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DEVELOPMENT OF EDIBLE FILMS CONTAINING ARONIA (*Aronia melanocarpa*) AND PROBIOTIC

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ABSTRACT

The application of herbal extracts and probiotics to edible films has the potential to improve food safety. This study aims to investigate the usage possibility of food packaging film prepared by combining aronia fruit ethanol extract (AEE) and probiotic candidate strain *Limosilactobacillus fermentum* MA-7 in the food industry. Firstly, the antimicrobial activity of AEE was determined. The extract showed antimicrobial activities against all tested microorganisms except *Listeria monocytogenes* ATCC 7644. Minimum inhibitory concentration (MIC) and minimal bactericidal or fungicidal (MBC or MFC) concentration values of the extract against test microorganisms were determined between 12.5 mg/mL and 50 mg/mL. Secondly, inhibitory activity of AEE on lactic acid bacteria (LAB) strains from human milk was determined. Then, the antimicrobial activity of the films obtained by adding AEE (10%) or *L. fermentum* MA-7 separately or together was determined. The results showed that the AEE and probiotic mixture showed a synergistic effect and the highest antimicrobial activity was determined to be in the Gum-Extract-Probiotic (GEP) group. The thickness, density, moisture content, transparency, swelling degree, water solubility and light transmittance of the developed edible film were also determined. The mechanical properties of the films have improved the quality in food applications and prevented food spoilage. The GEP group extended the shelf life of its fruits by reducing mass loss by 35.88% at +4°C and 40.96% at +25°C compared to the control. The developed film can be used as bioactive antimicrobial food packaging as an alternative to synthetic packaging in the food industry.

Keywords: Antimicrobial, Aronia, Edible film, Extract, Food spoilage, *Limosilactobacillus fermentum*, Probiotic

INTRODUCTION

Packaging is a technology that has been used for a long time to prevent food spoilage **(Ribeiro *et al.*, 2021)**. Food packaging is used to prevent microorganisms, chemicals and undesirable factors or conditions such as oxygen, humidity, light, external force, extend the shelf life of food, and the safety and quality of food during transportation and storage **(Rhim *et al.*, 2013)**. Food spoiled as a result of deformations in food packages and unpackaged foods cause food-borne diseases and shorten the shelf life of foods **(Sung *et al.*, 2013; Rhim *et al.*, 2013; Realini and Marcos, 2014)**. Paper or cardboard, glass and metal are among the materials frequently used in food packaging. Plastic, on the other hand, is the preferred packaging type because it is cheap and lightweight **(Jeevahan *et al.*, 2020)**. However, since it is not biodegradable, it creates serious problems in the environment **(Parreidt *et al.*, 2018)**. Various harmful substances (hydrochloric acid, carbon monoxide, benzenes etc.) released during the production of these packages also cause various health problems **(Amin *et al.*, 2021; Mangaraj *et al.*, 2019)**. Therefore, the use of renewable and natural biopolymers is gaining importance **(Parreidt *et al.*, 2018).**

Edible film is defined as a coating material that can be consumed with foods and is applied in the form of packaging and coating to extend the shelf life of foods **(Díaz-Montes and Castro-Muñoz, 2021)**. The world has begun to gain interest in edible films due to the numerous disadvantages and harmful effects of plastic packaging **(Gere *et al.*, 2019).** Edible films preserve the microbial, chemical and sensory properties of foods and extend their shelf life **(Hassanzadeh *et al.*, 2018; Ghorbanlou *et al.*, 2023)**. Edible films protect foods against unwanted microorganisms that selectively allow the exchange of gases in the atmosphere, prevent moisture and flavor losses **(Salgado *et al.*, 2015)**. Functional food packaging can be developed by potentially adding probiotics and/or prebiotics to edible films that can improve the health of consumers **(Sáez-Orviz *et al.*, 2023)**.

Probiotics are defined as "live microorganisms that provide health benefits to the host when administered in adequate amounts" **(Hill *et al.*, 2014)**. The most common probiotic microorganisms used in food products include lactic acid bacteria (LAB) such as *Bifidobacteria* and *Lactobacilli* **(Espitia *et al.*, 2016)**. *Lactobacillus* strains are used safely in foods for probiotic effect and lysates for prebiotic effect **(Lemos Junior *et al.*, 2020; Cizeikiene and Jagelaviciute, 2021)**. Since probiotic microorganisms and their lysates show inhibitory activity against food spoilage or pathogenic bacteria, their addition to edible films can improve food stability and food safety **(Hellebois *et al.*, 2020)**. Probiotics added to edible films can maintain their viability despite various physical and chemical factors affecting their viability during food production processes **(Mbye *et al.*, 2020)**. The foods packaged with films containing probiotics may be foods that provide more health benefits than their rich nutritional value if consumed regularly **(Majid *et al.*, 2018)**.

Aronia melanocarpa (Aronia), also known as chokeberry, is native to the eastern region of North America and Canada **(Mahoney *et al.*, 2023)**. Due to their antioxidant properties, high nutritional value and characteristic taste, currants grown in Türkiye and various parts of the world attract great attention from the world **(Şengül *et al.*, 2023; Şahin and Erdoğan, 2022)**. The main nutritional benefit of fruits and vegetables in preventing degenerative diseases has drawn the attention of scientists and consumers to the use of various mulberry fruits and their components **(Dhalaria *et al.*, 2020)**. Aronia is a type of fruit that attracts worldwide attention due to its wide usage area and high marketing opportunities. Aronia fruits are used in the fruit juice industry, pharmaceutical industry, diet menus and milk and dairy products technology **(TVOB, 2022)**. The food industry is still looking for plant-based natural substances as an alternative to synthetic dyes, which have been discredited due to their harmful side effects on health of human. Due to the limited stability of plant pigments, the situation needs to be evaluated when applying to food. A variety of exotic fruits and vegetables, often unknown, may be a promising source of new pigments **(Brauch, 2016)**. Many of these berries, including aronia, have been used in European and North American folk medicine for many years **(Gurčík *et al.*, 2023)**.

The objective of our study was to determine the potential for use of aronia ethanol extract (AEE) and probiotics as natural and bioactive additives in the food industry. For this purpose, the antimicrobial effect of the extract was first investigated. Then, it was aimed to develop coating material in edible form with the *L. fermentum* MA-7 probiotic strain to prevent food deterioration during storage and to extend the shelf life of foods. The antimicrobial activity of the developed coating materials on test microorganisms was investigated. In addition, some mechanical properties of the prepared films and their ability to extend the shelf life of the coated strawberries were evaluated and their usability as a coating material in foods was supported.

MATERIAL AND METHODS

Plant material

Aronia fruits were purchased in September 2022 as fresh fruit from Aronia Food and Health I. C. (Yalova-Türkiye) (Figure 1A).

Preparation of aronia ethanol extract

The fruits were dried in an airy and sunlight-free environment after washing (Figure 1B). The dried fruits were powdered with a Waring blender and then stored in dry and dark conditions until use. Then, the powdered fruits were extracted with ethanol solvent (99.9%) for 10 minutes (3 days) every day using a sonicator device (Amplitude, 100%; Cycle, 1) (Figure 1C). The ethanol was then evaporated from the extract. The AEE was dissolved with Dimethyl-Sulfoxide (DMSO) and sterilized with a 0.45 µm filter.

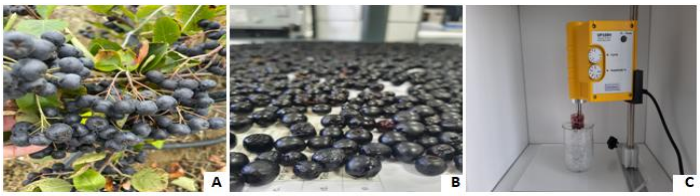

Figure 1 Aronia fruits and extraction with sonicator device.

Determination of antibacterial and antifungal activities of aronia ethanol extract

Disc diffusion assay

The antimicrobial activity of the AEE was determined using disc diffusion test **(Saglam and Asan-Ozusaglam, 2023).** The test microorganism, food-borne Gram (+) bacteria *Listeria monocytogenes* ATCC 7644 (Tryptic-Soy-Broth (TSB)/Agar), fish-pathogenic Gram (-) bacteria *Aeromonas hydrophila* ATCC 19570 (Nutrient-Broth (NB)/Agar), *Yersinia ruckeri* (TSB/Agar) and *Vibrio anguillarum* A4 (TSB/NaCl) and fungal pathogen *Candida glabrata* RSKK 04019 (Yeast-Extract-Peptone Dextrose (YPD)/Agar) were used. *Lactobacillus gasseri* MA-1, *Limosilactobacillus fermentum* MA-7, *Lactobacillus delbrueckii* MA-9, *Limosilactobacillus vaginalis* MA-10 (De-Man, Rogosa and Sharpe (MRS)/Agar) and *Streptococcus thermophilus* MAS-1 (M17/Agar) were used as probiotic candidate LAB strains from human milk. The test microorganisms (100 µL) prepared at a concentration of 0.5 McFarland ($1x10^8$ CFU/mL) were spread onto the solid medium. Sterile discs (Whatman No: 1, Diameter: 6 mm) were placed on the solid media and AEE (20 µL, 2 mg/disc) was dripped onto the discs. For the positive control, Ampicillin (AM: 10 µg/disc), Kanamycin (K: 30 µg/disc), Ofloxacin (OFX: 10 µg/disc) and Cloxacillin (CLOX: 5µg/disc) were used as standard antibiotics against bacteria and Fluconazole (FCA: 25 µg/disc) against fungi. The plates were incubated for 24 hours at 37°C for bacteria and 30°C for yeast. After incubation, the clear areas around the discs were recorded. It was repeated three times to obtain the mean values and standard deviation of the inhibition zone diameter of the extract.

Micro-broth dilution

Micro-broth dilution test was used to determine MIC and MBC or MFC values of AEE **(Saglam and Asan-Ozusaglam, 2023)**. Two-fold serial dilutions of the extract were carried out in the appropriate medium for each microorganism (TSB, NB or TSB/NaCl). The suspension containing 0.5 McFarland was then added to the diluted tubes. The mixture was incubated for 24 hours at appropriate temperatures for the microorganisms as mentioned above. The AEE concentration at which no microbial growth occurred after incubation was recorded as MIC values. Afterward, the samples taken from the tubes were inoculated sequentially onto the solid medium using the spot drop method. The plates were incubated at suitable temperatures for each microorganism for 24 hours. After incubation, the extract concentration without microbial growth on the solid medium was evaluated as MBC or MFC values.

Food coating material with aronia ethanol extract and probiotic content

Preparation of edible food coating material

The ethanol extract of aronia and probiotic candidate strain *L. fermentum* MA-7 obtained from human milk **(Asan-Ozusaglam and Gunyakti, 2019)** were used as natural additives to develop edible and biodegradable food coating material. The new food coating material was obtained by modifying the methods of **Gimenez *et al.* (2013)** and **Kılınç *et al.* (2018)**. The coating material groups contained Gum (Xantana) (1% w/v) and Glycerin (3% w/v). AEE was used as 10% w/v. The gum (G) group without extract and probiotic was used as the control of the coating test groups. The groups without probiotics were completed with pure water. *L. fermentum* MA-7 active culture was added to the probiotic-containing groups after 18 hours of incubation and then 15 minutes of sonication (30 kHz, 100% amplitude). The prepared film groups are shown in Figure 2.

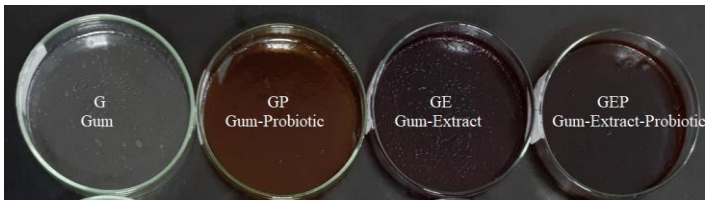

Figure 2 Film groups prepared with AEE and/or *L. fermentum* MA-7

Determination of antimicrobial activities of edible food coating material

The agar diffusion assay was used to test the biological activity of four different food coating materials **(Kanmani and Rhim, 2014).** First, 100 µL of 0.5 McFarland suspension ($1x10^8$ CFU/mL) was spread over the surface of the agar plates. Then, 6 mm diameter wells were drilled on the agar and the wells were filled with 100 µL of film solutions. After incubation (24 h), the inhibition zone diameter (mm) around the well was measured to obtain the antimicrobial activities of the prepared coating materials. The test was repeated three times to obtain the mean values of the inhibition zone diameter.

Mechanical properties of edible food coating material

Thickness and density

The thickness of the developed films was determined with a digital-micrometer **(Mahajan *et al.*, 2021)**. Film density is the ratio of film weight to film volume (Formula 1) **(Ghiasi and Golmakani, 2023)**. The film density was determined with 3 different film samples:

$$Density = \text{Surface Area} \times \text{Thickness} \quad (1)$$

Moisture content

The differential weighing was used to obtain the moisture content of the prepared films before and after drying by using the oven-drying method. Three different film samples from each group were oven dried at 70°C until constant weight. Three replications were made for each film formulation. The moisture content of the film was calculated using the formula (2) below **(Socaciu *et al.*, 2020)**:

$$Moisture\ Content\ (\%) = \frac{wi - wd}{wi} \times 100 \quad (2)$$

In the formula, w_i is the initial weight (g) and w_d is the oven-dried film weight (g).

Transparency

The relative transparency of the film strip was obtained using a spectrophotometer (OD 600 nm) (Beckman Coulter, USA) and calculated by the following formula (3) **(Ramos *et al.*, 2013)**:

$$Transparency = \left(\frac{A600nm}{mm}\right) = \frac{A600nm}{X} \quad (3)$$

In the formula, X is the film thickness (mm) and A_{600} is the absorbance value. Three measurements were made for each formulation of film.

Swelling degree and water solubility

The swelling degree and water solubility of the developed films were determined by the modified method of **Wang *et al.* (2010) (Socaciu *et al.* 2020).** Each film sample was cut into square pieces. The film pieces were weighed (approximately 0.1 g) in the beaker and then distilled water (40 mL) was added. The prepared film samples were kept at room temperature (approximately +25°C) for 24 hours, and the residue was filtered with paper (Whatman No.1). The filtrate was weighed to determine the degree of swelling. The water solubility of the films was obtained and weighed to constant weight using the oven-dried filtrate at 70°C. Three replications were made for each film formulation. The degree of swelling (4) and solubility in water (5) were calculated using the formulas:

$$Swelling\ Degree\ (\%) = \frac{wi - wf}{wi} \times 100 \quad (4)$$

$$Solubility\ in\ Water\ (\%) = \frac{wi - wd}{wi} \times 100 \quad (5)$$

In the formula, w_i is the initial weight (g); w_f, filtrate weight (g); w_d is the dry weight of the filtered film in the oven.

Light transmission

The light transmittance was determined using a UV-VIS (dual beam) spectrophotometer. (Beckman Coulter, USA) at wavelengths between 200-800 nm at 5 nm intervals in 3 repetitions **(Socaciu *et al.*, 2020)**. The percent transmittance of the absorbance value was calculated using the Lambert Beer Equation (6):

$$\textit{Light Transmission}\ (\%) = \text{Antilog}_{10}(2 - \text{A}) \quad (6)$$

In the formula, A is the absorbance value of the film strip recorded in the spectrophotometer.

Composite film coating of strawberry

Fresh strawberry (*Fragaria*) fruits were purchased from a local market in Aksaray/Türkiye province to be coated with the edible coating material. The strawberries were selected uniformly in size, color and without defects. The test groups were stored at +4°C and +25°C to observe changes in storage conditions. A total of 24 strawberries were used, 3 in each film group. The strawberry fruits immersed in 1% sodium hypochlorite solution were sterilized for 2 minutes. They were then washed with distilled water and dried **(Robles-Flores *et al.*, 2018)**. For the experimental group, the fruits were immersed in the film solution for 2 minutes, and for the control group, they were washed with distilled water only and dried. The fruits immersed in the film were drained and dried at room temperature (approximately +25°C) for 4 hours. The control sample was kept in the same environment. The fruits stored in the dark in +4°C and +25°C environments were weighed for 3 days and the results were recorded. The fruit samples were weighed on each day of storage to calculate weight loss. The results were evaluated with the following % weight loss formula (6) **(Liu *et al.*, 2017)**:

$$\textit{Weight Loss}\ (\%) = \frac{w1 - w0}{w0} \times 100 \quad (6)$$

In the formula, w_0 is the initial fruit weight (g); w_1, fruit weight (g) at any time period.

Statistical analysis

Data were analyzed using GNU SPSS version software and statistical significance was confirmed by One-Way analysis of variance (ANOVA) with Tukey's post-hoc test. The difference between means was considered statistically significant at the $p<0.05$ level.

RESULTS AND DISCUSSION

In recent years, pathogenic microorganisms have been developing resistance to commercial antimicrobials and antifungals and adversely affect human health **(Lucien *et al.*, 2021)**. Some *Candida* species may cause spoilage of food. Also, these species may be transmitted to humans through food and cause nosocomial infections **(Yurdakul *et al.*, 2018)**. *L. monocytogenes* can spoil all ready-to-eat foods and cause Listeriosis, which can lead to death **(FDA, 2018)**. Consumption of fish and marine animals can cause many diseases such as infections caused by fish pathogens **(Ina-Salwany *et al.*, 2019)**. Therefore, safe bioactive compounds are needed to control the growth of pathogenic microorganisms. Contamination of these pathogenic microorganisms into food causes food spoilage. Renewable films and coatings, on the other hand, is thin-layer coating materials that play an important role in extending shelf life, food preservation, and marketing. They are also used to protect food from microbiological, physical and chemical damage **(Yelda and Doğruer, 2022)**.

Antimicrobial activity

The biological activity of AEE against foodborne and fish pathogenic test microorganisms was determined (Table 1). The disc diffusion test results showed that the highest inhibition zone of AEE was determined as 11.79 mm against *C. glabrata* RSKK 04019 and the statistical difference between the test microorganisms was significant ($p<0.05$). The difference between the antimicrobial activity results of aronia extract against *A. hydrophila* ATCC 19570 (8.73 mm) and *Y. ruckeri* (8.32 mm) is statistically insignificant ($p>0.05$). The positive control antibiotics at the indicated concentrations exhibited stronger inhibitory activity than AEE against all pathogens. DMSO did not show inhibition zones on the test microorganisms.

Table 1 Antimicrobial activity of AEE

	Inhibition Zone Diameter (mm±SD)			
Test Microorganisms	**AEE**	**AM (10 µg/disc)**	**K (30 µg/disc)**	**FCA (25 µg/disc)**
Clinical fungal pathogen				
C. glabrata RSKK 04019	11.79±0.25[a]	NA	NA[a]	25.35±0.10
Food-borne bacterial pathogen				
L. monocytogenes ATCC 7644	NA [b]	17.76±0	NA[a]	NA
Fish bacterial pathogens				
A. hydrophila ATCC 19570	8.73±0.39[c]	NA	16.16±0.05[b]	NA
V. anguillarum A4	7.61±0.14[d]	NA	13.76±0.03[c]	NA
Y. ruckeri	8.32±0.20[c]	NA	17.54±0.03[d]	NA

Legend: AEE: Aronia Ethanol Extract, AM: Ampicillin, K: Kanamycin, FCA: Fluconazole, NA: No Activity. Different superscript values in the columns indicate significantly different ($p<0.05$) by one-way ANOVA followed by Tukey's post-hoc test. F (1011.505) Sig.(0.000) for AEE. F(2050.532) Sig(0.000) for Kanamycin. The inhibition zones in the table include disc diameter.

A previous study on the antimicrobial properties of aronia berries investigated the antimicrobial activity of the water extract against nine different pathogens (*Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 8739, *Salmonella enterica* ssp. ATCC BAA-2162, *S. aureus* ATCC 25923, *L. monocytogenes* I, *L. monocytogenes*, *Proteus vulgaris* G, *C. albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 9027). It was determined that the extract did not show any antimicrobial activity (disc diffusion and MIC assays) on the test microorganisms **(Denev *et al.*, 2019)**. In the research on the antimicrobial activity of aronia **(Nowak *et al.*, 2022)**, they determined that it has an antibacterial effect on Gram (+) bacteria (*L. monocytogenes*: 24 mm). Contrary to our findings in this study, they determined that Gram (-) bacteria were resistant to aronia fruit juice. In our study, *L. monocytogenes* ATCC 7644 (Gram (+)) was resistant to the extract. Additionally, variable antimicrobial activity against Gram (-) microorganisms was detected. The differences between our results and the studies in the literature may be due to the differences in the climate and soil conditions in which the aronia fruit is grown, the solvent used to obtain the fruit extracts and the extraction method.

The antimicrobial activity of AEE was determined against LAB strains (Table 2). AEE showed either very low or no inhibitory effect on LAB strains. For example, the difference between the antimicrobial effect of the extract on *L. fermentum* MA-7 and *S. thermophilus* MAS-1 is statistically insignificant ($p>0.05$), but is significant when compared to other microorganisms ($p<0.05$). The low inhibitory activity or lack of activity of AEE indicates that the extract can be used as a natural substance together with these probiotic LAB strains.

Table 2 Antimicrobial activity of AEE against LAB strains

	Inhibition Zone Diameter (mm±SD)			
Lactic Acid Bacteria Strains	**AEE**	**OFX (10 µg/disc)**	**K (30 µg/disc)**	**CLOX (5µg/disc)**
L. fermentum MA-7	6.77±0.14[a]	7.31±0.16[a]	NA	12.60±0.46[a]
L. delbrueckii MA-9	8.02±0.21[b]	NA[b]	NA	21.22±1.28[b]
L. vaginalis MA-10	NA[c]	NA[b]	NA	19.85±0.86[b]
L. gasseri MA-1	7.26±0.24[d]	NA[b]	NA	23.78±0.[85c]
S. thermophilus MAS-1	6.78±0.14[a]	19.96±0.51[c]	10.69±1.15	NA[d]

Legend: AEE: Aronia Ethanol Extract, OFX: Ofloxacin, K: Kanamycin, CLOX: Cloxacillin, NA: No Activity. Different superscript values in the columns differ significantly ($p<0.05$) by one-way ANOVA followed by Tukey's post-hoc test. AEE: F(1125.767) Sig(0.000). OFX: F(3954.261) Sig(0.000). CLOX: F(413.939) Sig(0.000).
The inhibiti on zones in the table include disc diameter.

Micro-broth dilution

The minimum antimicrobial concentration that can inhibit the microorganism was determined. The lowest concentration at which bacterial growth is inhibited is reported as the MIC of the extract, and the lowest concentration at which bacterial growth on solid media is completely inhibited is reported as the MBC or MFC of the extract. The results are shown in Table 3. Among test microorganisms, the lowest MIC and MFC values of the extract were obtained as 12.5 mg/mL against *C. glabrata* RSKK 04019. *L. monocytogenes* ATCC 7644 is the most resistant microorganism to AEE.

Table 3 MIC and MBC or MBC values of AEE.

Test Microorganisms	MIC (mg/mL)	MBC or MFC (mg/mL)
C. glabrata RSKK 04019	12.5	12.5
L. monocytogenes ATCC 7644	50	50
A. hydrophila ATCC 19570	25	25
V. anguillarum A4	25	25
Y. ruckeri	25	25

In a study, the antimicrobial effect of the extract obtained from aronia fruits with 60% ethanol on *P. aeruginosa, S. aureus, E. coli* and *Streptococcus mutans* was examined **(Peng *et al.*, 2022)**. They determined that the MIC values of the ethanol extract were 10 mg/mL on *E. coli* and 5 mg/mL on other bacteria. They determined that the MBC value of the extract was >20 mg/mL on *S. mutans* and *E. coli* and 20 mg/mL on *S. aureus and P. aeruginosa* **(Peng *et al.*, 2022)**. Differences from our current study may be due to differences in the solvent used in the extraction, the location where it is grown, or the test microorganisms used.

Antimicrobial activities of edible food coating material

The use of herbal extracts in the development of edible films is an approach used to protect food from microorganisms. The use of AEE in food coatings has the potential to be implemented as one of the alternative uses in the food industry. Covering foods with edible films can be considered as an alternative that can reduce the use of more costly methods such as packaging using synthetic materials, controlled and modified atmospheres **(Passos *et al.*, 2016; Gümüş and Kızıl, 2022)**. In our study, edible food coating material with natural content was prepared by using AEE which has high antimicrobial activity, probiotic microorganism and microbial gum. The antimicrobial activity of the prepared coating material against pathogenic microorganisms that cause food spoilage was obtained. The group (G) without aronia extract and probiotic had no antimicrobial effect ($p>0.05$). All film groups showed increased antimicrobial activity against *C. glabrata* RSKK 04019 and *Y. ruckeri* ($p<0.05$) (Table 4). The antimicrobial activity of the film groups prepared on *C.glabrata* RSKK 04019 is shown in Figure 3. *L. monocytogenes* ATCC 7644 was resistant to the mixture of gum and aronia extract (GE) ($p>0.05$), however, GEP group showed a synergistic effect that significantly increased antimicrobial activity ($p<0.05$). For *V. anguillarum* A4 and *A. hydrophila* ATCC 19570, the statistical difference between the GE and GP groups was not significant ($p>0.05$), but all groups showed a statistically significant difference compared to the control ($p<0.05$).

Table 4 Antimicrobial activity of food coating material containing AEE and probiotic.

Test Microorganisms	Inhibition Zone Diameter (mm±SD)				F(Sig)
	G	GE	GP	GEP	
C. glabrata RSKK 04019	NA[a]	7.83±1.00[b]	12.24±0.30[c]	18.86±0.95[d]	373.698(0.000)
L. monocytogenes ATCC 7644	NA[a]	NA[a]	4.58±0.61[b]	15.97±0.60[c]	912.450(0.000)
A. hydrophila ATCC 19570	NA[a]	7.82±0.74[b]	7.96±0.84[b]	12.70±0.11[c]	261.882(0.000)
V. anguillarum A4	NA[a]	5.72±1.37[b]	5.48±1.55[b]	10.24±1.73[c]	28.831(0.000)
Y. ruckeri	NA[a]	9.94±0.46[b]	4.67±0.73[c]	12.74±0.41[d]	413.838(0.000)

Legend: G: Gum, GE: Gum-Extract, GP: Gum-Probiotic, GEP: Gum-Extract-Probiotic. Different superscript values in rows differ significantly with one-way ANOVA followed by Tukey's post-hoc test ($p<0.05$).

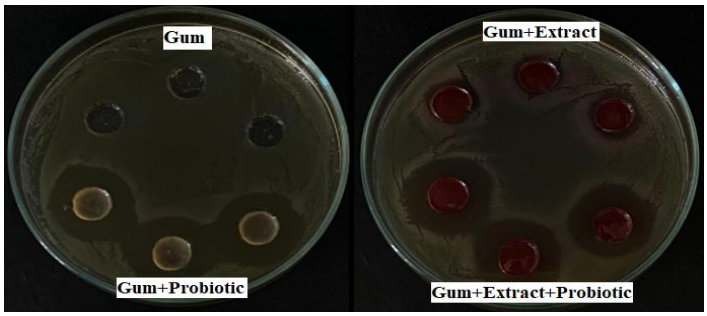

Figure 3 Antimicrobial activity of prepared film against *C.glabrata* RSKK 04019

In a study using aronia fruit powder, the antimicrobial activity of the food coating material prepared at various concentrations (25% and 50% w/v) on Gram (-) (*E. coli*) and Gram (+) (*S. aureus*) bacteria was investigated. The coating materials are more effective against *S. aureus* **(Lee *et al.*, 2020)**. **Ozcan and Arman Kandirmaz (2022)** obtained color pigment from aronia fruits using 50% ethanol. The biological activity of films prepared with pigment against *E. coli* and *S. aureus* were determined by the disc diffusion method. The films containing aronia color pigment showed antimicrobial activity against *S. aureus* in the range of 17 - 22 mm and against *E. coli* in the range of 4 - 9.9 mm. As in our current study and the literature, films prepared with aronia fruits have high antimicrobial activity on Gram (+) bacteria. In conclusion, the films developed with aronia may be a potential agent in protecting foods against pathogens.

Mechanical properties of food coating material

The thickness and density of the film groups were determined and the results are given in Table 5. The thickness and density of the control group was the lowest among all groups (0.17 mm and 0.71 g/cm^3) ($p>0.05$). Probiotic bacteria added to the film significantly increased the density of the film (1.11 g/cm^3 and 1.17 g/cm^3). As the film thickness and density increase, the amount of dissolved solids in the film increases **(Arham *et al.*, 2016)**. Increasing film thickness and density by adding extracts and probiotics to the film will be more effective in protecting foods. The moisture content of the film indicates the volume occupied by the water molecules in the micro-network of the film **(Wang *et al.*, 2010)**. The moisture content values of the film samples are shown in Table 5. The G group has the highest moisture content (96.21%) and the difference between other film groups is statistically significant ($p<0.05$). Probiotic bacteria and aronia extract added to the films reduced the moisture content of the GEB film by 8.34%. Among the prepared films, the G and GE groups have a rough appearance, while the GB and GEB groups have a bright, smooth and complete appearance. The transparency of the films increased with the addition of bacteria and extract. GEB group showed the highest transparency with 3.94.

Table 5 Mechanical properties of film groups

Film Groups	Thickness (mm)	Density (g/cm^3)	Moisture Content (%)	Transparency (A_{600}/mm)
G	0.17±0.01[a]	0.71±0.0	96.21±0.15[a]	0.70±0.0
GE	0.34±0.01[a]	0.88±0.0	92.56±0.02[b]	3.34±0.0
GP	0.35±0.0[a]	1.11±0.0	91.99±0.22[c]	0.98±0.0
GEP	0.33±0.0[a]	1.17±0.0	87.87±0.14[d]	3.94±0.0

Legend: G: Gum, GE: Gum-Extract, GP: Gum-Probiotic, GEP: Gum-Extract-Probiotic. Different superscript values in the columns differ significantly ($p<0.05$) by one-way ANOVA followed by Tukey's post-hoc test. F (0.011) Sig.(0.998) for thickness, F (1505.920) Sig.(0.000) for moisture content.

Socaciu *et al.* (2020) prepared edible films with whey protein isolate containing tarragon essential oil. The mechanical properties of the prepared films were examined and the thickness of the films in translucent form was measured between 0.43 mm and 0.46 mm. The moisture content of the films between 40.4% to 56.4% and transparency measurements in the range of 0.5 to 1.3 degrees were determined. The densities of pectin-based films containing mulberry leaf extract prepared by **Shivangi *et al.* (2021)** were determined between 5.46 g/cm^3 and 6.33 g/cm^3. The mechanical properties of aronia extract and probiotic-containing films are similar in both studies.

The high degree of swelling for the edible film is undesirable for packaging foods with high moisture content **(Šuput *et al.*, 2017)**. The water solubility of the films is one of the important parameters of the hydrophilicity of the films **(Jiang *et al.*, 2010)**. The degree of swelling degree and water solubility of the film formulations are given in Figure 4. Swelling degrees were found to be close to each other in the film groups, between 101.25% and 101.73% ($p>0.05$). Among the film groups, the GEB group had the highest water resolution capacity (70.55%). GE had a statistically significant difference and the lowest water resolution capacity (31.73%) ($p<0.05$).

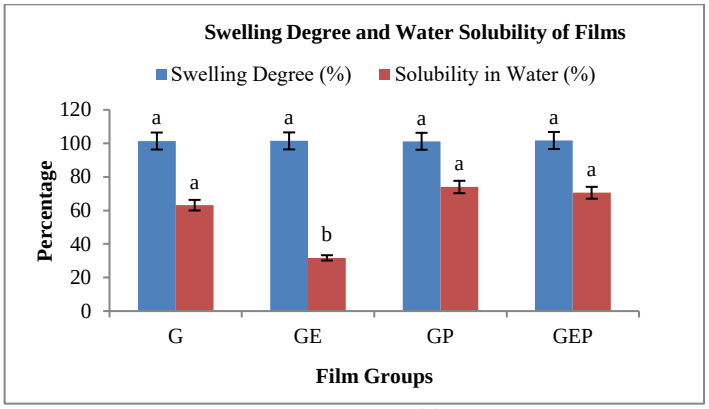

Figure 4 Swelling degree and water solubility of films. **Legend:** G: Gum, GE: Gum-Extract, GP: Gum-Probiotic, GEP: Gum-Extract-Probiotic. Different superscript values on the bars differ significantly ($p<0.05$) by one-way ANOVA followed by Tukey's post-hoc test.

A film prepared with whey protein isolate by **Wang *et al.* (2010)** has a much higher swelling degree than the film groups prepared in our study, with 350%. **Socaciu *et al.* (2020)** the films we prepared are between 100.4% and 101.9% swelling degree and have close values with the films prepared in our study. In addition, the film containing aronia extract and probiotics have a lower resolution percentage than the films prepared by **Socaciu *et al.* (2020)**.

The light transmittance of films prepared with AEE and/or *L. fermentum* MA-7 was determined in the range of 200-800 nm. The light transmittance percentages of the films in the wavelength range are shown in Figure 5. Light transmittance was determined between 51.88% and 71.28% in the G group. The GEP group was determined between 0.05% and 100%. GE, GP and GEP groups provide excellent protection in the UV light range compared to the G group. Visible light transmission percentages are close in all groups. The extract and *L. fermentum* MA-7 keep the light transmittance in the films at the desired level. **Socaciu *et al.* (2020)** determined that the light transmittance of the films containing whey protein isolate and glycerol ranged from 0.01 to 70.65%. In the food market, packaging is important for preserving the nutritional value of the food and for its appeal. Therefore, the film used in food coating should not change the appearance, taste and smell of the food **(Pająk *et al.*, 2019)**.

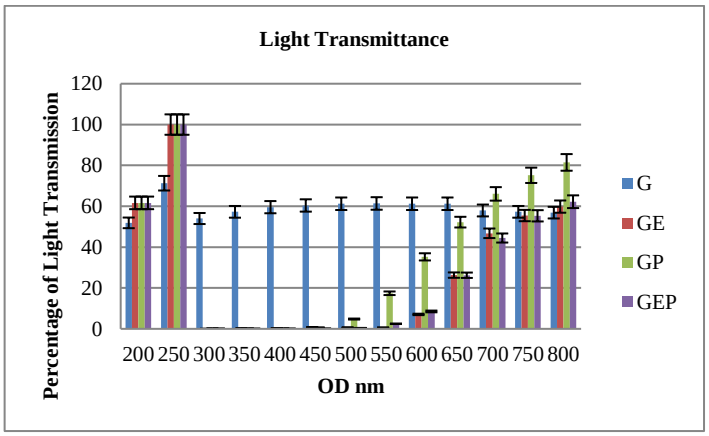

Figure 5 Light transmittance of edible films.
Legend: G: Gum, GE: Gum-Extract, GP: Gum-Probiotic, GEP: Gum-Extract-Probiotic.

Composite film coating of strawberry

The appearance of strawberry fruits coated with composite films prepared with AEE and/or *L. fermentum* MA-7 on days 0 and 3 is given in Figure 6. The appearance of the film-covered strawberry fruits was better than uncoated (control) strawberry fruit samples in both storage conditions. The protective feature of the film ensured that the physical appearance of the samples on days 0, 1, 2 and 3 was better preserved during storage. It was determined that the percentage of weight loss in uncoated samples was higher than in coated samples each day of storage.

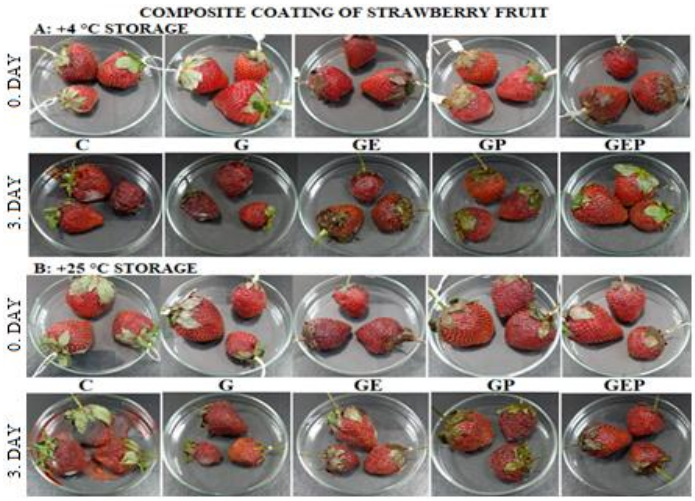

Figure 6 Images of strawberry fruits coated and uncoated with film groups on day 0 and day 3

Tables 6 and 7 show the percentage weight loss with storage for both control and coated samples. At the end of 3 days, the highest weight loss among strawberry fruits stored at +4°C belonged to the control group (48.49%). The least weight loss among coated fruits stored at +4°C was determined as 12.61% for the GEP group. The difference between the control group and GE, GP and GEP for day 1 was not significant ($p>0.05$). For the 2nd day, the difference between the control group and G, and between GE and GP was not statistically significant ($p>0.05$). The difference was statistically significant after 3 days compared to the control group and other groups ($p<0.05$).

Table 6 Percentage of weight loss of strawberry samples on days 0, 1, 2 and 3 under +4°C storage conditions

	Weight Loss of Strawberry Samples (%)			
+4°C Storage	**0. DAY**	**1. DAY**	**2. DAY**	**3. DAY**
C	0	25.16 ± 1.83^{a}	32.31 ± 1.06^{a}	48.49 ± 2.37^{a}
G	0	10.29 ± 2.15^{b}	$14.26\pm4.02^{b,c,d}$	$22.73\pm1.28^{b,c,d}$
GE	0	3.75 ± 2.27^{c}	$12.73\pm3.81^{c,b,d,e}$	$19.99\pm5.70^{c,b,d,e}$
GP	0	$4.13\pm1.42^{d,c}$	$10.63\pm1.23^{d,b,c,d,e}$	$15.08\pm1.35^{d,b,c,e}$
GEP	0	$2.35\pm1.22^{e,c}$	$6.31\pm2.04^{e,c,d}$	$12.61\pm2.08^{e,d}$
F(Sig)		80.379(0.000)	39.778(0.000)	67.805(0.000)

Legend: G: Gum, GE: Gum-Extract, GP: Gum-Probiotic, GEP: Gum-Extract-Probiotic. Different superscript values in the columns differ significantly ($P<0.05$) by one-way ANOVA followed by Tukey's post-hoc test.

The percentage weight loss of all groups was greater at +25°C than at +4°C. The highest weight loss among strawberry fruits stored at +25°C belongs to the control (61.62%) and the least weight loss among the coated fruits belongs to the GEP group (20.66%). For all days, the difference between control and GEP and other groups is statistically significant ($p<0.05$).

Table 7 Percentage of weight loss of strawberry samples on days 0, 1, 2 and 3 under +25°C storage conditions

	Weight Loss of Strawberry Samples (%)			
+25°C Storage	**0. DAY**	**1. DAY**	**2. DAY**	**3. DAY**
C	0	36.87 ± 1.32^{a}	50.10 ± 1.21^{a}	61.62 ± 1.66^{a}
G	0	26.81 ± 1.53^{b}	38.76 ± 1.35^{b}	50.18 ± 0.71^{b}
GE	0	17.90 ± 3.20^{c}	29.41 ± 0.74^{c}	36.34 ± 0.42^{c}
GP	0	12.62 ± 0.83^{d}	23.13 ± 3.16^{d}	31.41 ± 1.26^{d}
GEP	0	3.61 ± 1.01^{e}	15.34 ± 2.83^{e}	20.66 ± 2.16^{e}
F(Sig)		153.079(0.000)	125.486(0.000)	396.724(0.000)

Legend: G: Gum, GE: Gum-Extract, GP: Gum-Probiotic, GEP: Gum-Extract-Probiotic. Different superscript values in the columns differ significantly ($P<0.05$) by one-way ANOVA followed by Tukey's post-hoc test.

Robles-Flores *et al.* (2018) determined that after 10 days in the refrigerator, strawberry samples coated with the film obtained from *Cajanus cajan* seeds had a mass loss of 39.3 g, and uncoated strawberry samples had a mass loss of 49.2 g. **Piechowiak *et al.* (2022)** stored blueberries covered with composite films prepared with cinnamon oil at +4°C for 10 days. They determined that weight loss was 33-43% lower than the control. In our study, the GEP group provided high food preservation under both storage conditions tested. In a study, the antimicrobial film contained postbiotic was developed as natural preservatives to extend the shelf life of ground meat. They found that the film prepared with lyophilized postbiotics obtained from *Lactobacillus rhamnosus* at various concentrations (10% and 4%) significantly reduced the number of *S. aureus* in ground meat for 9 days **(Ansari**

***et al.*, 2024).** In a study, chitosan and grape seed extract were combined to determine the changes in the microbial properties of rainbow trout fillets after storing them at 4 °C for 15 days. According to their results, they determined that there was a significant reduction in the number of mesophilic and psychrophilic bacteria in the coated fillet **(Hassanzadeh *et al.*, 2018)**.

CONCLUSION

Since food packaging, which cannot be degraded in nature for many years, negatively affects the environment and our lives, people are becoming aware of degradable and environmentally friendly packaging. In our study, the use of aronia extract (AEE) and *L. fermentum* MA-7 as natural additive sources in the production of a quality, edible and biodegradable film for food preservation was comprehensively examined. The AEE prepared for use in the film showed a good biological effect on the test microorganisms. AEE could have potential use as a natural antimicrobial agent in food additives and coating materials. AEE improved the physical properties of the prepared coating material and also provided a food preservative effect. The developed films preserved the physical properties of strawberry fruits by extending their shelf life. Additionally, the films minimized the loss of their mass during storage. The synergistic effect of AEE and *L. fermentum* MA-7 probiotic strain revealed the potential of the coating material with high antimicrobial properties as bioactive materials in the food industry. The mechanical properties of the food coating material have shown that GEP may have the potential to be used commercially to extend the shelf life of foods.

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