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Research Article/Araştırma Makalesi

Sustainable edible films based on seaweed mucilage enriched with pomegranate peel extract

Nar kabuğu ekstraktı ile zenginleştirilmiş deniz yosunu müsilajı esaslı sürdürülebilir yenilebilir filmler

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ABSTRACT

The objective of this study was to develop sustainable edible films based on seaweed mucilage (*Chondrus crispus*) containing pomegranate peel extract (PPE). For this purpose, films containing different concentrations of PPE (%0, 0.25, 0.50, and 1; w/v) were evaluated for their thickness, mechanical properties, color, opacity, antioxidant capacity, and total phenolic content (TPC). The incorporation of PPE significantly affected the values of elongation at break, color, antioxidant capacity, and TPC ($p<0.05$). However, the effect of PPE incorporation on the thickness, tensile strength, and opacity of the films was not significant ($p>0.05$). The addition of PPE significantly ($p<0.05$) increased the elasticity of the films, with the highest elongation at break observed in the film containing 0.5% PPE (126.90%). The DPPH scavenging activity of the films containing PPE ranged from 0.13 to 0.23 mmol TEAC/g, and the TPC values ranged from 8.39 to 29.95 mg GAE/g film. The antioxidant capacity and TPC values of the films increased significantly ($p<0.05$) with higher concentrations of PPE. The addition of PPE resulted in a decrease in film brightness, but an increase in redness and yellowness. In conclusion, the developed films offer a promising alternative for sustainable edible film production.

Key Words: Edible film, Sustainable film, Seaweed, *Chondrus crispus*, Natural extract

ÖZ

Bu çalışmanın amacı, nar kabuğu ekstraktı (NKE) içeren deniz yosunu müsilajı (*Chondrus crispus*) esaslı sürdürülebilir yenilebilir filmler geliştirmekti. Bu amaçla, farklı konsantrasyonlarda (%0, %0.25, %0.50 ve %1; a/h) NKE içeren filmler kalınlık, mekanik özellikler, renk, opaklık, antioksidan aktivite ve toplam fenolik içeriği (TPC) açısından incelendi. NKE ilavesi filmlerin uzama katsayısı, renk, antioksidan aktivite ve TPC değerleri üzerinde önemli ($p<0.05$) bir etkiye sahipti. Ancak, NKE ilavesinin filmlerin kalınlığı, çekme direnci ve opaklık değerleri üzerindeki etkisi önemli değildi ($p>0.05$). NKE ilavesi filmlerin elastikiyetini önemli ($p<0.05$) düzeyde artırdı ve en yüksek uzama katsayısı değeri %0.5 NKE içeren filmde (% 126.90) belirlendi. NKE içeren filmlerin DPPH süpürme aktivitesi 0.13-0.23 mmol TEAC/g arasında değişirken, TPC değerleri 8.39-29.95 mg GAE/g film aralığında bulundu. Bunlara ek olarak, filmlerin antioksidan aktivite ve TPC değerleri, NKE konsantrasyonunun artmasıyla birlikte belirgin ($p<0.05$) düzeyde arttı. NKE ilavesi filmlerin parlaklığında azalmaya, ancak kırmızılık ve sarılıkta artışa neden oldu. Sonuç olarak, geliştirilen filmler sürdürülebilir yenilebilir film üretimi için umut verici bir alternatif sunmaktadır.

Anahtar Kelimeler: Yenilebilir film, Sürdürülebilir film, Deniz yosunu, *Chondrus crispus*, Doğal ekstrakt

Introduction

The impact of plastic waste is a major concern in the food industry due to the large proportion of traditional packaging materials (Briassoulis & Giannoulis, 2018). Environmental concerns about plastic packaging materials have led to these materials being replaced with bio-based alternatives (Briassoulis & Giannoulis, 2018). Edible bio-based films are emerging as primary alternatives to traditional fossil-based plastic films, especially for applications such as food packaging. Bio-based films exhibit superior properties compared to fossil-based films in terms of carbon footprint, biodegradability, and active properties (Sampaio et al., 2023).

Seaweeds have received considerable attention due to their rich bioactive components such as dietary fibers, minerals, vitamins, unsaturated fatty acids, polyphenols, carotenoids, and tocopherols (Albertos et al., 2019). Bio-based edible films derived from seaweeds represent one of the recent advances in bioplastic production. Seaweeds are considered a promising source for bioplastic production due to their film-forming ability (Lim et al., 2021). Several studies have been conducted on the use of seaweed derivatives or extracts in edible film and coating formulations (Yang et al., 2017; Augusto et al., 2018; Goma et al., 2018; Albertos et al., 2019). Chondrus crispus, like most seaweeds, has typical properties such as antioxidant capacity, vitamins, minerals, and fiber content (Collen et al., 2014). Despite the utilization of C. crispus derivatives or polysaccharides in edible film/coating formulations (Thakur et al., 2018; Daei et al., 2022), there is limited research available on the use of C. crispus mucilage in film formulations.

Food waste is used to produce active packaging materials, contributing to the development of sustainable packaging materials (Khalil et al., 2024). Food waste is used to produce active packaging materials, contributing to the development of sustainable packaging materials (Khalil et al., 2024). Pomegranate (Punica granatum L.) is a nutritious fruit that contains numerous bioactive compounds, particularly in its inedible parts such as the peel and seed. Pomegranate peel serves as a potential source of antioxidant compounds and exhibits antifungal, antimicrobial, and antibacterial activity (Bertolo et al., 2020). Recently, pomegranate peel has attracted the attention of researchers due to its proven therapeutic properties (Cui et al., 2020). Pomegranate peel extract has been incorporated into edible films in numerous studies to produce active films and improve film properties (Alsaggaf et al., 2017; Dai et al., 2022). The addition of pomegranate peel extract to edible films has been reported to increase the phenolic and antioxidant activities of the films (Kumar et al., 2019) and contribute to the improvement of film properties (Bertolo et al., 2020; Kumar et al., 2021; Munir et al., 2019). For all these reasons, the aim of this study was i) to produce sustainable edible films based on C. crispus mucilage containing different concentrations of pomegranate peel extract and ii) to investigate the properties of thickness, mechanical, optical, color, antioxidant, and total phenolic contents the films.

Materials and Methods

Materials

Chondrus crispus and pomegranates (Punica granatum) were supplied from a local market. Glycerol was sourced from Merck (Darmstadt, Germany). Chemicals including sodium thiosulfate, Folin-Ciocalteu reagent, gallic acid, sodium carbonate, methanol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich.

Preparation of C. crispus mucilage

The extraction of C. crispus mucilage was conducted with modifications to the method proposed by Albertos et al. (2019). For this, the seaweed sample was weighed and washed under running water. It was then diluted 1:10 with distilled water and stirred at a constant speed at 90 °C for 30 min using a magnetic stirrer. This was transferred to Falcon tubes and centrifuged

(Hettich Lab., Universal 32 R centrifuge, Tuttlingen, Germany) at 4500 rpm for 40 min at 4 °C. The mucilage phase was separated, and the extraction process was repeated twice. The obtained mucilage was frozen at -20 °C and then lyophilized (Biobase, Seoul, South Korea). The lyophilized mucilage was ground and stored in a sealed bag at -20 °C until film production.

Preparation of pomegranate peel extract

The pomegranate peel extract (PPE) was obtained through a modification of the methodology proposed by Kanatt et al. (2012). The pomegranates (Hicaz pomegranate) were washed under running water, and the outer peel was separated. The peels were cut into small pieces and dried at room temperature for 4 h in a fume hood. The dried peels were then ground and diluted with distilled water (1:10). This mixture was stirred at a constant speed at 35 °C for 3 h. Subsequently, it was filtered using coarse filter paper at 4 °C for 15 h. To increase extraction yield, this process was repeated twice. The obtained pomegranate peel extract (PPE) was centrifuged at 4500 rpm for 20 min at 4 °C. The moisture from the PPE was removed using a vacuum evaporator at 140 rpm and 75 °C, and the extract was then lyophilized. The lyophilized PPE was stored at -20 °C for film production.

Preparation of edible films

The production of edible films was carried out by modifying the method proposed by Hajivand et al. (2020). Seaweed mucilage (3%, w/v), glycerol (30% of the mucilage, w/w), and distilled water were mixed using a magnetic stirrer for 30 min. Then, PPE was added to the mixture containing 0, 0.25, 0.5, and 1% (w/v) PPE and mixed for 20 min. 8.5 mL of the film-forming solution was transferred to Petri dishes (85 mm diameter). The samples were dried at 40 °C for 16-18 hours. Subsequently, the dry films were peeled off and transferred to zipped bags. The films were conditioned in a desiccator containing silica gel at 25 ± 2 °C for 2 days before thickness, mechanical, color, and opacity analyses.

Determination of the properties of edible films

Thickness

The film thickness was determined using a digital micrometer (Insize 3108-25A, Germany). For this purpose, the thickness was measured at a minimum of 10 random points on the film, and the average film thickness was calculated (Ciurzynska et al., 2024).

Mechanical properties

The mechanical properties of the films were determined using a TA-XT Plus Texture Analyzer (Godalming, Surrey, UK). Samples were placed between AT/G Mini Tensile Grips and stretched to break. The instrument software was used to calculate the tensile strength (TS) and elongation at break (EAB) values of the films (Ceylan and Atasoy, 2022).

Total phenolic content

The total phenolic content (TPC) of the films was determined using the method proposed by Moghadam et al. (2020). First, 0.05 g of the film was mixed with 10 mL of distilled water and allowed to dissolve at ambient temperature for 2 h. The mixture was centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was analyzed using the Folin-Ciocalteu method. For this purpose, 100 µL of the extract (supernatant) was mixed with 2 mL of Folin-Ciocalteu reagent and 2.5 mL of Na_2CO_3 solution (7.5%, w/v), respectively. The mixture was incubated in the dark at ambient temperature for 30 min, and the absorbance was measured at 765 nm using a spectrophotometer (Biochrom Libra, S60, Cambridge, UK). The results were calculated as mg GAE/g film.

Antioxidant properties

0.05 g of the film was mixed with 10 mL of distilled water and allowed to dissolve at ambient temperature for 2 h. The mixture was centrifuged at 4000 rpm and 4 °C for 10 min. The supernatant was used in antioxidant capacity analysis. 100 µL of extract (supernatant) was added to 3.9 mL of DPPH solution and incubated at ambient temperature (30 min). Subsequently, the absorbance was measured at 517 nm. The results were calculated as mmol TEAC/g (Carpes et al., 2021).

Color and opacity

The color of the films was measured on a white background (L*: 95.17, a*: 3.69, b*: -6.29). The L*, a*, b*, and ΔE values were determined using a colorimeter (Spec HP 200, China) based on the CIELab color measurement system (Khalil et al., 2024). The values were calculated by averaging measurements taken from at least 10 random points on each film. In the calculation of the ΔE value, the color values of the white background were used as a reference.

The opacity of the films was determined using a UV/visible spectrophotometer (Biochrom Libra, S60, Cambridge, UK). For this purpose, the absorbance of the film was recorded at 600 nm. The opacity was calculated by dividing the absorbance at 600 nm by the thickness of the film (Ceylan and Atasoy, 2022).

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) with SPSS software (IBM Corp, Armonk, NY, USA). Differences between films were determined utilizing the Duncan multiple comparison test. Analyzes were conducted in two replicates and two parallels.

Results and Discussions

Thickness

The thickness of edible films is a highly critical characteristic that influences the properties of the film (Kanatt et al., 2012). The thickness values of the films are presented in Table 1. The thickness values of the films were found to be between 0.023-0.026 mm. Moreover, the thickness values of the films containing different concentrations of PPE and the control film were similar ($p>0.05$). Similar findings were reported by Kanatt et al. (2012) for chitosan-polyvinyl alcohol films enriched with natural extracts. Emam-Djomeh et al. (2015) stated that the addition of PPE had no statistically significant effect on the thickness of sodium caseinate-based films. Similarly, Kumar et al. (2021) reported that the incorporation of PPE did not affect the thickness of chitosan-based films.

Contrary to our results, Dai et al. (2022) reported that the addition of PPE increased the thickness in polylactic acid-based composite films. In another study (Hanani et al., 2019), it was reported that increasing the pomegranate peel powder content from 1% to 2% significantly increased the thickness of gelatin-based films.

Mechanical properties

The mechanical properties of edible films play a critical role in ensuring their structural integrity and stability during food processing and storage (He et al., 2019). The maximum resistance to the applied stress on edible films is represented by the tensile strength (TS), while the elongation at break (EAB) is represented by the flexibility (Mushtaq et al., 2018). The TS and EAB values of the films are shown in Table 1. TS values of films containing different concentrations of PPE ranged from 5.45 to 7.22 MPa, while the EAB values ranged from 104.04% to 126.90%.

Table 1. The value of thickness, tensile strength, and elongation at the break of edible films

Films	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
PPE-1	0.026±0.004^{a}	5.45±0.87^{a}	111.93±4.71^{b}
PPE-0.5	0.022±0.003^{a}	7.22±1.10^{a}	126.90±2.51^{a}
PPE-0.25	0.023±0.004^{a}	6.93±0.87^{a}	112.62±6.05^{b}
PPE-0	0.023±0.005^{a}	6.06±0.82^{a}	104.04±2.10^{c}

Different lowercase letters indicate significant differences between films containing different concentrations of PPE ($p<0.05$). Values are given as mean ± std deviation. PPE-0: control (containing 0% PPE), PPE-0.25: containing 0.25% (w/v) PPE, PPE-0.5: containing 0.50% (w/v) PPE, PPE-1: containing 1% (w/v) PPE, and PE1.50: containing 1.5% (w/v) PPE. PPE: pomegranate peel extract.

The addition of PPE had a significant ($p<0.05$) effect on the EAB values of the films, while the effect of PPE on the TS values was not significant ($p>0.05$). The addition of PPE resulted in a significant ($p<0.05$) increase in the EAB value of the films. The highest EAB value was observed in the PPE-0.5 film, followed by the PPE-0.25 and PPE-1 films. The increase in film elasticity due to the addition of PPE can be attributed to the interaction between the bioactive components of PPE and the film matrix (Kanatt et al., 2019). Several studies (He et al., 2019; Kumar et al., 2019; Kumar et al., 2021) have indicated that the mechanical properties of films can be influenced by the interaction between natural components and phenolic compounds in plant extracts with the film matrix. Furthermore, the higher elasticity value of the PPE-0.5 film compared to the PPE-1 film can be attributed to the expansion of the stress field in the PPE region due to the higher concentration of PPE (Dai et al., 2022). The results demonstrated that the elasticity of the film was affected by the concentration of PPE, depending on the phenolic components.

Consistent with our findings, He et al. (2019) reported that the addition of PPE increased the elasticity of the film. In similar, Cui et al. (2020) stated that the addition of PPE to zein films significantly increased the film elasticity. The researchers attributed the increase in film elasticity to the plasticizing effect of PPE due to its viscous nature. In contrast to our findings, He et al. (2019) reported that the addition of PPE weakened the mechanical properties of the film, and the highest TS was observed in the control film. Hanani et al. (2019) reported that the addition of 1% pomegranate peel powder (PPP) to the edible film caused a significant increase in the TS value, while 2% and 3% PPP had no significant effect on TS.

Antioxidant capacity and total phenolic content

The values of antioxidant capacity and total phenolic content (TPC) of edible films are provided in Table 2. The antioxidant capacity of the films was evaluated using DPPH scavenging activity. The DPPH scavenging activities of the films containing PPE ranged from 0.23 mmol TEAC/g to 0.13 mmol TEAC/g. The effect of PPE addition on the antioxidant capacity of the films was significant ($p<0.05$). The results demonstrated that the antioxidant capacity of the film increased with the concentration of PPE. The highest DPPH scavenging activity was observed in the PPE-1 film, while, the control film exhibited no antioxidant activity. Similarly, Mushtaq et al. (2018) reported that zein films without PPE exhibited no antioxidant capacity, whereas films containing PPE showed significant capacity, which increased with higher concentrations of PPE. Consistent with our results, Dai et al. (2022) observed a significant increase in the DPPH scavenging capacity of polyvinyl alcohol film with increasing PPE concentration.

Table 2. The values of antioxidant capacity and total phenolic content of edible films

Films	DPPH TEAC (mmol TEAC/g)	Total phenolic content (mg GAE g^{-1} film)
PPE-1	0.23±0.00^{a}	29.95±1.37^{a}
PPE-0.5	0.21±0.01^{b}	19.16±1.29^{b}
PPE-0.25	0.13±0.01^{c}	8.39±0.95^{c}
PPE-0	-	0.62±0.06^{d}

Calibration curves: DPPH (y=-493.11x+ 330.76, R^2: 0.99); TPC (y=92.776x-7.9843, R^2: 0.99).
Different lowercase letters indicate significant differences between films containing different concentrations of PPE (p<0.05). Values are given as mean ± std deviation. PPE-0: control (containing 0% PPE), PPE-0.25: containing 0.25% (w/v) PPE, PPE-0.5: containing 0.50% (w/v) PPE, PPE-1: containing 1% (w/v) PPE, and PE1.50: containing 1.5% (w/v) PPE. PPE: pomegranate peel extract.

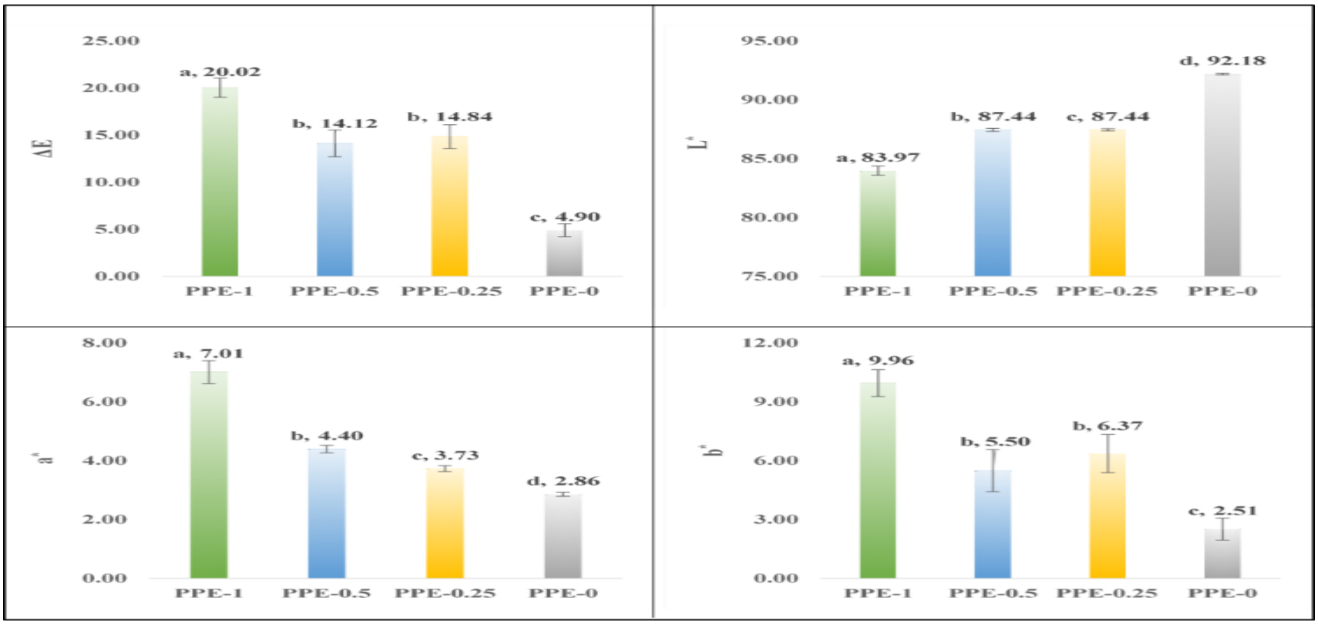

Figure 1. Color properties of edible films
Different lowercase letters indicate significant differences between films containing different concentrations of PPE (p<0.05). Values are given as mean ± std deviation. PPE-0: control (containing 0% PPE), PPE-0.25: containing 0.25% (w/v) PPE, PPE-0.5: containing 0.50% (w/v) PPE, PPE-1: containing 1% (w/v) PPE, and PE1.50: containing 1.5% (w/v) PPE. PPE: pomegranate peel extract.

The TPC values of the films ranged from 0.62 to 29.95 mg GAE g-1 film. The effect of PPE incorporation on the TPC values of the films was significant (p<0.05). The results indicated that the TPC in the films increased with the concentration of PPE. The highest TPC was observed in the PPE-1 sample, while the lowest TPC was detected in the control film (PPE-0). Similar findings were reported for zein films by Mushtaq et al. (2018). The antioxidant capacity of the films may be attributed to the phenolic components of PPE. PPE serves as a significant source of bioactive compounds, primarily polyphenols, and functions as a potential reservoir of antioxidant compounds (Bertolo et al., 2020; Vargas-Torrico et al., 2024). The antioxidant capacity and presence of phenolic content are attributed to the presence of compounds such as hydrolyzable tannins, phenolic acids, flavonoids, ellagic acid, citric acid, derivatives of caffeic acid, quinic acid, quercetin-3-O-glucoside, and gallocatechin Vargas-Torrico et al. (2024). In addition to these, tocopherols, carbohydrates, terpenes, carotenoids, vitamin C, and pigments also can contribute to the antioxidant capacity of films (Saberi et al., 2017).

Color and opacity

The color of edible films is an important factor in consumer acceptance (Nayak et al., 2024). L*, a*, b*, and ΔE values of edible films are shown in Figure 1. A high L* value indicates lightness, whereas a low L value indicates darkness. A positive a* value represents redness, while a positive b* value represents yellowness (Nayak et al., 2024). The effect of PPE addition on the color values of the films was significant (p<0.05). As expected, the control film had significantly ($p<0.05$) higher L* values and lower a* and b* values compared to the films containing PPE. As the amount of PPE increased, the lightness decreased and the intensities of the yellow and red colors increased. In addition, the incorporation of PPE resulted in a significant increase in the ΔE values of the films, indicating an overall difference in the color properties of these films. Similar findings have been reported by Nayak et al. (2024) for films incorporating corn starch/moringa gum with pine cone extract. In line with our findings, Munir et al. (2019) indicated that the control film without PPE had the highest L* value, the lowest a* and b*, and the ΔE value. The observed change in the color of the films with the addition of PPE may be due to the presence of antioxidants and anthocyanin pigments (Kumari et al., 2017). Furthermore, the increase in film redness and yellowness at higher concentrations of PPE is due to the increased concentration of color pigments present in PPE (Munir et al., 2019).

The opacity values of films containing PPE at different concentrations were in the range of 3.27 - 3.67 A/mm (Figure 2). The opacity of edible films increased slightly with the addition of PPE, but this increase was not statistically significant ($p>0.05$). The fact that the addition of PPE did not affect film opacity may be due to the thin nature of the produced films. Another possible reason could be the concentration of PPE. Emam-Djomeh et al. (2015) found that the addition of PPE significantly reduced the transparency of sodium caseinate-based films, but this reduction was not significant at lower extract concentrations. Contrary to our results, Munir et al. (2019) reported that the transparency of films increased with the concentration of plant extracts, which was attributed to the type and level of phenolic compounds. In another study (Dai et al., 2022), it was stated that the addition of PPE increased the transparency of edible films. Vargas-Torrico et al. (2024) reported that the opacity of gelatin-carboxymethylcellulose films increased with increasing PPE concentration.

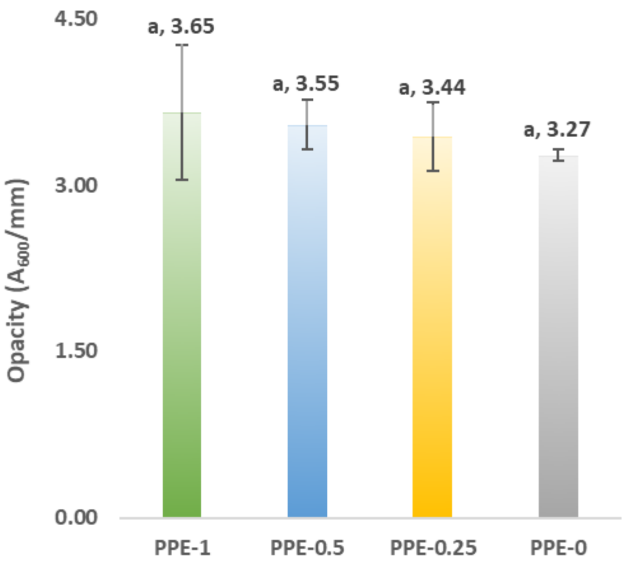

Figure 2. The opacity values of edible films
Different lowercase letters indicate significant differences between films containing different concentrations of PPE ($p<0.05$). Values are given as mean ± std deviation. PPE-0: control (containing 0% PPE), PPE-0.25: containing 0.25% (w/v) PPE, PPE-0.5: containing 0.50% (w/v) PPE, PPE-1: containing 1% (w/v) PPE, and PE1.50: containing 1.5% (w/v) PPE. PPE: pomegranate peel extract.

Conclusions

In this study, edible films containing pomegranate peel extract (PPE) based on C. crispus seaweed mucilage were developed and characterized. The thickness, color, opacity, mechanical properties, antioxidant capacity, and total phenolic content (TPC) of the obtained films were determined. The addition of PPE improved the elasticity of the edible films. The obtained films were a significant source of phenolic compounds and exhibited antioxidant activity. The incorporation of PPE resulted in an increase in the antioxidant activity and TPC of the films. Films containing PPE exhibited darker, red, and yellow color intensities compared to the control film.

The tested films can be considered an important alternative to sustainable edible films. To enhance the water resistance of the obtained films (data not shown), future studies could explore the incorporation of hydrophobic components into the formulation or the development of composite films using different polymers. However, for a comprehensive understanding of the film properties, future studies should also evaluate the barrier, morphological, and structural properties of the films. Furthermore, investigating the ultraviolet (UV) light transmittance properties of the films could provide valuable insights for their application in light-sensitive food packaging. To better understand their potential as edible packaging materials, it would be beneficial to test these films in food applications.

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Conflict of interest:

The author declares that they have no conflict of interest.

Author contributions:

Huriye Gözde CEYLAN Conceptualization, Investigation, Methodology, Validation, Formal analysis, Writing - Original Draft, Visualization.

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