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ORIGINAL RESEARCH PAPER

ANTIMICROBIAL ACTIVITY AND MECHANICAL PROPERTIES OF EDIBLE FILM ENRICHED WITH FINGERED CITRON WATER EXTRACT AND PROBIOTIC

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Abstract: Food consumption is vital, so edible and biodegradable materials are being developed against environmental factors. The aim of the study was to develop edible film containing fingered citron leaf extract and determine its biological activities and mechanical properties. In the study, the antimicrobial effect of fingered citron water (FCW) extract against pathogens was determined by disc diffusion, micro and macro dilution tests. Additionally, the antimicrobial activity of edible films containing the extract and *Limosilactobacillus fermentum* MA-7 was determined by well diffusion assay. The highest and lowest inhibition diameter of extract against pathogens was detected for *Escherichia coli* O157:H7 (11.61 mm) and *Vibrio anguillarum* A4 (9.09 mm). Minimal inhibition and bactericidal concentration (MIC and MBC) values ranged between 5 - 40 $\mu g \cdot \mu L^{-1}$. Also, it was determined that FCW extract prepared at 50 and 100 $mg \cdot mL^{-1}$ concentration had an inhibitory effect against *E. coli* O157:H7 at 0, 24 and 48^{th} hours by viable cell count. Then, the antimicrobial activity of the prepared films was evaluated and the highest and lowest inhibition zone diameters against *E. coli* O157:H7 (12.85 mm) and *Streptococcus agalactiae* Pas. Ins. (4.02 mm) were determined. It was determined that the edible film prepared with FCW extract and probiotics has the potential to protect against food spoilage.

Keywords: *antibacterial activity, carrageenan gum, Citrus medica L. var. sarcodactylis, lactic acid bacteria, leaf, pathogen*

INTRODUCTION

Food is an important product for people to survive [1]. However, the increase in color, taste, texture deterioration and toxicity in foods worries the consumer [2]. In addition, this situation causes losses in the country's economic development and resources [3]. As problems arise as a result of food consumption, edible resources are being investigated to develop natural and biodegradable materials [4]. Edible films and coatings, which are among the edible resources, are thin layers created to increase the safety and quality of foods. Edible films and coatings can be applied to protect the flora against spoilage of many foods such as fruits, meat, vegetables, and seafood, to minimize the risk of reproduction of pathogens and to extend the shelf life [1, 5].

Herbal products are preferred worldwide due to their high nutritional values. They also contain bioactive components that have strong effects on health [6]. For this reason, research on obtaining bioactive substances from different sources to prevent various diseases is rapidly increasing [7]. Bioactive components are generally known as secondary metabolites. The secondary metabolites such as alkaloids, tannins, lectins, flavones, polypeptides, flavonoids, coumarin, flavonols, terpenoids, essential oils are also known to be beneficial for their antibacterial and antifungal effects [8]. The fruit known as fingered citron (FC, *Citrus medica* L. var. *sarcodactylis*) belongs to the Rutaceae family and is among the subspecies of *Citrus medica* L. It is a fruit that attracts attention with its unique shape and appearance. FC, which is rewarded with its beautiful smell, has an important place in Chinese culture. Southwestern China is an important center of diversity for FC. The internal structure of the fruit has a whitish color and does not have a seed [9]. It is reported in the literature that FC contains various bioactive components including phenolic acids, flavonoids, essential oil, polysaccharide, and coumarin, etc. [10]. The FC essential oil is used in food fields thanks to its pleasant smell and anti-inflammatory and anti-cancer activities [11].

Probiotics are a group of microorganisms that, when taken in adequate amounts, have beneficial properties for host health and protective properties against pathogens [12]. Their positive effects on human health have become more evident today and the use of probiotics is attracting attention in the food industry [13]. Changes in food products and eating habits lead to new demands from consumers [14]. For these reasons, it is stated that it can be an alternative antimicrobial agent for health and food safety. In recent years, coating materials obtained by combining probiotics and plant extracts have been extensively researched in order to improve the safety and shelf life of products [15].

In the study, the potential of the edible films prepared with fingered citron water (FCW) extract and/or probiotics to protect food safety and quality was evaluated. First, the biological activity of the extract against microbial strains was investigated. Then, the antimicrobial activity of edible film mixtures obtained using carrageenan gum, FCW extract and/or probiotic was evaluated and the potential of these mixtures as 'natural agents' was examined. Furthermore, the mechanical properties of the prepared edible films were investigated.

MATERIALS AND METHODS

Supply of samples and preparation of extract

FC leaves were obtained from 'Muğla Köyceğiz Plant World' (Türkiye) in October 2022 (Figure 1). Then, the leaves were kept in a ventilated and dark environment for drying. The dried leaf samples, which were pulverized with a grinder, were subjected to extraction using water as a solvent. The homogeneous mixtures prepared with powder (10 g) and solvent (30 mL) were subjected to extraction for 24 hours (2 days, 80 °C) using a water bath (Memmert WNB 14, Germany). As a result of this process, the remaining solvent was evaporated. The storage conditions of the prepared extract are +4 °C.

A B

***Figure 1.** FC production area (A) and leaves (B)*

Growth conditions of bacteria

The antimicrobial activity of FCW extract was determined against six pathogen bacteria. *Escherichia coli* O157:H7 and *Aeromonas hydrophila* ATCC 19570 in Nutrient Broth (NB) at 37 °C, *Streptococcus agalactiae* Pas. Ins. 55118 and *Yersinia ruckeri* were incubated in Tryptic Soy Broth (TSB) at 37 °C, *Vibrio anguillarum* A4, *V. anguillarum* M1 were incubated in Tryptic Soy Broth-NaCl (2 %) medium at 25 °C.

Biological activity of FCW extract against test microorganisms

The antimicrobial activities of FCW extract were determined by disc diffusion test [16]. 100 µL of the microbial suspension prepared at a density of 0.5 McFarland (1×10^8 CFU·mL^{-1}) was spread on the solid medium. The sterilized discs (6 mm) in triplicate were placed on solid medium. 20 µL (2 µg/disc) of the extract prepared at a concentration of 100 mg·mL^{-1} was dropped onto each disc for pathogens. Petri dishes were left to incubate (24 h) at the appropriate temperature and medium specified above. Kanamycin (K; 30 µg/disc) standard antibiotic disc was used as the positive control group.

Micro-dilution assay

The micro-dilution assay was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of FCW extract against the pathogens [16]. Appropriate medium, extract and microbial suspension were added into the tubes. The tubes were incubated in the appropriate temperature and nutrient environment mentioned above. After overnight incubation, the lowest concentration of the FCW extract that inhibits visible growth of the microorganism in liquid media was determined as the MIC value [17]. As for the MBC value, the sample from each tube at various concentrations was spot-dropping into solid medium. The solid media were incubated for 24 hours at the appropriate temperatures. The extract concentration at which microbial growth stopped was determined as MBC value.

Macro-dilution assay

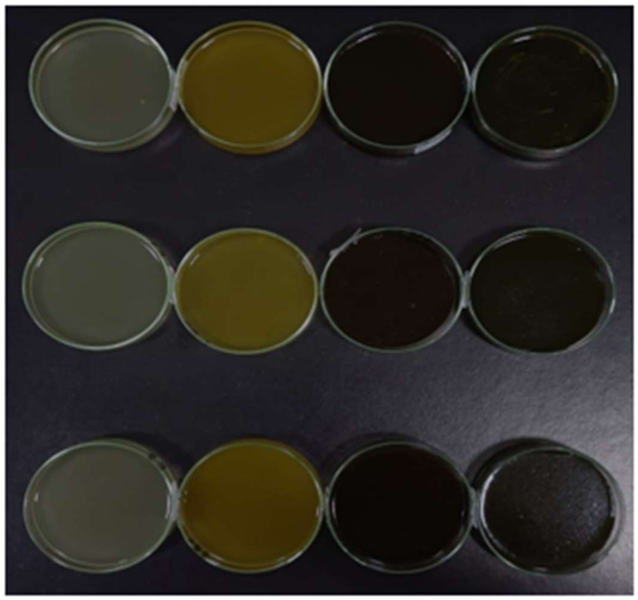
The antimicrobial activity of FCW extract against *E. coli* O157:H7, an important food-borne pathogen, was determined using the macro-dilution assay [18]. The microbial suspension (0.5 McFarland) was added onto the FCW extract prepared at concentrations of 50 and 100 mg·mL^{-1}. The test and control groups were incubated in the environment and temperature specified above for *E. coli* O157:H7. In the prepared groups, it was diluted 10 times in saline solution (SF) at the 0, 24 and 48th hours of incubation and spread cultivation was done by dropping 100 μL into the solid medium. After the incubation period of the Petri dishes, colonies were counted, and the results were determined as $\log_{10}$ (CFU·mL^{-1}). The % antimicrobial activity of the calculated cells [19] were evaluated using the equation given below (1):

$$\%\,Dead = \frac{w_k - w_t}{w_k} \times 100 \tag{1}$$

where: w_k - number of living cells counted in the control group ($\log_{10}$) and w_t - number of live cells counted at various concentrations ($\log_{10}$).

Biological activity of and edible film with FCW extract and probiotic

Edible films containing extract and/or probiotic were developed with the protocol used by Venkatachalam *et al*. [20]. FCW extract (10 % w/v), active culture lysate of *Limosilactobacillus fermentum* MA-7 isolated from human-milk (OD: 1.6), carrageenan gum (1 % w/v) and glycerol (3 % w/v) were used in the edible film formulation. The current study has 4 different experimental groups. These are Gum (G/Control), Gum-Probiotic (GP), Gum-FCW Extract (GF) and Gum-FCW Extract-Probiotic (GFP). Among the groups, the final volume of the groups without probiotics was completed with water. The final volume of the probiotic-containing groups was completed with *L. fermentum* MA-7 lysate. The film mixtures were mixed homogeneously at 90 °C for 30 minutes using a magnetic stirrer (Velp Scientifica, India). Firstly, the antimicrobial activity of the films was determined. Then, to determine the mechanical properties, the films were dried in the Pasteur oven (Elektro-Mag M 3025 P, Turkey) until they reached constant weight (Figure 2). For antimicrobial activity; homogeneous film mixtures were sterilized using a 0.45 μm filter. 100 μL of bacterial suspension was added onto solid agar medium. Then, 100 µL of the film mixture was added into the wells (6 mm). The Petri dishes were incubated in the environment and temperature specified above for the pathogens. After incubation, the inhibition zone diameters were measured with a caliper.



A B C D

Figure 2. *Prepared edible films: carrageenan gum (A), carrageenan gum-probiotic (B), carrageenan gum-FCW extract (C) and carrageenan gum-FCW extract-probiotic (D)*

Determination of mechanical properties of edible film developed using FCW leaf extract and/or probiotic

Mechanical properties such as film thickness, density, light transmittance, moisture content, swelling degree and water solubility of edible films prepared using FCW extract and/or probiotic lysate were determined [21 – 25].

Thickness and density of edible films

The thickness of the prepared edible films was determined using a digital caliper (Mitutoyo 500-181-30, Japan). Random areas were taken from the films, and the results were determined in millimeters. The density of the films was calculated as the ratio of film weight to volume.

Moisture content of edible films

The moisture content of the prepared edible film samples was determined by weighing and drying method. The film samples, divided into equal parts, were analyzed using their initial weight (W_0) and their weight after drying at 90 °C (W_1). The obtained weights were calculated using the following equation (2):

$$Moisture\ content\ (\%) = \frac{W_0 - W_1}{W_0} \quad (2)$$

Transparency and light transmittance of edible films

A spectrophotometer (Beckman Coulter DU730, USA) was used to determine the transparency and light transmittance of the prepared edible film samples. The film samples cut into strips for transparency were measured at a wavelength of OD_{600} nm. To determine the light transmittance, it was measured at 50 nm intervals at a wavelength of $OD_{200-800}$ nm. Measurement results were calculated using the equations (3) and (4) stated below:

$$Transparency = \frac{A\ (600\ nm)}{thickness\ of\ the\ film\ (mm)} \quad (3)$$

$$Lighy\ transmittance\ (\%) = antilog_{10}(2 - A) \quad (4)$$

where: A - absorbance value of the film sample.

Swelling degree and water solubility of edible films

The square-shaped pieces were cut from the prepared edible film samples to determine their swelling degree. The film pieces were placed in glass beakers and weighed (W_i). Then, 40 mL of water was added into it. The films were kept until they reached a constant weight and were filtered and weighed (W_f). For water solubility, it was kept at Pasteur
(90 °C) until it reached a constant weight and weighed (W_d). Each experimental group was conducted in triplicate. Values were calculated using the equations (5) and (6) below:

$$Swelling\ degree\ (\%) = \frac{W_i - W_f}{W_i} \times 100 \quad (5)$$

$$Solubility\ in\ water\ (\%) = \frac{W_i - W_d}{W_i} \times 100 \quad (6)$$

Statistical analysis

One-way ANOVA was performed using the SPSS program (GNU) to determine significant differences between antimicrobial activity values. The significance of differences between mean values was evaluated with the Tukey test ($P < 0.05$).

RESULTS AND DISCUSSION

Microorganisms are one of the main building blocks of life [26]. Proper control of microorganisms is an important issue. Their uncontrolled proliferation can lead to various dangers [27]. In study, the biological activity of the natural component FCW extract against pathogens was determined. The inhibition zone diameters obtained as a result of the disc diffusion method are given in Table 1. The highest two inhibition zone diameters were determined as 11.61 and 10.33 mm against the food pathogen *E. coli* O157:H7 and the fish pathogen *A. hydrophila* ATCC 19570. It has been determined that while these pathogens are not statistically significant among themselves ($P > 0.05$), there is a significant difference against other pathogens ($P < 0.05$). *V. anguillarum* A4 has the lowest inhibition zone diameter of 9.09 mm. *E. coli* O157:H7 is a dangerous pathogen that poses serious risks to public health [28]. It is also known that *A. hydrophila*, which causes diseases in marine and freshwater fish, is widespread worldwide [29]. In this context, the strong antimicrobial activity shown by FCW extract may offer a preventive alternative against infections seen in food and fish.

Table 1. *The inhibition zones of FCW extract on test microorganisms*

Microorganisms	Inhibition zone diameters (SD±mm)	
	Extract	Antibiotic
	FCW	K
E. coli O157:H7	11.61±0.16^{a}	19.33±0.40^{a}
S. agalactiae Pas. Ins. 55118	9.83±0.25^{b}	19.69±0.08^{a}
A. hydrophila ATCC 19570	10.33±0.61$^{a, b}$	16.16±0.42^{b}
Y. ruckeri	9.19±0.40$^{b, c}$	17.55±0.59^{c}
V. anguillarum A4	9.09±0.69$^{b, c}$	13.76±0.18^{d}
V. anguillarum M1	9.79±0.55$^{b, c}$	12.40±0.09^{e}

FCW: Fingered citron water extract, K: Kanamycin
*Different superscript values in the columns indicate significantly different (P < 0.05) by one-way ANOVA followed by Tukey's post-hoc test. F (Sig): 11.041(0.000) for FCW, F (Sig): 213.388(0.000) for K

In a study conducted by Akinnibosun and Edionwe [30], the extract was obtained from *C. aurantifolia* leaf using water as solvent. A water bath (100 °C/24 h) was used for extraction. The biological activity of leaf water extract against *E. coli* was determined by the well diffusion method. The results showed that the leaf water extract had an inhibition zone diameter of 9 mm against *E. coli*. The compared to our study, it was determined that FCW extract showed a higher inhibitory activity against *E. coli* O157:H7 (11.61 mm).
The biological activity of FCW extract against pathogens determined using the micro-dilution assay is given in Table 2. The MIC and MBC values of the extract on test microorganisms ranged from 5 $\mu g \cdot \mu L^{-1}$ to 40 $\mu g \cdot \mu L^{-1}$. The lowest MIC values were determined as 5 $\mu g \cdot \mu L^{-1}$ against fish pathogens (*A. hydrophila* ATCC 19570, *Y. ruckeri* and *V. anguillarum* A4). The lowest MBC value of FCW extract is 5 $\mu g \cdot \mu L^{-1}$ against *V. anguillarum* M1.

Table 2. *MIC and MBC values of FCW extract on test microorganisms*

Microorganisms	MIC [$\mu g \cdot \mu L^{-1}$]	MBC [$\mu g \cdot \mu L^{-1}$]
E. coli O157:H7	10	20
S. agalactiae Pas. Ins. 55118	20	20
A. hydrophila ATCC 19570	5	10
Y. ruckeri	5	20
V. anguillarum A4	5	5
V. anguillarum M1	40	40

MIC and MBC: Minimal inhibition and minimal bactericidal concentrations

In the study conducted by Shojaemehr *et al.* [31], the MIC and MBC values of *C. hystrix* leaf water extract obtained with a Soxhlet extractor were determined. The concentration of the water extract was determined as 400 $mg \cdot mL^{-1}$. The results obtained using the micro-dilution assay showed that no inhibitory activity was determined for the water extract against *E. coli* ATCC 25922 when considering the MIC and MBC values

[31]. In our study, the MIC and MBC values of FCW extract on *E. coli* O157:H7 were determined as 10 and 20 μg·μL^{-1}.
E. coli O157:H7 is an important food-borne microorganism that causes serious diseases in humans [32]. Although there is no specific treatment for infections caused by *E. coli* O157:H7, the use of available antibiotics is generally not recommended. This is because antibiotics are thought to increase the production of toxins, which may cause the disease to progress [33, 34]. In this study, the biological activity of FCW extract, which has the potential to create an alternative innovative approach, was also tested by the macro-dilution assay. As shown in Figure 3, antimicrobial activity (%) of *E. coli* O157:H7 were determined.

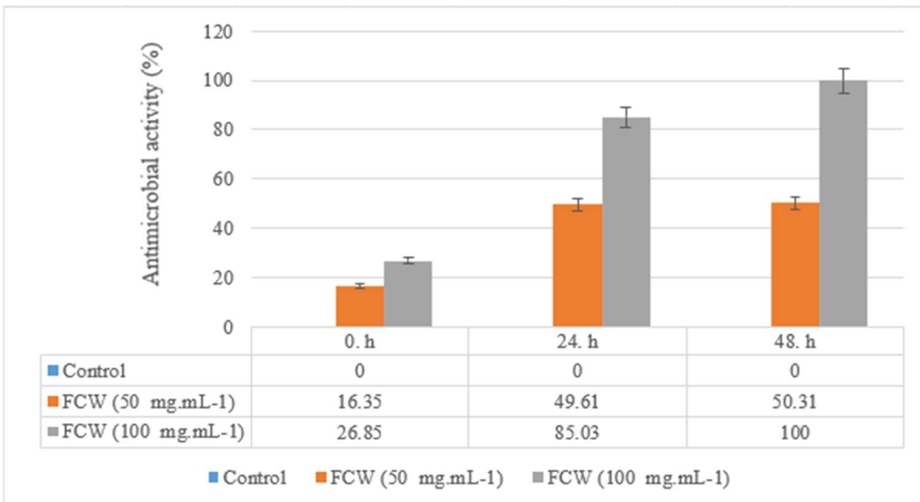


***Figure 3.** Antimicrobial activity rates based on the effect of FCW extract at different concentrations on the food pathogen E. coli O157:H7*

It was determined that FCW extract inhibited the growth of *E. coli* O157:H7 by 49.61 % in 24th hours at a concentration of 50 mg·mL^{-1}. It was observed that this rate increased in the 48th hour and reached 50.31 %. It was determined that the 100 mg·mL^{-1} concentration of FCW extract significantly inhibited the growth of *E. coli* O157:H7 by 85.03 % and 100 % at the 24 and 48th hours. It was determined that FCW extract prepared at different concentrations effectively inhibited the growth of the test microorganism compared to the control group during all incubation periods (0, 24 and 48th hours).
In a study conducted by Nicolo *et al*. [35], the antimicrobial activities of fruit juice prepared with *C. paradisi* on *E. coli* O157:H7 were determined using the live cell counting method at 0, 24, and 48th hours. *E. coli* O157:H7 cultures prepared with low, and high inoculum sizes were incubated with *C. paradisi* water extract. The results showed that after 24th hours live cells retained their viability, but after 48th hours both culturability, and viability were lost. In our study, it is seen that FCW extract shows a significant antibacterial activity on *E. coli* O157:H7 at 0, 24, and 48th hours, and its effect increases over time.
The antimicrobial activities of the edible film were determined using FCW extract and/or probiotic candidate strain. Table 3 shows the inhibition zone diameters against

the tested strains. While no inhibitory activity was noted against the tested strains in the gum group (control), inhibitory activity was observed against all strains (except *Y. ruckeri*) in the gum-probiotic (GP) group. The highest inhibition zone diameters were determined in the GP and gum-FCW extract (GF) groups for *A. hydrophila* ATCC 19570 (10.11 mm) and *E. coli* O157:H7 (2.43 mm). Statistical analysis showed that while there was no significant difference between the G group and the GF group in *S. agalactiae* Pas. Ins. 55118, *A. hydrophila* ATCC 19570 and *V. anguillarum* M1 ($P > 0.05$), there was a significant difference between the GP and GFP groups ($P < 0.05$). Moreover, G and GFP groups showed significant increase in inhibitory activity against all test microorganisms. These results indicate that the edible film may offer a natural solution against food and fish pathogens, and its rich bioactive component content may have potential for use in the food and pharmaceutical industries.

Table 3. *Inhibition zones of the edible film prepared with FCW extract and probiotic candidate strain on test microorganisms*

Microorganisms	**Inhibition Zone Diameters (SD±mm)**				
	G	**GP**	**GF**	**GFP**	**F (Sig)**
E. coli O157:H7	-^{a}	3.27±0.10^{b}	2.43±0.18^{c}	12.85±0.08^{d}	7677.218(0.000)
S. agalactiae Pas. Ins. 55118	-^{a}	2.36±0.12^{b}	-^{a}	4.02±0.22^{c}	731.259(0.000)
A. hydrophila ATCC 19570	-^{a}	10.11± 0.14^{b}	-^{a}	10.07±0.35^{b}	2830.654(0.000)
Y. ruckeri	-^{a}	-^{a}	-^{a}	2.90±0.11^{b}	2252.679(0.000)
V. anguillarum A4	-^{a}	2.73±0.11^{b}	2.09±0.03^{c}	4.47±0.21^{d}	713.799(0.000)
V. anguillarum M1	-^{a}	4.24±0.33^{b}	-^{a}	5.94±0.29^{c}	568.453(0.000)

G: Carrageenan Gum, GF: Carrageenan Gum-FCW Extract, GP: Carrageenan Gum-Probiotic, GFP: Carrageenan Gum-FCW Extract-Probiotic

*Different superscript values in lines differ significantly with one-way ANOVA followed by Tukey's post-hoc test ($P < 0.05$)

In the study of Deb Majumder and Sarathi Ganguly [36], edible film enriched with 2 % chitosan, *C. lemon* peel extract and *Ocimum tenuiflorum* leaf extract was prepared, and the quality changes of bananas were evaluated. The results showed that the edible film significantly delayed the ripening process in bananas [36]. A different study [37] shows that fruit peel (*C. limon* and *C. sinensis*) extracts can be used effectively to produce edible films with antimicrobial properties and that these films are successful in extending the shelf life of fishery products. In particular, it has been determined that edible films produced by combining *C. limon* peel extract with carob and carrageenan significantly delay the microbial deterioration of squid ring [37]. In our study, it was determined that the edible film obtained with FCW extract and probiotics showed significant biological activity against food-borne and fish pathogens. The mechanical properties of edible films containing FCW extract and/or probiotic are listed in Table 4.

As a result of adding the extract and probiotic, the thickness of the films increased significantly ($P < 0.05$). The thinnest film was observed in the control group (0.38 mm), while the thickest film was detected in the GFP group (1.92 mm). This may have increased the durability, particularly by contributing to the film structure of the FCW extract and probiotic. At the same time, a significant difference was determined between the moisture content of the control group and the groups containing FCW extract and/or probiotic ($P < 0.05$). It is the GFP group with the lowest moisture content at 74.97 %.

Table 4. *Mechanical properties of edible film developed with FCW extract and/or probiotic*

Film groups	Thickness [mm]	Density [g·cm^{-3}]	Moisture content [%]	Transparency (A_{600}/mm)	Swelling degree [%]	Solubility in water [%]
G	0.38±0.05^{a}	0.83±0.0	90.38±0.42^{a}	0.16±0.05^{a}	100.59^{a}	87.50^{a}
GP	0.74±0.19^{b}	1.01±0.0	82.50±0.85^{b}	0.52±0.19^{b}	100.53^{a}	72.57^{b}
GF	1.14±0.04^{c}	0.70±0.0	79.88±0.11^{c}	2.32±0.02^{c}	100.28^{b}	70.01^{c}
GFP	1.92±0.15^{d}	0.54±0.0	74.97±0.97^{d}	2.33±0.01^{c}	100.25^{b}	72.94^{b}

G: Carrageenan Gum, GF: Carrageenan Gum-FCW Extract, GP: Carrageenan Gum-Probiotic, GFP: Carrageenan Gum-FCW 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(Sig): 79.479 (0.000) for thickness, 264.300(0.000) for moisture content, 365.601(0.000) for transparency, 19.957(000) for swelling degree and 948.053(0.000) for solubility in water.

According to the density data, probiotic addition increased the density of the films, while FCW extract slightly decreased the density, indicating that the films can be optimized according to different usage areas. A statistically significant difference was determined between the transparency values of the control group and the groups containing FCW extract and/or probiotic (P < 0.05). This suggests that the addition of the extract and probiotic can improve the visual quality of food products by differentiating the optical properties of films. Small differences were observed between groups in the degree of swelling. In addition, it was shown that the G group (87.50 %) had the highest solubility in water and the GF group (70.01 %) had the lowest solubility (P < 0.05). It shows that the extract and probiotic increase the water resistance of the film. Overall, our study shows that edible films developed with FCW extract and probiotic exhibit good qualities in terms of both mechanical and functional properties. These films may have the potential to offer innovative and effective solutions, especially in the fields of food preservation and packaging. In the study of Bhatia *et al.* [38], the food packaging potential of the edible film obtained by using *C. reticulata* L. essential oil and pectin was determined. It was determined that the thickness of the films obtained varied between 0.04 - 0.07 mm and the moisture content varied between 20.25 - 26.05 % [38]. In our study, while FCW increased the thickness of the extract and probiotic films, it was determined that the films had a higher moisture content. Additionally, moisture exchange between food and the environment is a critical factor in the selection of food coatings and packaging materials. Since each type of food has different moisture transfer requirements, the most appropriate material must be selected for each product [39]. Transparency in films is an important parameter and opacity is a criterion used to determine this transparency [40, 41]. In a study conducted by Asfaw *et al.* [42], edible films were obtained by using pectin obtained with citron peel, and the transparency of the films varied between 6.03 and 14.09. In our study, it was determined that the transparency of the film groups was between 0.16 and 2.33. The degree of swelling in edible films is undesirable, especially when packaging foods with high moisture content [43]. This determined that the water absorption capacities of the edible film groups in our study were minimal and similar between them. In addition, in our study, the water solubility of the GF group prepared with FCW extract was found to be similar to the group prepared with gelatin (100 %) and lemongrass essential oil (0.5 %) in the study conducted by Azizah *et al.* [44].

The light barrier properties of food films determine the ability of the packaging to protect food from visible and ultraviolet light. This property plays an important role in preventing deterioration of the nutritional value and flavor of food [45]. Light transmittance properties of film groups containing FCW extract and/or probiotics were determined in the range of 200 - 800 nm (Figure 4). The light transmittance at wavelengths of 200 and 800 nm was determined as 57.01 - 73.45 % of the G group and 52.11 - 68.70 % of the GP group. While the light transmittance decreased when probiotics were added to the films, it lost its light transmittance feature as a result of the addition of the extract. This may be due to the color of FCW leaf extract. Plant extracts create opacity and reduce transparency in composite materials. However, this helps protect light-sensitive components by providing an effective barrier against light. This feature is critical to maintain the quality of packaged products [46].

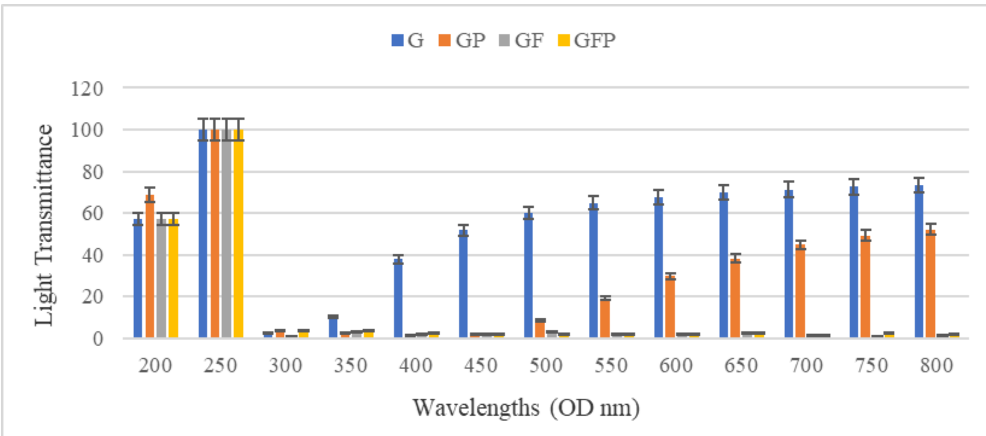


Figure 4. *Light transmittance of film groups containing FCW extract and/or probiotics*

Umeohia and Olapade [47] revealed in their study that edible films can function as a UV barrier against nutrient loss, lipid oxidation, and loss of color and taste of foods. The addition of leaf extract increased the resistance to UV light by reducing light transmission and increasing light scattering. These findings are compatible with our study. Additionally, Firdaus *et al.* [46] found in their study that the addition of the extract significantly reduced the light transmittance of the film and was similar to our study.

CONCLUSIONS

The potential for use of water extract obtained from FC leaf as a natural antimicrobial agent and the applicability of edible films prepared with this extract in the food industry were investigated. The increasing consumer demand for 'natural' and 'additive-free' products has heightened interest in natural preservatives that can replace chemical additives. Our study results indicate that FCW extract and/or probiotic-containing edible films have significant inhibitory properties against food-borne and fish pathogens. This suggests a solution for developing natural and antimicrobial-effective films that can extend the shelf life of foods by preventing spoilage. Additionally, these natural substances may contribute to the development of new plant-based products. The data obtained from in vitro studies of FCW extract indicate that this extract has the potential to be used in various industries such as food, pharmaceutical and feed after in vivo studies.

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