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Formulating edible films with red pitahaya extract and probiotic

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Abstract:
Preventing food spoilage and prolonging its shelf life are of great importance to meet the increasing food demand. Dietary fibers in red pitahaya are known to help maintain food freshness. Lactic acid bacteria have probiotic properties and can be a good alternative to additives in food production. Therefore, we aimed to investigate the potential use of gum-based edible films containing red pitahaya extract and probiotic as a coating material in the food industry.

Firstly, we determined the antimicrobial activity of red pitahaya peel and flesh extracts against pathogenic microorganisms and probiotic strains. Then, we employed the well diffusion method to determine the antimicrobial activity of the edible films containing red pitahaya extracts and *Limosilactobacillus fermentum* MA-7 used as a probiotic strain.

The largest inhibition zone diameters of peel and flesh extracts were 12.97 and 13.32 mm, respectively, against *Candida albicans* ATCC 10231. The inhibition of the growth of lactic acid bacteria was lower as the extract concentration decreased. The gum-based films with flesh extract and probiotic had the largest inhibition zone diameters of 21.63 and 21.52 mm, respectively, against Aeromonas hydrophila ATCC19570 and *C. albicans* ATCC 10231.

The edible films containing red pitahaya extract and *L. fermentum* MA-7 may have the potential to prevent spoilage caused by microorganisms in the food industry and to extend the shelf life of foods.

Keywords: *Hylocereus polyrhizus*, pitahaya, lactic acid bacteria, guar gum, coating material, antimicrobial activity, plant extracts

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INTRODUCTION

Today, the growing world population is increasing the demand for high-quality food. Extending the shelf life of foods is one of the ways to meet this demand. There is a lot of current research into packaging systems that prevent food spoilage. One of them, active packaging, is a system that maintains the product's quality and extends its shelf life through the interaction between packaging, the product, and the environment [1, 2]. This interaction has become of great importance recently due to the problem of hazardous waste and the environmental damage caused by non-biodegradable materials [3–5]. Coating processes are commonly used in various industries, such as food, agricultural, pharmaceutical, cosmetic, and textile industries. Products are generally coated for protective, decorative, or functional purposes [6, 7]. Edible film coatings, for example, have a number of advantages. They are biodegradable and therefore do not pollute the environment. They also serve as a nutritional supplement for consumers and as flavoring and dyeing agents for the product. Finally, edible film coatings exhibit antimicrobial and antioxidant properties due to essential oils, nisin, and plant extracts that they contain [8–11].

Edible film solutions can be prepared from gums of natural origin since they are inexpensive, biocompatible, non-toxic, and readily available [12]. Among natural biopolymers, guar gum is receiving a lot of attention in the field of food packaging due to its good film-forming and biological properties [13]. Guar gum is a hydrophilic nonionic macromolecule of polysaccharides with a high molecular weight. It is of low cost and has excellent biodegradability and biocompatibility [14]. Guar gum is one of the most essential thickeners and a flexible ingredient for a variety of food applications [15].

Pitahaya belongs to the genus *Hylocereus* of the *Cactaceae* family and is commonly known as the dragon fruit [16]. Fifteen years ago, the pitahaya fruit was unheard of, but today it has gained popularity in the European market and such countries as Colombia, Costa Rica, Vietnam, Mexico, the USA (Florida and California),

and Nicaragua [17]. In Turkey, pitahaya is grown in the Mediterranean region, especially in Mersin, Antalya and partially in Adana [18]. Pitahaya is considered a promising fruit with antioxidant, anticancerous, and antimicrobial properties, as well as prebiotic effects [19]. Dietary fibers in red pitahaya are important for maintaining the fruit's freshness. Therefore, red pitahaya can be potentially used to preserve food freshness [20].

Probiotics are non-pathogenic living microorganisms [21]. They can be found in various types of products such as foods, medicines, and dietary supplements [22]. Recently, probiotics have been increasingly used as a biocontrol agent in the food industry. In particular, lactic acid bacteria are excellent biocontrol agents due to their probiotic potential. Various methods have been developed to preserve the biological activities of probiotics during food processing and storage [23]. One of them is the use of edible films and coatings as potential carriers for probiotics [24]. The inclusion of probiotics in edible film solutions or coatings promotes the survival of these microorganisms [25]. This can also contribute to better food stability and safety due to the antimicrobial activity of probiotics against spoilage or pathogenic bacteria [26].

Unlike synthetic additives, new natural coating materials can inhibit the growth of pathogenic and food spoilage microorganisms without having any negative effects on health. In this regard, we aimed to study the potential use of gum-based edible films containing red pitahaya extracts and the probiotic candidate strain *Limosilactobacillus fermentum* MA-7 in the food industry. First, we investigated the antimicrobial activity of red pitahaya extracts against pathogenic test microorganisms. Then, the extracts were tested on the probiotic candidate strains. Finally, we determined the antagonistic effect of the film solutions prepared with red pitahaya extracts and *L. fermentum* MA-7 as natural biocontrol agents against pathogenic test microorganisms.

STUDY OBJECTS AND METHODS

Preparation of red pitahaya methanol extracts. Red pitahaya fruits were obtained from Kumluca (Antalya, Turkey) in October 2021 (Fig. 1). Then, their flesh was separated from the peel, and they were left to dry. After grinding, the powder from red pitahaya peel and flesh (10 g) was extracted with 99.7% methanol (30 mL) in two repetitions for two days. For this, we sonicated the mixes on ice for 10 min every day using a sonication device (Hielscher, 30 kHz, 100% amplitude). The crude red pitahaya methanol extracts were stored at 4°C until used.

Microorganisms and growth conditions. *Candida albicans* ATCC 10231 was cultured at 30°C for 24 h in YPD (Yeast Peptone Dextrose). *Aeromonas hydrophila* ATCC 19570 and *Listeria monocytogenes* ATCC 7644 were cultured at 37°C for 24 h in NB (Nutrient Broth) and TSB (Tryptic Soy Broth). *Yersinia ruckeri* and *Vibrio anguillarum* A4 were cultured in TSB and TSB/NaCl medium at 25°C for 24 h. *Limosilactobacillus fermentum* MA-7, *Lactobacillus gasseri* MA-1, *Limosilactobacillus vaginalis* MA-10, and *Lactobacillus delbrueckii* MA-9 were cultured at 37°C in MRS (Man, Rogosa, and Sharpe) for 24 h. *Streptococcus thermophilus* MAS-1 was cultured at 37°C in M17 broth medium for 24 h.

a b c

Figure 1 Pitahaya: (a) production greenhouse and (b, c) red pitahaya

Disc diffusion susceptibility test. The disc diffusion susceptibility test was used to determine the inhibitory effect of the red pitahaya peel and flesh methanol extracts against pathogenic test microorganisms and probiotic lactic acid bacteria. The prepared culture suspension (adjusted to 0.5 McFarland) was inoculated on an agar medium using the spread method and sterile discs (6 mm in diameter) were placed on the agar. The red pitahaya methanol extracts dissolved in dimethyl sulfoxide were dripped onto the sterile discs. Kanamycin (K; 30 µg/disc) and Ampicillin (AM; 10 µg/disc) antibiotic discs were used as controls for pathogenic microorganism strains, and Fluconazole (FCA; 25 µg/disc) was used for yeast. The culture dishes were incubated for 24 h at the suitable temperatures indicated previously. Then, the inhibition zone around the discs was measured using a caliper.

Micro-dilution assay. The micro-dilution assay was used to determine minimum inhibitory concentrations, as well as minimum fungicidal or bactericidal concentrations of the red pitahaya extracts. For this, the extracts were added to the growth medium and diluted by a two-fold serial dilution method to obtain a final concentration of 80–5 µg/µL. The culture suspension (0.5 McFarland) was added to each tube and then incubated under the conditions required for each microorganism as mentioned above. After incubation, the extract's concentration in the tube without microbial growth was determined according to turbidity and the lowest concentration was recorded as a minimum inhibitory concentrations value. Minimum bactericidal or fungicidal concentrations values were determined by inoculating samples from the mixture onto an agar medium. The culture dishes were incubated at the appropriate temperature for 24 h. The lowest concentration without growth at the end of incubation was defined as minimum bactericidal or fungicidal concentrations values.

Microbial and physicochemical characterization of edible film solutions containing red pitahaya and *L. fermentum* MA-7. *Preparation of edible film solutions.* An edible film formulation was designed by modifying

a b c d e f

Figure 2 Preparation of edible film solutions: (a) guar gum; (b) guar gum + *Limosilactobacillus fermentum* MA-7; (c) guar gum + pitahaya flesh extract; (d) guar gum + pitahaya peel extract; (e) guar gum + *Limosilactobacillus fermentum* MA-7 + pitahaya flesh extract and (f) guar gum + *Limosilactobacillus* MA-7 + pitahaya peel extract

the methods of Kılınç *et al.* and Bambace *et al.* from commercially available guar gum, the pitahaya methanol extract, and the human milk-originated probiotic candidate strain *L. fermentum* MA-7 [27, 28]. This study included a control group and three different experimental test groups. The control group contained guar gum (1%, w/v) adjusted to the final volume with distilled water. The edible film formulation test groups included: guar gum (1%, w/v) with *L. fermentum* MA-7, guar gum (1%, w/v) with red pitahaya extract (10%, w/v), and guar gum (1%, w/v) with red pitahaya extract (10%, w/v) and *L. fermentum* MA-7. Glycerol (3%, w/v) was used as a plasticizer in all the groups. First, we determined the antimicrobial activity of the edible film solutions. Then, the solutions were dried in a Pasteur oven until they reached constant weight, to be used in characterization tests (Fig. 2).

Antimicrobial activity of edible film solutions. The antifungal and antibacterial activities of the edible film formulation test groups were determined using the well diffusion assay. The test microorganisms included *C. albicans* ATCC 10231, *A. hydrophila* ATCC 19570, *L. monocytogenes* ATCC 7644, *Y. ruckeri*, and *V. anguillarum* A4. The culture suspensions (0.5 McFarland, 100 µL) were spread on the surface of the medium. Then, 100 µL of the mixture from the test groups and the control were added to each well (6 mm diameter, 0.1 cm^3 volume). The experiment was carried out in triplicate. After incubation of the petri dishes for 24 h at appropriate temperatures, the inhibition zone diameters were obtained using a caliper.

Thickness and density of edible film solutions. The thickness of the films was determined with a digital micrometer. The density of the films was reported as the ratio of the cut mass of the film to its volume (Thickness × Surface Area) [29].

Moisture content of edible film solutions. The moisture content, %, was determined using the oven-drying method by differential weighing of a film sample before and after drying. Three different film samples from each group were oven-dried at 90°C to constant weight. The film content was calculated using Eq. (1):

$$\text{Moisture content} = \frac{w_i - w_d}{w_i} \times 100 \quad (1)$$

where w_i is the initial weight of the film sample, g; w_d is the weight of the oven-dried film sample, g. Each of the groups was tested in triplicate [30].

Transparency and light transmission of edible film solutions. The transparency and light transmission values were determined using a UV-VIS spectrophotometer (Beckman Coulter, USA) by reading the absorbance of the number of films at wavelengths in the range of 200–800 nm [30]. The film samples were cut into three strips (0.7×3 cm). Each of the strips was placed in a quartz cuvette and its absorbance was read against an empty cuvette. The relative transparency, A_{600}/mm, of the film strip was measured at 600 nm and calculated using Eq. (2):

$$\text{Transparency} = \frac{A_{600}}{X} \quad (2)$$

where A_{600} is the absorbance value at 600 nm and X is the film thickness, mm. Triplicate readings were made for each film formulation.

The light transmittance, %, of the film groups was recorded by making spectrophotometric readings at

50 nm intervals at 200–800 nm and calculated using the Lambert-Beer equation:

$$\text{Lidht transmittance} = antilog_{10}(2 - A) \quad (3)$$

where A is the absorbance value of the film strip.

Water solubility of edible film solutions. The film samples were cut into square pieces in triplicate. The films were weighed in glass Petri dishes and then 30 mL of distilled water was added. After immersion at room temperature (~ 25°C) for 24 h, the residues were filtered and weighed to determine the degree of swelling or dried in an oven at 70°C to constant weight to determine their solubility [30]. The solubility in water, %, was calculated using Eq. (4):

$$\text{Solubility in water} = \frac{w_i - w_d}{w_i} \times 100 \quad (4)$$

where w_i is the initial weight of the film sample, g; w_d is the weight of the oven-dried film sample, g. Triplicate readings were made for each of the film solution groups.

Statistical analysis. The analysis of variance (one-way ANOVA) was performed using the SPSS program (GNU) to determine significant differences between antimicrobial activity values. Tukey's post-hoc test was used for multiple comparisons between different groups with 5% statistical significance ($p < 0.05$).

RESULTS AND DISCUSSION

Increasing antibiotic resistance in the world has led researchers to look for plant-based natural alternatives to control pathogenic microorganisms instead of synthetic preservatives [31]. In this study, we determined the biological activity of red pitahaya extracts against food-borne, fish, and yeast microorganisms by using the disc diffusion susceptibility and micro-dilution methods (Table 1). The largest inhibition zone diameters in the peel and flesh extracts were determined as 12.97 and 13.32 mm, respectively, against *Candida albicans* ATCC 10231. The smallest inhibition zone diameters in the peel and flesh extracts were determined as 9.09 and 10.62 mm, respectively, against *Listeria monocytogenes* ATCC 7644. The difference in antimicrobial activity between the *C. albicans* ATCC 10231 and *L. monocytogenes* ATCC 7644 strains was statistically significant ($p < 0.05$) in both extracts.

In our previous study, where we investigated the biological activity of fruit and peel methanol extracts from white pitahaya, the inhibition zone diameters were determined against *L. monocytogenes* ATCC 7644 (6.30 and 6.35 mm, respectively) and against *C. albicans* ATCC 10231 (11.66 and 13.15 mm, respectively) [32]. The differences in the phenolic content, especially betalain, of fruits may cause different results in antimicrobial activity against the same test microorganisms [33, 34].

Antimicrobial agents may have a static or cidal effect. The static effect has the ability to prevent the growth or reproduction of microorganisms, while the cidal effect has the ability to kill microorganisms [35]. The disc diffusion assay alone is not sufficient to determine whether the antimicrobial activity is a static or a cidal effect [36]. For this reason, it is necessary to determine minimum inhibitory concentrations, as well as bactericidal or fungicidal concentrations of the extracts. The micro-dilution assay results for red pitahaya extracts are presented in Table 2. As can be seen, the minimum inhibitory

Table 1 Biological activity of red pitahaya extracts

Microorganism strains	Inhibition zone diameters, mm ± SD				
	Extracts		Antibiotics		
	Red pitahaya peel methanol	Red pitahaya flesh methanol	Kanamycin	Ampicillin	Fluconazole
Candida albicans ATCC 10231	12.97 ± 0.46[a]	13.32 ± 0.51[a]	n.d.	n.d.	21.85 ± 1.76
Listeria monocytogenes ATCC 7644	9.09 ± 0.65[b]	10.62 ± 0.30[b]	14.77 ± 0.05	30.60 ± 0.11	n.d.
Aeromonas hydrophila ATCC19570	12.93 ± 0.21[a]	12.77 ± 0.84[a]	25.40 ± 1.30	29.57 ± 0.10	n.d.
Yersinia ruckeri	10.65 ± 1.06[b]	12.26 ± 0.44[a]	18.90 ± 0.05	18.70 ± 0.12	n.d.
Vibrio anguillarum A4	9.64 ± 0.64[b]	10.76 ± 0.33[b]	12.10 ± 0.13	15.13 ± 0.15	n.d.

n.d. – is the no inhibition zone diameter. Different letters in the same column show significance ($p < 0.05$)

Table 2 Micro-dilution assay of red pitahaya methanol extracts

Microorganism strains	Minimum inhibitory concentrations, μg/μL		Minimum bactericidal or fungicidal concentrations, μg/μL	
	Red pitahaya peel methanol	Red pitahaya flesh methanol	Red pitahaya peel methanol	Red pitahaya flesh methanol
Candida albicans ATCC 10231	40	40	80	80
Listeria monocytogenes ATCC 7644	40	40	80	> 80
Aeromonas hydrophila ATCC19570	40	20	80	> 80
Yersinia ruckeri	40	40	40	80
Vibrio anguillarum A4	20	40	40	80

concentrations value for the peel extract was determined as 40 μg/μL against all the test microorganisms, except *V. anguillarum* A4 (20 μg/μL). The flesh extract had a minimum inhibitory concentrations value of 40 μg/μL against all the test microorganisms, except *A. hydrophila* ATCC19570 (20 μg/μL). The minimum bactericidal concentrations values were determined in the range of 40–80 μg/μL in the peel extract and 80 and higher μg/μL in the flesh extract. The minimum fungicidal concentrations value was 80 μg/μL for both extracts.

Lactic acid bacteria are an important group of probiotic microorganisms. One of them is *Limosilactobacillus fermentum*, a generally recognized as safe bacterium used for food fermentation [25–38]. Table 3 shows the inhibition zone diameters of red pitahaya extracts against the probiotic candidate lactic acid bacteria strains at 1 and 2 mg/disc concentrations. As can be seen, the inhibitory zone diameters of 6.23 and 6.36 mm were determined in 1 mg/disc peel extract against *L. fermentum* MA-7 and *Lactobacillus delbrueckii* MA-9, respectively. Similarly, low inhibition activities against *L. fermentum* MA-7 (6.35 mm), *Lactobacillus gasseri* MA-1 (6.31 mm), and *L. delbrueckii* MA-9 (6.65 mm) were observed for 1 mg/disc flesh extract. At a concentration of 1 mg/disc, both extracts had a statistically insignificant antibacterial effect against the tested lactic acid bacteria ($p > 0.05$). As the extract's concentration decreased, its inhibitory activity against the lactic acid bacteria also decreased (Table 3).

A study by Siregar and Julianti showed no antibacterial activity of water, ethanol, and ethyl acetate extracts of red pitahaya peel against *Lactobacillus acidophilus* [39]. The differences in antimicrobial activity may be due to the environmental conditions in which pitahaya is grown, as well as the solvent, extraction method, and microorganism strains.

In our study, the minimum inhibitory and bactericidal concentrations values of the extracts against the tested lactic acid bacteria strains were determined in the range of 20 to > 80 μg/μL (Table 4). The highest minimum bactericidal concentrations value in the flesh extract was > 80 μg/μL against *L. fermentum* MA-7. The high minimum inhibitory and bactericidal concentrations values indicate that the red pitahaya extracts may have lower inhibitory activity against the lactic acid bacteria strains tested.

Edible film coatings with antimicrobial properties have been developed to provide consumers with foods that preserve high quality, do not spoil easily, keep microorganism growth under control, and have a long shelf life [40]. Literature has reported that *L. fermentum* strains produce various food-preservative antimicrobial peptides (fermenticins) and bacteriocins that can be used as an alternative to antibiotics [41]. These natural compounds are involved in antimicrobial activity in food bio-preservation and biomedicine [42]. For this reason, we used *L. fermentum* MA-7, which meets the criteria for being a good probiotic, to develop an edible film solution [43]. In addition, the tested red pitahaya extracts had relatively high minimum inhibitory and bactericidal concentrations values against *L. fermentum* MA-7.

Table 5 shows the biological activity of gum-based edible film solutions containing red pitahaya extracts and *L. fermentum* MA-7 (GEL) against the test microorganism. In most of the GEL groups, the antimicrobial activity was higher than in the other test groups, indicating a synergistic effect of pitahaya extracts with the probiotic. The antimicrobial activity of the GEL groups was statistically significant when compared to the gum-based film solutions without red pitahaya extract or *L. fermentum* MA-7 G ($p < 0.05$).

Table 3 Disc diffusion values for red pitahaya peel and flesh methanol extracts against lactic acid bacteria

Microorganism strains	Red pitahaya peel methanol		Red pitahaya flesh methanol	
	1 mg/disc	2 mg/disc	1 mg/disc	2 mg/disc
Limosilactobacillus fermentum MA-7	6.23 ± 0.13[a]	8.20 ± 0.15[a]	6.35 ± 0.07[a]	8.37 ± 0.19[a]
Lactobacillus gasseri MA-1	n.d.[b]	8.26 ± 0.09[a]	6.31 ± 0.46[a]	8.18 ± 0.07[a]
Limosilactobacillus vaginalis MA-10	n.d.[b]	6.27 ± 0.13[b]	n.d.[b]	7.22 ± 0.20[b]
Lactobacillus delbrueckii MA-9	6.36 ± 0.08[a]	7.62 ± 0.35[c]	6.65 ± 0.68[a]	8.92 ± 0.35[c]
Streptococcus thermophilus MAS-1	n.d.[b]	6.12 ± 0.07[b]	n.d.[b]	n.d.[d]

n.d. – is the no inhibition zone diameter. Different letters in the same column show statistical significance ($p < 0.05$)

Table 4 Micro-dilution assay of red pitahaya methanol extracts against probiotic strains

Microorganism strains	Minimum inhibitory concentrations, μg/μL		Minimum bactericidal concentrations, μg/μL	
	Red pitahaya peel methanol	Red pitahaya flesh methanol	Red pitahaya peel methanol	Red pitahaya flesh methanol
Limosilactobacillus fermentum MA-7	40	80	40	> 80
Lactobacillus gasseri MA-1	40	40	40	40
Limosilactobacillus vaginalis MA-10	40	40	40	40
Lactobacillus delbrueckii MA-9	40	40	40	40
Streptococcus thermophilus MAS-1	80	20	80	20

Table 5 Biological activity of edible film solutions containing red pitahaya extracts

Microorganism strains	Inhibition zone of edible film solutions containing red pitahaya peel and flesh extracts, mm					
	Gum	Gum + *L. fermentum* MA-7	Peel		Flesh	
			Gum + extract	Gum + extract + *L. fermentum* MA-7	Gum + extract	Gum + extract + *L. fermentum* MA-7
Candida albicans ATCC 10231	n.d.[a]	13.41 ± 0.45[b]	n.d.[a]	11.36 ± 0.76[c]	n.d.[a]	21.52 ± 0.08[d]
Listeria monocytogenes ATCC 7644	n.d.[a]	3.52 ± 0.32[b]	n.d.[a]	4.07 ± 0.28[b]	2.62 ± 0.09[c]	9.24 ± 0.46[d]
Aeromonas hydrophila ATCC19570	n.d.[a]	5.33 ± 0.77[b]	3.36 ± 0.53[c]	14.62 ± 0.61[d]	3.99 ± 0.97[b,c]	21.63 ± 0.28[e]
Yersinia ruckeri	n.d.[a]	4.74 ± 0.90[b]	n.d.[a]	8.74 ± 0.42[c]	n.d.[a]	11.00 ± 0.65[d]
Vibrio anguillarum A4	n.d.[a]	6.18 ± 0.47[b]	n.d.[a]	7.91 ± 0.33[c]	n.d.[a]	11.42 ± 0.75[d]

n.d. – is the no inhibition zone diameter. Different letters on the same line show statistical significance ($p < 0.05$)

Table 6 Physicochemical characterization of edible films containing red pitahaya peel extracts and *Limosilactobacillus fermentum* MA-7

Film groups	Thickness, mm	Density, g/cm^3	Moisture content, %	Transparency, A_{600}/mm	Solubility in water, %
Gum	0.14 ± 0.02[a]	0.73 ± 0.26	94.84 ± 0.02[a]	0.27 ± 0.16[a]	76.45 ± 0.61[a]
Gum + *L. fermentum* MA-7	0.24 ± 0.20[b]	2.92 ± 3.08	90.31 ± 0.27[b]	1.95 ± 0.06[b]	86.00 ± 1.06[b]
Gum + extract	0.18 ± 0.02[a,b]	0.74 ± 0.04	94.50 ± 0.08[a]	3.21 ± 0.50[c]	81.15 ± 0.79[c]
Gum + extract + *L. fermentum* MA-7	0.30 ± 0.04[c,b]	1.06 ± 0.33	89.28 ± 0.11[c]	5.67 ± 0.76[d]	79.36 ± 0.89[e]
F(Sig)	17.178(0.001)	1.359(0.323)	994.712(0.000)	71.321(0.000)	65.383(0.000)

Different letters on the same line show statistical significance ($p < 0.05$)

The GEL film solutions containing red pitahaya peel and flesh extracts showed inhibition zone diameters of 11.36 and 21.52 mm, respectively, against *C. albicans* ATCC 10231. Yeasts are important contaminants that enter the food chain during food processing, storage, and transportation [44]. Restricting yeast and fungi growth in food remains of high priority in the food and agricultural industries [45–47]. Edible films containing antimicrobials have the potential to prevent food spoilage caused by yeasts [48]. We found that our GEL film formulation has the potential to be used in preventing yeast-induced spoilage.

The GEL group showed statistically significant ($p < 0.05$) antimicrobial activity against *A. hydrophila* ATCC 19570, with inhibition zone diameters of 14.62 and 21.63 mm for the samples with the peel and flesh extracts, respectively. The use of antimicrobial film coatings in meat, fish, and seafood shows promising results for maintaining microbial stability during storage and ultimately increasing shelf life [49]. Our study indicated that the film formulations containing red pitahaya extract and *L. fermentum* MA-7 may had the potential to extend the shelf life of meat, fish, and seafood.

Qin *et al.* determined the antimicrobial activity of different film formulations obtained from red pitahaya peel extract against *Staphylococcus aureus*, *L. monocytogenes*, *Escherichia coli*, and *Salmonella* by the well diffusion method [50]. They found that the film solutions with large inhibition zones had the potential to be used not only as active packaging to extend the shelf life of foods, but also as smart packaging to preserve the freshness of protein-rich animal foods. Further, in a more recent study, edible films containing 0.5 and 1% concentrations of red pitahaya peel extract showed inhibition zone diameters of 1.24 and 1.69 mm, respectively, against *S. aureus* [51].

Table 6 shows the physicochemical characterization of the films with the red pitahaya peel extract. As can be seen, the thickness and density of the control group were the lowest compared to the films with *L. fermentum* MA-7 and the films with both the peel extract and the probiotic ($p < 0.05$). The difference between the control group and the group with the peel extract was not statistically significant ($p > 0.05$). The moisture contents of the control films and the ones with the extract were 94.84 and 94.50%, respectively. These values were higher compared to the films with *L. fermentum* MA-7 or the films with a combination of the red pitahaya peel extract and the probiotic. The addition of the extract and the probiotic changed the moisture content of the film by 5.56%, as well as decreased its transparency. The water solubility of the control group was 76.45%, while the group with *L. fermentum* MA-7 had the highest water solubility among the test groups ($p < 0.05$).

Table 7 presents the physicochemical characterization of the films containing the red pitahaya flesh extract. The highest thickness was detected in the films with *L. fermentum* MA-7. The films with both the flesh extract and the probiotic had a higher density (1.93 g/cm^3) compared to the control group (0.73 g/cm^3), whereas their moisture content was lower compared to the control group ($p < 0.05$). We found a statistically significant ($p < 0.05$) difference in transparency between the control films and the ones containing the flesh extract and

Table 7 Physicochemical characterization of edible films containing red pitahaya flesh extracts and *Limosilactobacillus fermentum* MA-7

Film groups	Thickness, mm	Density, g/cm^3	Moisture content, %	Transparency, A_{600}/mm	Solubility in water, %
Gum	0.14 ± 0.02^a	0.73 ± 0.26	94.84 ± 0.02^a	0.27 ± 0.16^a	76.45 ± 0.61^a
Gum + *L. fermentum* MA-7	0.24 ± 0.02^b	2.92 ± 3.08	90.31 ± 0.27^b	1.95 ± 0.06^b	86.00 ± 1.06^b
Gum + extract	0.18 ± 0.02^{a,b}	1.29 ± 0.04	92.76 ± 0.03^c	1.62 ± 0.21^b	61.63 ± 1.94^c
Gum + extract + *L. fermentum* MA-7	0.20 ± 0.02^{a,b}	1.93 ± 0.06	90.36 ± 1.04^b	3.44 ± 0.02^c	80.89 ± 1.55^d
F(Sig)	8.128(0.008)	1.139(0.390)	48.660(0.000)	156.996(0.000)	171.774(0.000)

Different letters on the same line show statistical significance ($p < 0.05$)

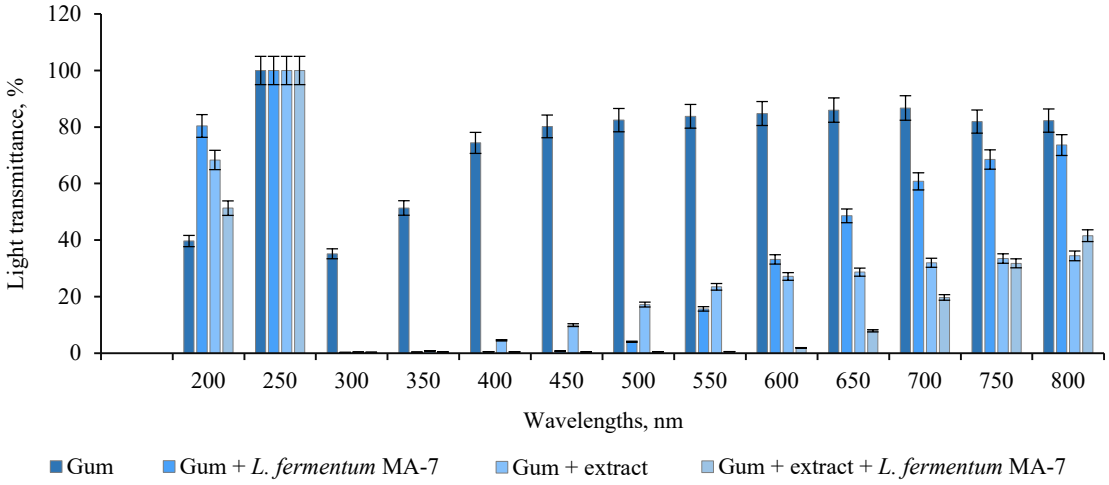

Figure 3 Light transmittance of films with red pitahaya peel extract

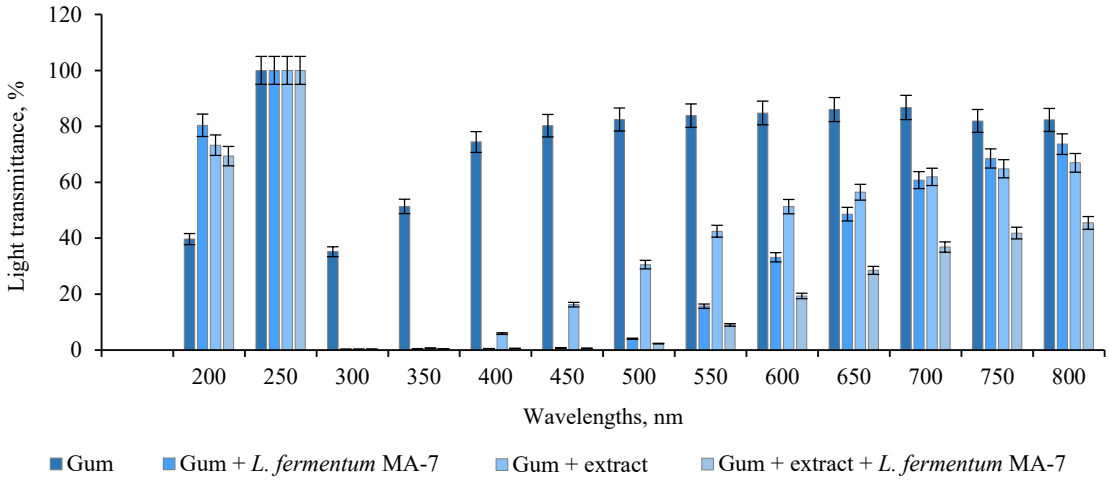

Figure 4 Light transmittance of films with red pitahaya flesh extract

L. fermentum MA-7. The lowest water solubility was determined as 61.63% in the films with the extract. The difference in water solubility among the film test groups was statistically significant ($p < 0.05$).

In a study by López-Díaz *et al.*, the films with red pitahaya had thickness values between 0.037 and 0.060 mm, whereas their moisture content ranged between 21.3 and 32.4% [52]. In a study by Azlim *et al.*, the films with red pitahaya peel extract had a moisture content between 0.24 and 0.28%, while their water solubility varied between 30.63 and 52.73% [53].

Thickness is one of the properties of edible films that affects the shelf life and biological structure of foods. The optimal thickness for edible films or coatings is ≤ 0.25 mm [54, 55]. In our study, the films with red pitahaya flesh and *L. fermentum* MA-7 were 0.20 mm thick, which was 0.05 mm thicker than the desired value stated in literature. High moisture content is a desirable

criterion for coating foods. In our study, the moisture content of the films with red pitahaya extracts and *L. fermentum* MA-7 was found to be higher than in the study by Šuput *et al.* [56]. High-resolution films are materials that dissolve easily but do not have the ability to hold water [55]. High water resistance is preferable for coatings, since water sensitivity of some products may lead to a loss of quality. For this reason, edible films need high solubility and rapid dissolution in water [57]. In our study, the films with red pitahaya extracts and *L. fermentum* MA-7 had high solubility in accordance with literature.

Figures 3 and 4 present the light transmittance of the films containing the red pitahaya peel and flesh extracts. As can be seen, the light transmittance of the control group (82.28–39.68%) was higher than in the test groups with the extract and/or *L. fermentum* MA-7 (51.31–41.57%).

The light transmittance of the extract-containing films ranged between 73.26 and 66.94%, while in the films with both the extract and *L. fermentum* MA-7, it varied between 69.34 and 45.47%. We found that light transmittance increased as the wavelength increased.

Socaciu *et al.* reported the light transmittance of films in the range of 0.01 to 70.65% [30]. The appearance of a product is important for presenting its quality and appeal to the consumer. Therefore, the transparency of films should not change the appearance, taste, or smell of the food [58]. The interaction of food with light depends on the relationship between packaging material and light. In this respect, it is important to know the optical properties of the packaging material. The interaction between the food material and light may cause unwanted photochemical reactions in the food depending on its composition [59]. In our study, the addition of red pitahaya extract and *L. fermentum* MA-7 to the films reduced their light transmittance.

CONCLUSION

We investigated the use of extracts from red pitahaya grown in Turkey as a natural antimicrobial agent and the potential use of edible films prepared with these extracts as a coating material in the food industry. Consumers are concerned about the potential dangers of synthetic preservatives for human health. Therefore, there is an increasing tendency toward using natural antimicrobial agents. According to our results, the gum-based edible films containing red pitahaya extract and *Limosilactobacillus fermentum* MA-7 had a cidal/static effect against pathogenic microorganism strains. These film solutions had large inhibition zones against the bacteria and yeast. Thus, the use of edible film formulations with antimicrobial effects as a coating material can be an alternative solution to prevent the deterioration of foods and extend their shelf life. Our study proved that the gum-based film formulations with red pitahaya extract and *L. fermentum* MA-7 have high biological activity and may be used as a coating material in the food industry. Since literature offers limited studies on pitahaya, there is a need for more research and our results study can be used in further *in vivo* studies. However, since literature offers limited studies on pitahaya, there is a need for more research and our results study can be used in further *in vivo* studies.

CONTRIBUTION

All authors have contributed equally to this project.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the authorship and publication of this article.

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