thickness in chitosan-based films. While the thickness increased regularly with increasing rhubarb extract in chitosan-based films, the thickness in starch-gelatin based films varied more widely, especially depending on the amount of gelatin and lecithin.

When the water solubility (WS) values of the F1–F7 samples were statistically evaluated, the formulations were found to have a significant effect on solubility ($p \leq 0.05$) (Table 1). While the F1 sample was classified in group "b", all other samples containing rhubarb were included in the same statistical group ("a") and did not show significant differences among themselves. This result indicated that the addition of rhubarb reduced the solubility of the films. The fibrous structure of rhubarb and the phenolic compounds present in its composition may have contributed to the formation of a denser and more compact film matrix, thereby limiting the penetration of water molecules into the film structure. In contrast, the addition of lecithin and gelatin did not produce a statistically significant effect on water solubility. When the water solubility (WS) values of the chitosan-based films belonging to the F8–F12 samples were statistically evaluated, significant differences were determined among the formulations ($p \leq 0.05$) (Table 1). In general, a decrease in water solubility values was observed with increasing rhubarb extract incorporation. This result may be attributed to the fibrous structure, phenolic compounds, and solid matter content of *Rheum ribes*, which likely contributed to the formation of a more compact film matrix. In addition, the incorporation of *Rheum ribes* may have enhanced the interactions between chitosan chains, thereby limiting the penetration of water molecules into the film structure and consequently increasing the water resistance of the films.

When the water vapor permeability (WVP) values of the F1–F7 samples were statistically evaluated, the formulations were found to have significant effects on WVP ($p \leq 0.05$) (Table 1). F2, F3, and F7 samples were classified within the same statistical group ("abc"), while the F5 sample was included in group "a" and the F4 sample in group "c", showing statistically significant differences compared to some other samples. The results indicated that the presence of lecithin at low concentrations contributed to a reduction in water vapor permeability, whereas increasing amounts of gelatin and lecithin resulted in higher WVP values. In addition, the incorporation of *Rheum ribes* was considered to contribute to the reduction of water vapor permeability, particularly in the presence of low gelatin and lecithin concentrations, by promoting the formation of a more compact film matrix. When the water vapor permeability (WVP) values of the F8–F12 samples were statistically evaluated, all samples were found to be included in the same statistical group ("a"), and no significant differences were determined among the samples ($p > 0.05$). This result indicated that the formulation modifications and the incorporation of rhubarb extract did not produce a statistically significant effect on the water vapor permeability of the chitosan-based films. The findings suggested that the barrier properties of the chitosan matrix were largely preserved and that the applied additive concentrations did not substantially alter water vapor transfer.

Table 1. Results of film thickness (FT), water solubility (WS), elongation at break (EB), tensile strength (TS), and water vapor permeability (WVP) analyses of the film samples

Film samples	FT (mm)	WS (%)	WVP (g mm $h^{-1}cm^{-2}$ $Pa^{-1}x10^{-6}$) 24h	EB (%)	TS (MPa)
F1	0.164 ± 0.0048^{a}	95.47 ± 0.50^{b}	0.35 ± 0.05^{bc}	66.78 ± 2.77^{a}	0.53 ± 0.002^{c}
F2	0.163 ± 0.0050^{a}	93.50 ± 0.50^{a}	0.32 ± 0.01^{abc}	59.94 ± 1.90^{a}	0.34 ± 0.009^{a}
F3	0.165 ± 0.0056^{a}	93.08 ± 0.50^{a}	0.30 ± 0.01^{abc}	59.14 ± 2.35^{a}	0.60 ± 0.024^{d}
F4	0.258 ± 0.011^{a}	93.38 ± 0.50^{a}	0.37 ± 0.02^{c}	65.17 ± 2.31^{a}	0.64 ± 0.009^{d}
F5	0.172 ± 0.007^{a}	92.49 ± 0.50^{a}	0.26 ± 0.05^{a}	66.39 ± 14.3^{a}	0.41 ± 0.025^{b}
F6	0.185 ± 0.006^{b}	93.54 ± 0.50^{a}	0.28 ± 0.01^{ab}	66.58 ± 1.46^{a}	0.51 ± 0.014^{c}
F7	0.277 ± 0.016^{c}	91.78 ± 0.50^{a}	0.30 ± 0.06^{abc}	67.94 ± 0.29^{a}	0.51 ± 0.041^{c}
F8	0.162 ± 0.0019^{a}	88.96 ± 0.50^{d}	0.2 ± 0.01^{a}	88.33 ± 11.8^{b}	2.17 ± 0.189^{b}
F9	0.210 ± 0.0044^{b}	84.92 ± 0.50^{c}	0.4 ± 0.09^{a}	63.85 ± 2.71^{a}	1.20 ± 0.013^{a}
F10	0.213 ± 0.0088^{b}	83.75 ± 0.50^{bc}	0.2 ± 0.03^{a}	57.60 ± 3.09^{a}	1.17 ± 0.096^{a}
F11	0.218 ± 0.0011^{bc}	81.60 ± 0.50^{ab}	0.3 ± 0.02^{a}	54.93 ± 1.27^{a}	1.21 ± 0.005^{a}
F12	0.225 ± 0.0044^{c}	81.08 ± 0.50^{a}	0.3 ± 0.1^{a}	52.07 ± 0.84^{a}	1.16 ± 0.011^{a}

*Different letters (a–e) in the table indicate a statistically significant difference between samples within each polymer group/method ($p < 0.05$). Data are presented as mean ± standard deviation, and statistical analysis was performed using one-way analysis of variance (ANOVA) and the Duncan multiple comparison test. Both methods were subjected to independent statistical evaluation within their respective groups.

When the elongation at break (%EB) and tensile strength (TS) values of F1–F7 samples were statistically evaluated, it was determined that the formulations did not have a significant effect on %EB values ($p>0.05$), whereas significant differences were observed in TS values ($p\leq0.05$) (Table 1). The fact that all samples were grouped within the same statistical category ("a") in terms of %EB indicated that gelatine, lecithin, and *Rheum ribes* incorporation did not significantly affect the elasticity of the films ($p>0.05$). In contrast, the presence of different statistical groups in TS values demonstrated that formulation changes had a considerable effect on film strength. In particular, lecithin addition was thought to reduce tensile strength in some samples, whereas increasing gelatine concentration strengthened the film matrix and contributed to higher TS values. When the elongation at break (%EB) and tensile strength (TS) values of F8–F12 samples were statistically evaluated, significant differences were determined among the samples ($p\leq0.05$) (Table 1). The F8 sample was found to be statistically different from all other samples in terms of both TS and %EB values and was classified in group "b". In contrast, the F9–F12 samples were included in the same statistical group ("a") and did not exhibit significant differences among themselves. The results indicated that increasing rhubarb extract incorporation led to a reduction in the mechanical strength and flexibility of the film structure. In particular, the decrease in TS values may be associated with the weakening effect of the plant-based additive on the integrity of the chitosan matrix and the partial disruption of the regular interactions between polymer chains. Similarly, the reduction in %EB values may be attributed to the development of a more brittle and less elastic film structure. Nevertheless, the inclusion of the F9–F12 samples within the same statistical group suggested that, beyond a certain incorporation level, further increases in rhubarb extract concentration did not produce an additional significant effect on the mechanical properties of the films. TS values in the chitosan group ranged from 1.16 to 2.17 MPa, approximately 2-4 times higher than those of starch-based films. These results indicated that the polymer structure of chitosan-based films exhibited a stronger network compared to the starch–gelatin-based films, and that increasing the proportion of rhubarb concentration reduced elasticity in chitosan films while maintaining strength within a certain range. In general, chitosan-based films offered higher mechanical strength, while starch–gelatin-based films exhibited a more flexible but lower-strength structure.

When the FRAP and DPPH antioxidant activity values of F1–F7 samples were statistically evaluated, significant differences were determined among the samples ($p\leq0.05$) (Table 2). According to the FRAP results, the F1 sample exhibited the lowest antioxidant capacity and was classified in group "a".

A marked increase in FRAP values was observed in all film samples containing *Rheum ribes*, with the F4 sample showing the highest value and being classified in group “d”. While F3, F5, and F6 samples were included in the same statistical group (“c”), F2 and F7 samples were classified in group “b”. A similar trend was observed in the DPPH results, where the F4 sample exhibited the highest radical scavenging capacity and was classified in group “c”. F2 and F3 samples were included in group “b”, whereas F5, F6, and F7 samples were grouped together with F1 in group “ab”, with no statistically significant differences determined among them. Overall, the results indicated that the incorporation of *Rheum ribes* enhanced the antioxidant capacity of the film-forming mixtures. However, higher gelatin and lecithin concentrations may have influenced the distribution or interactions of antioxidant compounds within the film matrix, thereby leading to a reduction in antioxidant activity in some samples.

Table 2. FRAP and DPPH results of film-forming mixtures and color parameters of dried film samples

Film samples	FRAP (µg BHA eq /mL)	DPPH (µg ascorbic acid eq/mL)	*L**	*a**	*b**
F1	25.10±1.78^{a}	229.48±51.06^{a}	86.31±3.33^{c}	-4.21±1.69^{a}	11.70±5.00^{a}
F2	72.79±9.16^{b}	431.37±108.94^{b}	80.68±2.06^{bc}	-4.63±0.27^{a}	13.17±1.34^{a}
F3	101.66±1.73^{c}	489.96±83.64^{b}	79.07±3.77^{abc}	-4.31±1.44^{a}	12.66±4.14^{a}
F4	135.84±13.09^{d}	637.50±99.51^{c}	74.91±4.79^{ab}	-5.16±0.92^{a}	15.83±3.49^{a}
F5	105.02±1.58^{c}	359.97±76.89^{ab}	81.63±2.34^{bc}	-6.29±1.07^{a}	18.44±4.25^{a}
F6	98.86±9.80^{c}	368.42±71.77^{ab}	79.07±3.77^{abc}	-4.31±1.44^{a}	13.00±4.71^{a}
F7	72.56±7.78^{b}	360.86±41.29^{ab}	69.66±10.84^{a}	-6.21±1.32^{a}	20.20±6.35^{a}
F8	19.52±0.98^{a}	221.64±46.92^{a}	93.40±5.53^{d}	-3.70±0.29^{ab}	10.56±1.55^{a}
F9	85.20±3.70^{b}	466.27±53.70^{b}	57.73±7.00^{c}	-6.23±0.88^{a}	33.93±2.05^{d}
F10	91.40±31.25^{b}	787.85± 70.93^{c}	57.53±5.90^{c}	-4.44±0.92^{ab}	36.57±0.04^{d}
F11	154.88±15.55^{d}	853.667±113.04^{c}	19.94±5.59^{a}	-0.79±0.98^{c}	24.03±5.28^{c}
F12	187.26±16^{e}	893.63±23.92^{c}	31.00±8.01^{b}	-2.86±0.06^{bc}	16.76±8.54^{b}

*Different letters (a–e) in the table indicate a statistically significant difference between samples within each polymer group/method ($p<0.05$). Data are presented as mean ± standard deviation, and statistical analysis was performed using one-way analysis of variance (ANOVA) and the Duncan multiple comparison test. Both methods were subjected to independent statistical evaluation within their respective groups.

When the FRAP and DPPH antioxidant activity values of F8–F12 samples were statistically evaluated, significant differences were determined among the samples ($p\leq0.05$) (Table 2). According to the FRAP results, the F8 sample exhibited the lowest antioxidant capacity and was classified in

group “a”. A significant increase in FRAP values was observed with the incorporation of rhubarb extract, while the F11 and F12 samples exhibited the highest antioxidant capacities and were classified in groups “d” and “e”, respectively. In contrast, the F9 and F10 samples were included in the same statistical group (“b”) and did not show significant differences between each other. Evaluation of the DPPH results revealed that the F8 sample had the lowest radical scavenging capacity and was classified in group “a”. While the F9 sample was classified in group “b”, the F10, F11, and F12 samples were included in the same statistical group (“c”) and no significant differences were determined among them. Overall, the obtained results indicated that the incorporation of rhubarb extract significantly enhanced the antioxidant capacity of the film-forming mixtures. This increase may be attributed to the phenolic compounds, and other bioactive components present in the structure of *Rheum ribes*. Furthermore, increasing rhubarb extract concentration was considered to enrich the film matrix with greater amounts of antioxidant compounds, thereby enhancing both reducing power and free radical scavenging capacity. It should also be noted that the antioxidant activity analyses were performed on the film-forming mixtures before the drying process. Therefore, the FRAP and DPPH values obtained in this study should be interpreted as indicators of the antioxidant potential of the precursor film-forming systems rather than as direct measurements of the antioxidant performance of the final dried films. Drying conditions, polymer–phenolic interactions, and possible changes in phenolic stability and availability within the dried film matrix may influence the actual functional performance of the films.

A reduction in antioxidant activity was observed following the addition of soy lecithin. Although rhubarb addition significantly enhanced antioxidant activity, the incorporation of soy lecithin slightly decreased the antioxidant performance in starch–gelatin-based films. This reduction may be attributed to the entrapment of antioxidant compounds within the emulsion matrix formed by soy lecithin, thereby limiting their accessibility. Furthermore, the aggregation of phenolic compounds at high concentrations or their non-homogeneous distribution within the film matrix may also explain the decrease in antioxidant activity. In a study conducted on polyvinyl alcohol/starch-based films enriched with rosemary extract, antioxidant activity analyses similarly reported that rosemary extract enhanced antioxidant activity, whereas the subsequent addition of kaolin reduced this activity. The decrease was attributed to synergistic interactions arising from the simultaneous presence of additives, negatively affecting antioxidant performance [38]. Moreover, Laguerre et al. (2007), in their study investigating the relationship between antioxidants and lipids, reported that various synergistic effects may occur

between antioxidants and lipids, including interactions of antioxidants distributed in multiphase environments with different polarities, phases, or solvents. Despite this decrease, the antioxidant activity of rhubarb- and soy lecithin-containing samples remained higher than that of the control sample [39]. Similarly, studies conducted on films produced using gelatin, soy polysaccharides, and tea polyphenols, as well as another study on chitosan–starch-based films enriched with thyme extract, reported that the incorporation of phenolic compounds significantly enhanced antioxidant activity [40, 41]. Overall, the FRAP and DPPH values obtained in the present study indicate a higher antioxidant capacity compared with many edible film systems reported in the literature. This may be explained by the high phenolic compound content of rhubarb extract, its strong interactions with the chitosan matrix, and the enhanced stability of antioxidant compounds resulting from these interactions. The results represent the antioxidant capacity imparted by rhubarb extracts obtained through two different methods within the respective film matrices. In another study, DPPH analyses performed on chitosan–gelatin-based films enriched with ferulic acid, caffeic acid, and tyrosol revealed that although radical scavenging capacity reached up to 90%, the release rate decreased [42]. These findings suggest that rhubarb extract possesses a richer phenolic composition and a stronger redox potential.

When the color parameters (*L*, a*,* and *b**) of F1–F7 samples were statistically evaluated, significant differences were determined among the samples, particularly in terms of *L** values ($p \leq 0.05$) (Table 2). According to the *L** results, the F1 sample exhibited the highest brightness value and was classified in group “c”, whereas the F7 sample showed the lowest *L** value and was classified in group “a”. The F2 and F5 samples were included in group “bc”, the F3 and F6 samples in group “abc”, and the F4 sample in group “ab”. The results indicated that the incorporation of *Rheum ribes* led to a decrease in film brightness, resulting in a darker film appearance. In particular, the combined effect of higher gelatin and lecithin concentrations together with *Rheum ribes* incorporation may have increased color intensity within the film matrix, thereby reducing light transmission. In contrast, evaluation of the *a** and *b** values revealed that all samples were included in the same statistical group (“a”), and no significant differences were determined among the samples ($p > 0.05$). Nevertheless, numerical evaluation indicated that increasing *Rheum ribes* incorporation caused the *a** values to shift toward more negative values, while the *b** values increased. This effect may be attributed to the natural pigments and phenolic compounds present in *Rheum ribes*, which likely imparted more greenish-yellow tones to the films.

When the color parameters (*L*, a*,* and *b**) of the F8–F12 film samples were statistically evaluated, the formulations were found to exert significant effects on all color parameters ($p \leq 0.05$) (Table 2). Evaluation of the *L** values revealed that the F8 sample exhibited the highest brightness value and was classified in group “d”. In contrast, the F11 sample showed the lowest *L** value and was classified in group “a”. The F12 sample was included in group “b”, whereas the F9 and F10 samples were classified within the same statistical group (“c”). These results indicated that increasing Rheum ribes incorporation decreased the brightness of the films, resulting in a darker appearance. This effect may be attributed to the high phenolic compound and natural pigment contents of *Rheum ribes*, which likely increased color intensity within the film matrix. In terms of *a** values, the F11 sample exhibited the highest value and was classified in group “c”, whereas the F9 sample showed the lowest value and was classified in group “a”. The F8 and F10 samples were included in the same statistical group (“ab”), while the F12 sample was classified in group “bc”. The results suggested that Rheum ribes incorporation altered the green color tones of the films. In particular, more negative *a** values indicated the development of more pronounced greenish tones in the film structure. Evaluation of the *b** values demonstrated significant differences among all samples ($p \leq 0.05$). The F9 and F10 samples exhibited the highest *b** values and were classified within the same statistical group (“d”). The F11 sample was included in group “c”, the F12 sample in group “b”, and the F8 sample in group “a”. These findings indicated that the incorporation of rhubarb extract increased the yellowness values of the films and particularly contributed to the development of more intense yellow tones at moderate incorporation levels.

The SEM images (Figure 2) revealed that the F1 control film (a) in the starch-gelatin based films exhibited a smoother and more homogeneous surface structure, with a limited number of small particles observed in the matrix. This indicated that the base formulation without rhubarb extract forms a more compact structure. With the addition of rhubarb extract, the F2 film (b) featured significant irregularities, aggregates, and larger particles on the surface, indicating that the extract components are not fully dispersed within the polymer matrix. Although sample F3 (c) showed a more uniform structure compared to F2, irregular particles attached to the extract were still present on the surface. It could be said that the matrix integrity was partially improved with increasing gelatin content. Formulation F4 (d) exhibited the most pronounced surface roughness and heterogeneous distribution. It was observed that increasing amounts of gelatin and rhubarb led to denser aggregations and a more irregular morphology. In general, the addition of

rhubarb extract disrupted the surface morphology and increased surface unevenness, while the amount of gelatin partially moderated this effect but increased heterogeneous formations at high rates.

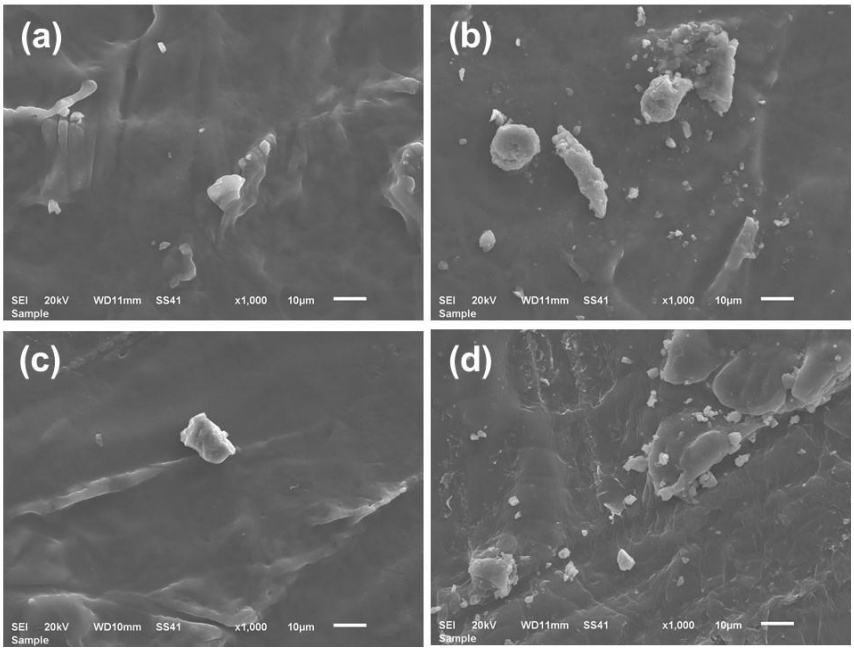

Figure 2. SEM images of (a) F1, (b) F2, (c) F3, and (d) F4 samples

SEM images in Figure 3 revealed that starch-containing gelatin-added rhubarb mixtures exhibited similar behavior. As the gelatin addition ratio increased with the addition of lecithin, a homogeneous distribution of particles on the surface was observed. The SEM images of chitosan-based films (Figure 4) showed that in the control sample (Figure 4a), particles of different sizes and shapes were formed on a homogeneous film, whereas in the rhubarb-added films (Figure 4 b-e), the chitosan-derived particles covered the surface more homogeneously. Furthermore, as the level of rhubarb addition increased, the size of the chitosan particles increased. This can be explained by the presence of a physical interaction in chitosan-rhubarb gel mixtures.

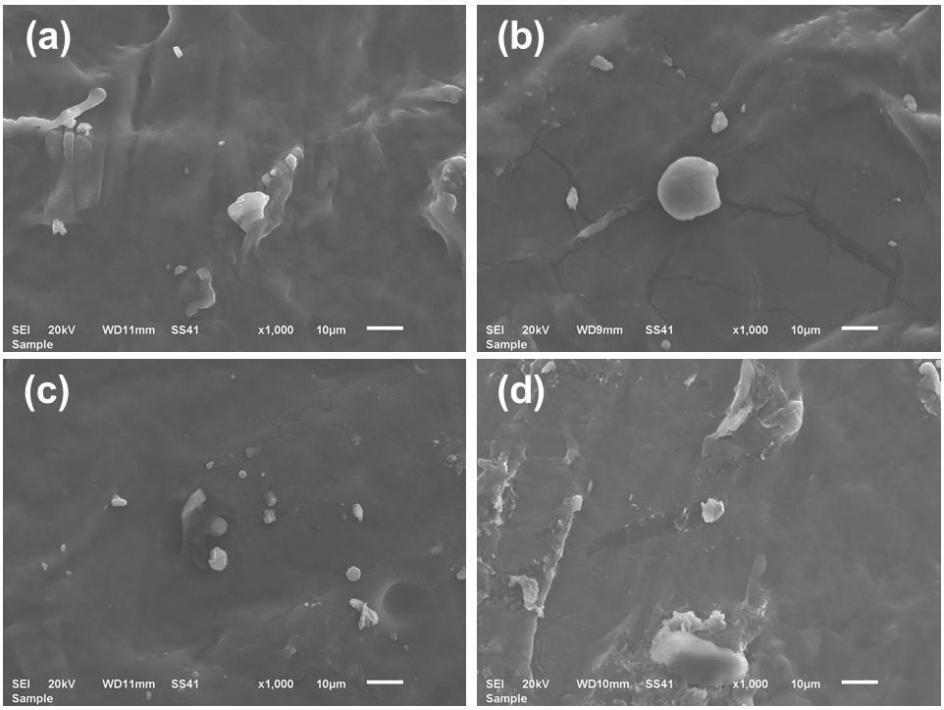

Figure 3. SEM images of a) F1, b) F5 c) F6, and d) F7 samples

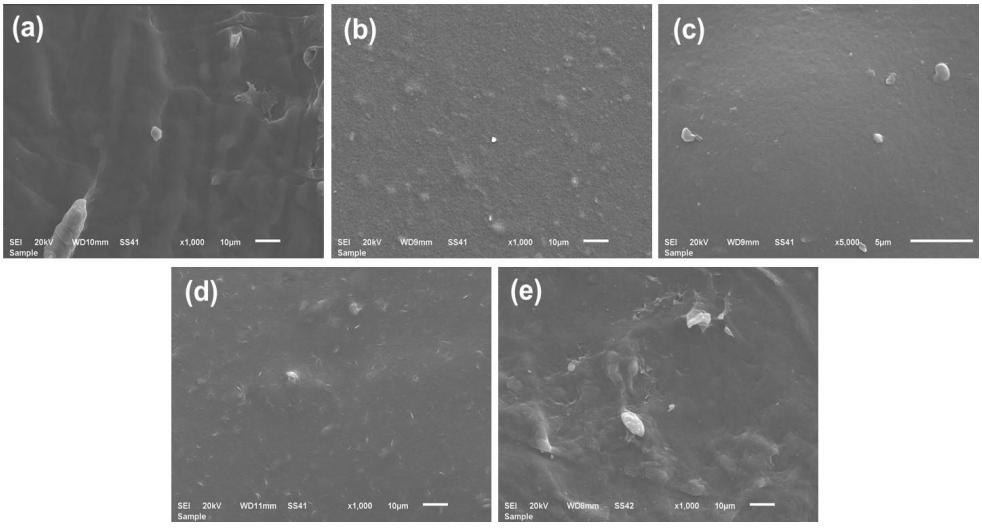

Figure 4. SEM images of a) F8, b) F9 c) F10 d) F11 and e) F12 samples

A comparison of the SEM films made with both methods showed that rhubarb addition increased surface roughness, but lecithin addition provided a more homogeneous image in starch-gelatin based films. It was determined that the amount of particles on the surface increased as the amount of gelatin increased. Overall, surface roughness increased slightly from the control samples to the other samples.

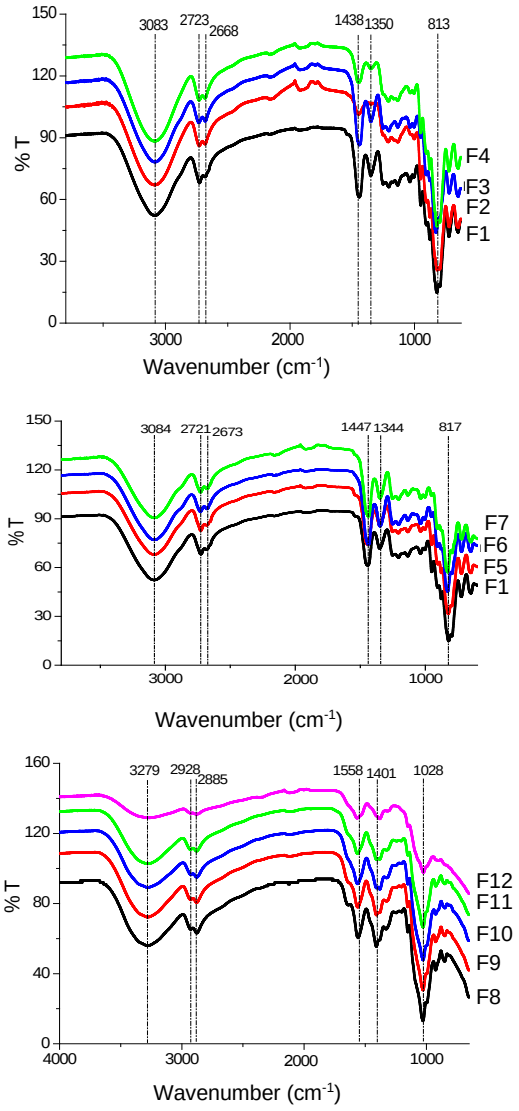

Figure 5. FT-IR spectra of the film samples

FT-IR spectra of samples produced by both methods are shown in Figure 5. In starch-gelatin based films, the band observed at the peak value of 3083 cm^{-1} in the spectra corresponds to the -O-H- bond strain in the -OH- structure. The bands observed at wavenumber values of 1152 and 1079 cm^{-1} belong to natural starch and correspond to the -C-O-- bond strain in the -C-O-H structure of starch. Furthermore, the peaks at 1160 and 1100 cm^{-1} belong to the -C-O-C- bond strain. The peaks at 1000-800 cm^{-1} originate from -C-H- bond strains [38]. In addition, the absorption band observed at approximately 2721 cm^{-1} can be attributed to the symmetric stretching vibration of CH_2 groups, while the band at 2668 cm^{-1} is associated with the vibration of $CH_3NH_3^+$ groups. In films prepared with starch-gelatin mixtures, it was observed that starch and gelatin had their own peaks in their spectra. Since there was no change in peaking or peak intensity, the structure did not deviate from the natural starch-gelatin structure and no chemical deformation occurred.

In the FT-IR spectra of chitosan-based films, characteristic bands of chitosan were present, and there was no change in band positions. These findings may indicate that no major chemical interaction or structural deformation occurred during film preparation. It was observed that there was no interaction between the functional groups of chitosan and the active groups of the added substance, and the bond structure of the films remained unchanged [43].

A comparison of the film samples produced by both methods showed that the spectra of film mixtures prepared with starch showed characteristic peaks of starch and gelatin. This indicated that the structure did not deviate from the natural mixture and that no chemical deformation occurred. A similar situation was observed in film mixtures prepared with chitosan.

The absorbance and transmittance values of the 12 films produced are given in Figure 6. Since the values were not within the 0-1 range when the samples were used undiluted, the samples were diluted 10-fold with distilled water before analysis. In the samples created with starch-gelatin based films, a decrease in absorbance values was observed among the samples with added rhubarb, except for sample F7. The transmittance values of the samples indicated that an increase was observed among the samples with added rhubarb, except for sample F7. In chitosan-based films, the absorbance values of the samples showed a regular increase towards the control sample F8 and the rhubarb-added samples (F9-F12). Transmittance values showed a regular decrease towards the control sample F8 and the rhubarb-added samples (F9-F12).

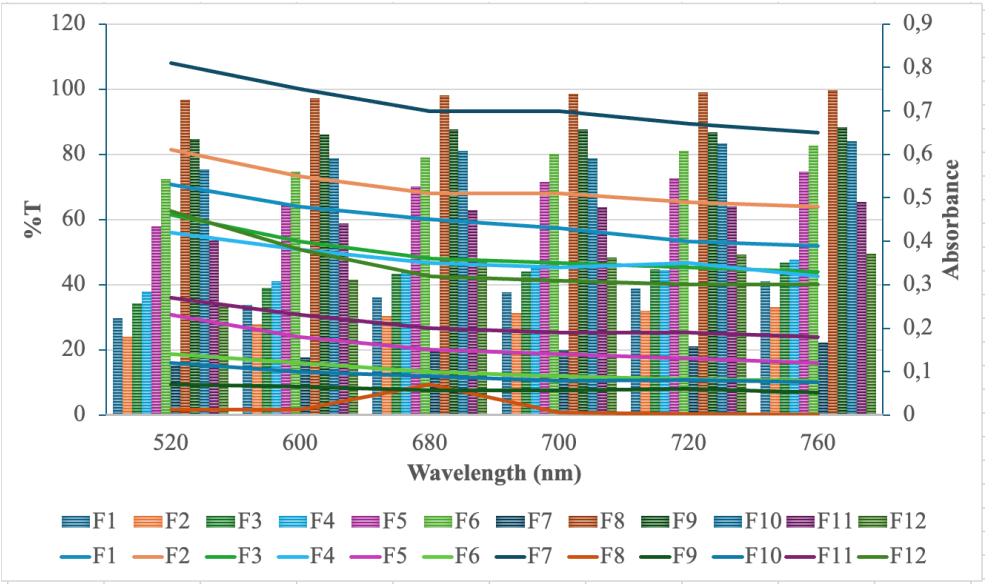

Figure 6. Absorbance–%Transmittance (%T) results of the film samples
*single-line absorbance, triple-line transmittance.

Figure 6 indicates that the absorbance and transmittance behavior of the 12 film samples produced showed significant differences depending on both wavelength and film matrix type. Performing measurements on samples diluted 10 times allowed for the analytical evaluation of absorbance values within a reliable range. In starch-gelatin based films, with the addition of rhubarb, absorbance curves generally remained lower across all wavelengths except for sample F7, while transmittance values increased. This can be attributed to the more homogeneous distribution of rhubarb within the starch-gelatin matrix and the resulting effect of reduced light scattering in the film structure. The exception of sample F7 to this general trend suggested the presence of a denser structure or components that absorbed light more in the film matrix. In chitosan-based films, on the other hand, absorbance values increased regularly across all wavelengths from the control sample F8 to the rhubarb-added samples (F9-F12), while transmittance values decreased significantly. This trend indicated that rhubarb-induced phenolic and colored components in the chitosan matrix increased the optical density of the film, thus limiting light transmittance. The consistent change in absorbance and transmittance curves, particularly across increasing wavelengths, revealed that rhubarb addition was structurally integrated into the film matrix and enhanced its optical barrier properties. Overall, the results demonstrated that rhubarb

addition had a matrix-specific effect on film optical properties and can provide advantages for packaging applications, especially in chitosan-based films, by reducing light transmittance.

The biodegradation behavior of the films was evaluated separately for each polymer system. Starch–gelatin-based films (F1–F7) exhibited biodegradability values ranging from 32.09% to 66.99% after 24 h of incubation. (24-hour; F1: 66.99±6.21, F2: 64.45±0.07%, F3: 56.96±4.42%, F4: 59.43±4.86%, F5: 61.37±12.01%, F6: 37.35±1.44% and, F7: 32.09±4.45%) At 72 hours of incubation, the biodegradability values of starch–gelatin-based films were determined as follows: F1: 90.44±1.58%, F2: 86.69±2.74%, F3: 87.20±2.48%, F4: 89.85±2.15%, F5: 89.74±3.25%, F6: 82.37±0.71% and F7: 84.26±0.94% indicating a high level of degradation within a relatively short time period. At 120 hours, F1–F7 exhibited complete biodegradation (100%), demonstrating that the degradation process was rapid and nearly completed within five days. These results highlight the fast degradation behavior of starch–gelatin matrices, which can be attributed to their hydrophilic nature and relatively loose polymer network. This can be explained by the hydrophilic structure of these biopolymers and the presence of glycosidic and peptidic bonds that are easily broken down by microorganisms. The film sample showing 90.44% biodegradability, in particular, revealed that the polymer matrix has a looser network structure and is susceptible to rapid degradation under environmental conditions. Chitosan-based films (F8–F12) showed a gradual increase in biodegradation over the incubation period. At day 5, the biodegradability values were determined as F8: 35.77%, F9: 29.00%, F10: 35.53%, F11: 40.88%, and F12: 37.43%. As the incubation period progressed, these values increased, and at day 15, biodegradability values were recorded as F8: 57.96%, F9: 62.17%, F10: 53.61%, F11: 59.89%, and F12: 53.36%. F8-F12 films, on the other hand, featured biodegradability values ranging from 78.33% to 97.79% after a 30-day incubation period (F8: 91.08±1.92%, F9: 97.79±3.13%, F10: 88.20±0.45%, F11: 83.40±0.04% and F12: 78.33±0.96%). The highest biodegradability rate of 97.79% can be attributed to the gradual hydrolysis of chitosan chains during long-term incubation and the decrease in the initially present antimicrobial effect over time. On the other hand, the film sample showing 78.33% biodegradability exhibited a denser and partially crystalline polymer network structure, displaying a relatively more resistant structure against microorganisms. It should be noted that the biodegradation behavior of starch–gelatin-based and chitosan-based films was evaluated over different incubation periods. Therefore, the results were interpreted separately within each polymer system rather than through direct quantitative comparison between the two film groups. This methodological difference should be considered when evaluating the biodegradation performance of the films.

Although the present study provides useful comparative information on rhubarb-enriched edible films prepared using starch–gelatin and chitosan matrices, it should be noted that the two experimental systems differed not only in polymer matrix type but also in rhubarb form, preparation procedure, and processing conditions. Therefore, the differences observed in physicochemical, mechanical, and functional properties cannot be attributed exclusively to the polymer matrix. Instead, these results should be interpreted as the combined effect of the matrix characteristics and the matrix-specific formulation and processing conditions required for successful film formation.

CONCLUSIONS

In the present study, the physicochemical, mechanical, optical, antioxidant, biodegradability, and structural properties of starch-gelatin and chitosan-based edible films with rhubarb (*Rheum ribes* L.) addition, produced by two different methods, were comparatively evaluated. The results revealed that film performance varied significantly depending on the production method and the polymer matrix used. Starch-gelatin-based films exhibited higher water solubility and flexibility, while chitosan-based films showed higher tensile strength and better water vapor barrier properties.

Rhubarb addition significantly increased the antioxidant potential of the film-forming mixtures in both systems. This increase was more pronounced in the chitosan-based film-forming mixtures. Color and optical analyses showed that increasing rhubarb concentration led to darkening of the film matrix and a decrease in light transmittance. SEM and FT-IR analyses revealed that rhubarb addition caused physical changes in the film structure but did not result in any degradation in the chemical structure of the polymers. Biodegradability results revealed distinct degradation patterns within each polymer system. Starch–gelatin-based films and chitosan-based films were evaluated over different incubation periods; therefore, the biodegradation findings were considered separately within each polymer matrix, and no direct quantitative comparison was made between the two systems. Overall, chitosan-based films with rhubarb addition appeared to exhibit improved functional and mechanical properties and may represent a promising alternative for active and sustainable food packaging applications. Under the experimental conditions of this study, chitosan-based films appeared to show relatively favorable mechanical and functional properties; however, these findings should be interpreted with caution because the two systems differed in rhubarb form, preparation conditions, and polymer matrix composition.

EXPERIMENTAL SECTION

Production of Edible Films

Method 1: The method described by Tulamandi et al. was revised and used [44]. The method and steps used are summarized in Table 3. In this context, first, the peel of the rhubarb stored at -18°C was removed, cut into small pieces, and pureed in distilled water (1/0.5-w/v) using a homogenizer (OVS-VELP Scientifical). A starch (BCCB9290, Sigma Aldrich) film solution was prepared at the concentrations given in Table 4. The solution was incubated at 75°C for 30 min on a magnetic stirrer (Heildolph MR Hei Standard-505-20000-00-2). Then, glycerol (SZBE030EV, Sigma Aldrich) was added. The mixture was cooled to 55-60°C while stirring continued for 30 min. Finally, gelatin (PE 19-00723-1, Sigma Aldrich) and soy lecithin (SIL29018-1, Sigma Aldrich) were added in the specified proportions. It was observed that soy lecithin did not achieve sufficient dissolution when added directly to the film solution. The best dissolution was achieved by heating 1 mL of glycerol and 1 mL of distilled water to 55°C and slowly adding soy lecithin. The produced film samples were spread in glass petri dishes. The samples spread in the petri dishes were left to dry in an oven at 40°C (POL-EKO-APARATURE-SLN-53-std) for 18 hours.

Table 3. Film formulation used in the first method

Film Samples	Rhubarb	**Starch**	**Gelatin**	**Glycerol**	**Lecithin**
F1	-	2%	2%	3%	0.5%
F2	8**%**	2%	1%	3%	-
F3	8**%**	2%	2%	3%	-
F4	8**%**	2%	3%	3%	-
F5	8**%**	2%	1%	3%	0.5%
F6	8**%**	2%	2%	3%	0.5%
F7	8**%**	2%	3%	3%	0.5%

Method 2: The method described by Ponce et al. was used with some modifications [45]. The method and its steps are summarized in Table 4. Firstly; the rhubarb (*Rheum ribes* L.) plant, which was kept at -18°C, was taken, after removing the peel it was powdered by evaporating the water by keeping it in a lyophilizer (LABCONCO-7670530) at -44°C for 48 hours. Solutions within the concentration range shown in Table 4 were stirred on a magnetic stirrer (Heildolph MR Hei Standard-505-20000-00-2) for 6-12 hours before the addition of rhubarb. The rhubarb concentrations specified in Table 4 were added to the mixtures after incubation and homogenized for 15 minutes using a homogenizer (OVS-VELP Scientifical). The resulting mixtures were spread into glass petri dishes and dried at 30°C for 18 hours to evaporate the water.

Table 4. Film formulation used in the second method

Film Samples	Rhubarb	Chitosan	Glycerol
F8	0%	2%	1%
F9	0.5%	2%	1%
F10	1%	2%	1%
F11	1.5%	2%	1%
F12	2%	2%	1%

Characterization Studies of Films

Film Thickness

Film thickness was determined using a digital micrometer. At least six measurements were taken from different points of the film, in at least three parallels, and the average thickness was determined [46].

Water Solubility

The method described by Tunç et al. was revised and used [47]. The sample, dried at 103°C for 24 hours, was weighed and the initial weight was determined. Ten times the weight of the weighed sample was added to distilled water. It was stirred with a magnetic stirrer at 100 RPM at room temperature for six hours. Then, the part that did not dissolve in water was passed through filter paper. The part remaining on the filter paper was dried at 103°C for 24 hours until it reached a constant weight and weighed at the end of the period.

Color Measurements of Films

Each film sample was measured on a white standard surface using a color analyzer (PenColorArt USB Model, 1L) for *L** (lightness), *a** (red-green), and *b** (yellow-blue) color parameters. The analysis was performed in three parallels for each film [44].

Determination of Tensile Strength and Elongation at Break (TS, %EB) of Films

The tensile strength (TS, tensile strength) and elongation at break (EB, elongation at break) values of the films were determined using the standard method of ASTM standard method 882 [48]. Results were obtained using a texture analyzer (TA. HD Plus Stable Micro Systems Texture Analyser, UK). Films (6 cm x 1 cm) were cut and attached to the ends of the tensile probe. The initial distance between the probe ends was determined as 25 mm, and the films were stretched at a speed of 10 mm/s. Tensile measurements were performed

using a 5 kg load cell. Measurements were performed at room temperature as two replicates [49]. Tensile strength (TS, MPa) and elongation at break (E, %) were calculated using Equations (1) and (2), respectively:

$$TS\ (MPa) = (RE \times g) / (W \times T \times 10^{-6}) \quad (1)$$

$$E\ (\%) = (L / L_0) \times 100 \quad (2)$$

where T is the average thickness of the film samples (m), W is the width (m), RE is the resistance to elongation (kg), g is the gravitational acceleration (9.81 m/s²), L is the final length of the film (m), and L_0 is the initial length of the film (m).

Absorbance and Transmittance Values

The absorbance and transmittance values of the film solutions were read using a UV spectrophotometer (Shimadzu, UV–1280, Kyoto, Japan) at wavelengths of 520 nm, 600 nm, 680 nm, 700 nm, 720 nm, and 760 nm [50].

Fourier Transform Infrared Spectroscopy (FT-IR)

The films were analyzed with an FT-IR instrument (Thermo/Nicolet iseon 912A0607) at a resolution of 4 cm^{-1}, each with ratios between 4000-400 cm^{-1} [44].

Scanning Electron Microscopy (SEM)

SEM experiments were carried out with a JEOL JSM 6510 SEM. Prior to analysis, the samples were coated with a thin layer of gold (Au) to enhance conductivity. The SEM observations were performed at an accelerating voltage of 20 kV, and a magnification of 1000x was used for cross-sectional imaging [51].

Antioxidant Activity Analyses

Reducing power of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) (FRAP method) was measured according to the method of Benzie and Strain [52]. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was performed according to the method of Blois [53]. Antioxidant activity analyses of the samples used in the study were performed by taking samples directly from the mixtures before drying the gels. All analyses were performed in triplicate.

Biodegradability

The method applied by Jaramillo et al. was revised and applied [54]. Soil samples taken from the same conditions were filled into pots as 1 kg each. The samples, dried in Petri dishes, were divided into six different parts.

The samples were weighed (G1). Six different wells were opened in the pots containing the soil, and the samples were buried and covered with soil. The pots were watered with 50 mL of water daily. Every five days, the samples were removed, cleaned, and weighed (G2). The analysis was conducted at room temperature for 30 days. All studies were performed as two parallel processes.

Biodegradability (%) = $((G1-G2)/G1)\times 100$.

Water Vapor Permeability

The water vapor permeability of the films was determined using the ASTM (2003) method [55]. Silica gel (1 g) was placed in tubes. To prevent moisture from remaining in the tubes and silica gel, they were kept at 103°C for 24 hours. Film samples were cut into 2.5 cm x 2.5 cm pieces and placed at the mouth of the tubes. The tubes were coated with paraffin to prevent moisture absorption. The bottom of the desiccator was filled with distilled water. The tubes were placed in the desiccator and kept at 25°C for 24 hours. Samples were weighed at 0, 3, 6, 9, 24, 27, 30 and 33 hours.

x = Film thickness (mm), w/t (Slope) = Calibration slope (g/hour),

A = Surface area of the film in contact with water vapor (cm^2),

ΔP = Applied pressure difference (Pa)

Water vapor permeability = $(w/t) \times [x / (\Delta P \times A)]$.

Statistical Analysis

All data obtained are expressed as mean ± standard deviation. Statistical differences between samples were evaluated using one-way analysis of variance (ANOVA), and the Duncan multiple comparison test was applied to determine the differences between groups. A p-value of ≤ 0.05 was considered statistically significant. Statistical analyses were performed using the IBM SPSS Statistics 20 software package.

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