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Psyllium (*Plantago ovata*) Müsilaj Tozu ve Probiyotik *Saccharomyces Boulardii* İçeren Sodyum Kazeinat Bazlı Yenilebilir Biyoaktif Filmler

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ÖZET

Bu çalışmada, sodyum kazeinat esaslı *S. boulardii* ve farklı oranlarda psyllium müsilaj tozu (PMP; %0, %0.125, %0.25 ve %0.5 (a/h)) içeren biyoaktif filmlerin geliştirilmesi amaçlanmıştır. Elde edilen filmler kalınlık, nem içeriği, suda çözünürlük (SÇ), su buharı geçirgenliği (SBG), opaklık, renk ve kurutma sonrası probiyotik canlılığı açısından incelenmiştir. PMP ilavesinin filmlerin kalınlık, nem ve SBG değerleri üzerindeki etkisi önemli ($p<0.05$) bulunmuştur. %0.5 PMP içeren filmlerin kalınlık, nem ve SBG değerlerinde önemli bir artış tespit edilmiştir. PMP konsantrasyonunun film opaklığı üzerindeki etkisi önemli ($p>0.05$) bulunmamıştır. Ancak, PMP konsantrasyonunun renk değerleri üzerindeki etkisi önemli ($p<0.05$) bulunmuştur. PMP ilavesi, probiyotiğin kuruma stabilitesini önemli ölçüde artırmıştır ($p<0.05$) ve en yüksek canlılık oranı %0.25 PMP içeren örnekte tespit edilmiştir. Çalışma sonuçlarımız, psyllium müsilajı içeren sodyum kazeinat filmlerin probiotik *S. boulardii* için taşıyıcı olarak umut verici bir potansiyele sahip olduğunu desteklemektedir.

Gıda Bilimi

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Biyoaktif yenilebilir filmler

Sodium Caseinate-Based Edible Bioactive Films With Psyllium (*Plantago ovata*) Mucilage Powder And Probiotic *Saccharomyces boulardii*

ABSTRACT

This study aimed to develop sodium caseinate-based films incorporating *S. boulardii* and different amounts of psyllium mucilage powder (PMP; 0%, 0.125%, 0.25%, and 0.5% (w/v)). The obtained films were characterized for thickness, moisture content (MC), water solubility (WS), water vapor permeability (WVP), opacity, color, and probiotic viability after the drying process. The addition of PMP had a significant effect ($p<0.05$) on the thickness, MC, and WVP values of the films. The incorporation of 0.5% PMP led to a significant increase in the thickness, MC, and WVP values of films. While the addition of PMP did not result in a statistically significant impact on film opacity ($p>0.05$), it did have a significant effect on color values ($p<0.05$). The incorporation of PMP significantly ($p<0.05$) increased the drying stability of the probiotic, with the highest viability observed in the sample containing 0.25% PMP. Our study results support the promising potential of sodium caseinate films incorporating psyllium mucilage as carriers for the probiotic *S. boulardii*.

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INTRODUCTION

Probiotic is a term used to define "live microorganisms that provide health benefits to the host when consumed in adequate amounts." (FAO/WHO, 2006). However, food products containing probiotics face several limiting factors during their production process, including the structure of the food, temperature, osmotic and mechanical stress, and acid-base changes. Additionally, stress factors such as lack of nutrients, oxygen and temperature requirements, pH, and competitive microorganisms restrict the viability of probiotics in the final product (Zoghi et al., 2020). Therefore, various methods have been developed to preserve the biological activities of probiotics during food processing and storage stages (Espitia et al., 2016). One recent advancement in this field is the use of edible films as potential carriers for probiotics (Ceylan & Atasoy, 2023).

Lactobacillus and *Bifidobacterium* species are commercially widely used probiotics. *Saccharomyces boulardii* is the only yeast with probiotic properties in the market due to its bio-therapeutic effects (Khodaei et al., 2020; Goktas et al., 2022). The therapeutic effects of *S. boulardii* strains are increasing the demand for products containing *S. boulardii* in the probiotic market (Goktas et al., 2022). Although *S. boulardii* has been the subject of much research (Menezes et al., 2018; Santana et al., 2020) in recent years, there are limited studies (Khodaei et al., 2020; de Oliveira Filho et al., 2023) on the use of this yeast in edible packaging formulations containing probiotics.

The health issues and advancements in research are encouraging researchers to explore and evaluate new prebiotics that have the potential to contribute to human health. Psyllium (*Plantago ovata*) is a widely used prebiotic. Its seeds are recognized as a powerful dietary fiber that improves intestinal performance. Psyllium seeds contain a high proportion of various monosaccharides, including arabinose and xylose. Psyllium seeds are rich in bioactive compounds including minerals such as potassium, sodium and phosphorus, fatty acids, amino acids, polyphenols, and flavonoids. For all these reasons, the importance of products containing fibers obtained from psyllium as a prebiotic and probiotics is becoming increasingly important (Martellet et al., 2022; Martellet et al., 2023). In recent years, psyllium seed mucilage has been the subject of some research as a new sustainable film source due to its low cost, biodegradability, and gel-forming properties (Hajivand et al., 2020; Halász et al., 2022).

There are limited studies on the use of plant seed mucilages in edible packaging formulations containing probiotics (Davachi et al., 2021; Rodrigues et al., 2018; Semwal et al., 2022). In these studies, it has been reported that plant seed mucilages increase probiotic viability in edible films and coatings (Davachi et al., 2021; Semwal et al., 2022). However, no study could be found on the use of psyllium (*Plantago ovata*) mucilage in edible films with probiotics. For these reasons, the objective of this study was to develop films containing *S. boulardii* with different amounts of psyllium seed mucilage. The study aimed to investigate the physicochemical and optical properties of the films, as well as the viability of probiotics after the drying process.

MATERIALS and METHOD

Materials

Sodium caseinate and Psyllium seed were purchased from Kimbiotek A.Ş. (Türkiye, Istanbul) and TOS Grup (Türkiye, Antalya), respectively. *S. boulardi* was isolated from Reflor (lyophilized powder; Biocodex, Gentilly, France). Sodium chloride, glycerol phosphate, and Dichloran Rose Bengal agar (DRBC) were purchased from Merck (Darmstadt, Germany) and YPD broth was obtained from Condalab (Madrid, Spain).

Obtaining psyllium mucilage powder (PMP)

Psyllium seeds were diluted 1:10 and mixed with a magnetic stirrer for 2 h at room temperature. Then, to increase the mucilage yield, it was transferred to 50 mL centrifuge tubes and homogenized in a sonicator (WiseStir, HS-30D, Korea) at 75% power for 2 min. It was then centrifuged at 7471 for 40 min. at 4 °C. The separated supernatant (water) was decanted and the mucilage in the middle layer was separated. The remaining seeds were rehydrated and the same procedures were repeated 2 times. The mucilages were frozen at -20 °C for 72 h and then dried in a lyophilizer. The resulting dry mucilage was homogenized with a grinder and kept at -20 °C until film production.

Obtaining *S. boulardii* pellets

S. boulardii cells were activated in YPD broth (Yeast extract, peptone, dextrose) by modifying the method proposed by Goktas et al. (2022). For this, a capsule containing *S. boulardii* was transferred to 10 mL of YPB broth and incubated at 30°C for 24 h. Then, 3ml of culture was transferred to 150ml of YPD broth and incubated at 30°C for 48 h. At the end of the incubation, the culture was centrifuged in 50 mL tubes at 4500 rpm at 4 °C for 10 min and the pellet was twice swashed with sterile physiological saline (0.85%, w v^{-1}).

Film production

Film production was carried out according to the steps in Fig. 1 based on the method suggested by Ceylan & Atasoy (2022a). In total four film-forming solutions were manufactured which were; control (P-0, without addition of PMP), film containing 0.125% PMP (P-0.125), film containing 0.25% PMP (P-0.250), and film containing 0.5% PMP (P-0.500). Pellets of the harvested cells (corresponding to 150 mL of culture) were added to the film-forming solution to reach a concentration of 10^8 cfu mL^{-1}. Aseptically, 8.5 mL of solution was transferred into sterile Petri dishes (inner diameter 8.5 cm) and they dried at room conditions for 16 h in a laminar flow cabinet at room temperature. The dry films were carefully removed from the Petri dishes and stored in sterile zippered bags at 4 °C until probiotic cell count.

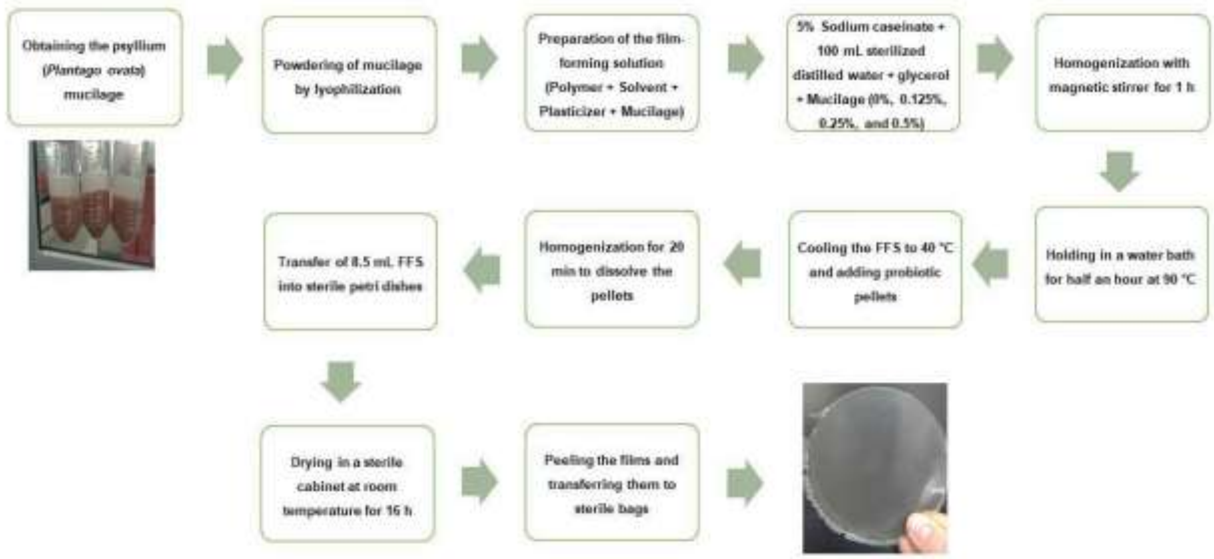

Figure 1. Flow chart of edible bioactive film production
Şekil 1. Yenilebilir biyoaktif film üretimi akış şeması

Determination of probiotic viability after drying process

The probiotics viability in films after the drying process was based on a method proposed by Khodaei et al. (2020) with modifications. One mL of film-forming solution was suspended in sterile 9 mL of NaCI (0.85%, w v^{-1}) and then serial dilutions were prepared. One gram of film samples was dissolved in sterile 99 mL of NACI at 37 °C and then serial dilutions were prepared. The number of colonies was counted on DRBC agar for yeast cells (incubation at 30 °C for 48 h). Additionally, the stability of probiotics in films after the drying process was calculated. The viability of the probiotics was calculated following the drying procedure of the film-forming solution using Eq. 1.

$$Viability\ (\%) = 100 \times N/N_0 \qquad (1)$$

where N and N_0 represent the number of viable cells in the film and the film solution, respectively.

Physicochemical analysis of films

The thickness, moisture content (MC, %), and water solubility (WS, %) values of the film samples were determined according to the method described by Ceylan & Atasoy (2022a). The thickness of the films was measured from at least 6 points randomly with a digital micrometer with the precision of 0.001 mm and the average film thickness was calculated. For MC and WS analysis, the films cut in 45 mm * 10 mm size were dried at 105 °C for 12 h. The moisture content was calculated using the percent weight loss after drying. To determine the WS value, the dry film was stirred in distilled water at room temperature for 3 min. And then the film was dried at 105 °C. The final weight of film was measured and the WS was calculated as a percentage based on the initial weight.

Water vapor permeability

For the water vapor permeability (WVP) of the films, silica gel dried at 105 °C was transferred into special containers and the mouth of the container was sealed with a film. Then, the samples were placed at 25 °C in a desiccator containing distilled water, and the weight changes were recorded hourly for 8 h. WVP values of films were calculated according to the ASTM E 96 (1995) procedure.

Opacity and color analysis of films

L*, a*, and b* values were determined in the films placed on white paper based on the CIE Lab color measurement system by colorimeter (Minolta CR-400, Japan). Also, the ΔE value of films was calculated using Eq. 2 (Khodaei et al., 2020). As a standard, the L*, a*, and b* values on the surface of the white paper were 90.04, 1.05, and -1.29, respectively.

$$\Delta E = \sqrt{(L^*_f - L^*_s)^2 + (a^*_f - a_s)^2 + (b^*_f - b^*_s)^2} \qquad (2)$$

Opacity analysis was performed with a UV/Visible spectrophotometer (Biochrom Libra, S60, Cambridge, UK) according to the method specified by Al-Hassan &

Norziah (2012). The opacity value was calculated by dividing absorbance at 600 nm wavelength by thickness (mm).

Statistical analysis

The obtained data were subjected to a one-way analysis of variance (ANOVA) using SPSS software (IBM Corp, Armonk, NY, USA). Differences between samples were determined using the Duncan multiple comparison test at a 95% confidence level. Edible bioactive film samples containing different levels of PMP were compared in terms of tested properties. The experiments were conducted in triplicate. The results were presented as mean ± standard deviation.

RESULTS and DISCUSSION

Physical properties of edible bioactive films

Fig. 2 presents the thicknesses, moisture content, and water solubility values of the bioactive edible films containing different concentrations of PMP. Thickness is one of the most important parameters affecting the optical, mechanical, and barrier properties of films (Ceylan & Atasoy, 2022b). The thickness range of the edible films was 0.068–0.094 mm. It was observed that the thickness of the edible films increased with the addition of PMP. The thickness value of the P-0.050 film was found to be significantly higher ($p<0.05$) than the control film. This was probably due to the amount of solid materials in the film-forming solution (Daei et al., 2022). Film thickness can be affected by the interaction between the ingredients as well as the content and amounts of the components in the film formulation (Zhang et al., 2020). Similar observations were reported by different researchers (Krystyjan et al., 2017; Daei et al., 2022) for edible films containing PMP.

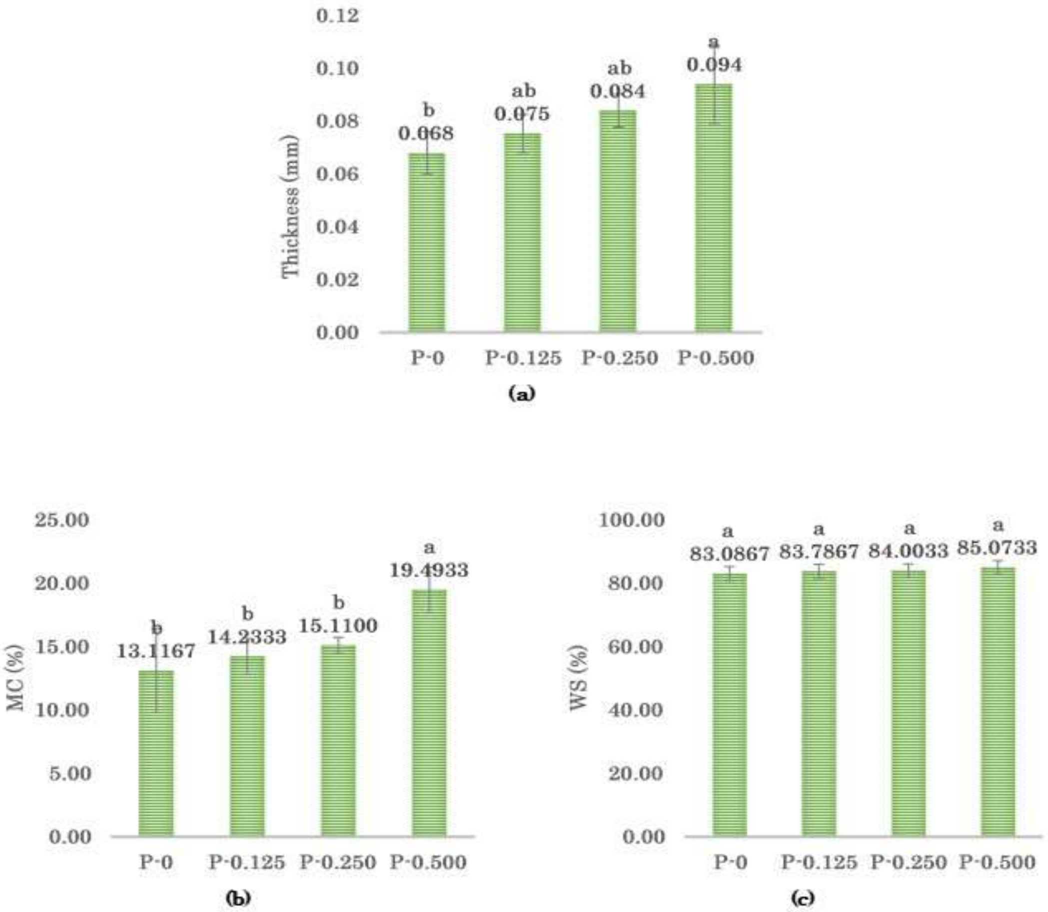

Figure 2. Physical properties of edible bioactive films

Şekil 2. Yenilebilir biyoaktif filmlerin fizikokimyasal özellikleri

Results are the as mean ± standard deviation (n=3). Different lowercase letters indicate statistically significant differences ($p<0.05$) between samples. MC, moisture content; WS, water solubility, P-0: control, P-0.125: film containing 0.125% PMP, P-0.250: film containing 0.25% PMP, P-0.500: film containing 0.5% PMP.

The melting of edible films is affected by factors such as moisture content (MC) and water solubility (WS) (Todhanakasem et al., 2022). In addition, the MC of the films after drying is of critical importance, as it affects the viability of probiotics in films (Davachi et al., 2021). As seen in Fig. 2, the MC value of films increased with the increase of the PMP content. The MC value of pure sodium caseinate film was the lowest (13.12%), while the MC value of the film containing 0.5% PMP was the highest (19.49%). However, the MC values of the films containing 0.125% and 0.25% PMP were not significantly different ($p>0.05$) from the control film. The addition of PMP causes an increase in the number of available active sites for water binding due to the hydrophilic nature of the mucilage (Badreddine et al., 2022). This can result in edible films becoming more hydrophilic and an increase in MC. Zhang et al. (2020) reported that the MC value increased depending on the PMP content in films containing different ratios of whey protein isolate and PMP.

Water solubility (WC) is a property that plays a significant role in determining the applicability of edible films in both the food and pharmaceutical industries. The solubility in water is influenced by the chemical composition of the films and serves as an indicator of their stability when exposed to water (Ceylan & Atasoy, 2022b). Furthermore, the solubility of probiotic films influences the release of probiotic cells, and higher WS leads to increased cell release (Kalantarmahdavi et al., 2021). The solubility of bioactive films ranged from 83.09% to 85.07% (Fig. 2). Semwal et al. (2022) reported the WS of probiotic films containing sodium caseinate and chia mucilage in the range of 35% and 92%. In another study (Krystyjan et al., 2017), the WS values of the films containing different ratios of psyllium mucilage and starch were found to be between 16.76% and 22.85%. The differences can be related to the polymers and their concentrations in the film formulation, as well as the differences in film preparation methods. In addition, the high solubility of bioactive films in this study may be attributed to the utilization of sonication during the production of psyllium mucilage. Sonication has the potential to decrease the molecular weight and increase the polydispersity to some extent, thereby enhancing solubility (Halász et al., 2022). When the film or coating is utilized as an edible covering and consumed along with the food, having a high level of water solubility can prove to be beneficial (Halász et al., 2022). While the WS values of the films increased with the increasing PMP content, the WS values of the films did not differ significantly ($p>0.05$). Previous studies (Krystyjan et al., 2017; Zhang et al., 2020) showed that psyllium mucilage added to edible films made from different polymers increased the water solubility of the films.

Water vapor permeability of edible bioactive films

The water vapor barrier (WVP) property of edible films is crucial for preserving food quality due to the significant role of water in food deterioration (Badreddine et al., 2022). Fig. 3 shows the WVP values found for films containing different concentrations of PMP. Bioactive films have a low permeability and WVP values were found in the range of 0.43 to 0.56 g mm m^{-2} h^{-1} kPa^{-1}. Zhang et al. (2020) reported the WVP values of films containing different ratios of WPI/psyllium mucilage in the range of 1.27 to 2.43 g m^{-1} s^{-1} Pa^{-1}. A low permeability implies resistance against water vapor interactions and hindered passage due to structural uniformity (Araújo et al. 2018). As the concentration of psyllium in the formulation increased, the water vapor permeability (WVP) generally increased. Films containing 0.5% (P-0.500 sample) psyllium had significantly higher ($p<0.05$) WVP values compared to the control film. However, films containing 0.125% and 0.25% psyllium had WVP values similar ($p>0.05$) to the control film. Water vapor transfer generally occurs along the hydrophilic portion of the film, and therefore, it varies depending on the hydrophilic-hydrophobic ratio of the film components (Rojas-Graü et al., 2006). Furthermore, the water vapor permeability (WVP) value of edible films is significantly influenced by factors such as film thickness, moisture content, biopolymer structure, and extraction method (Halász et al., 2022). Considering the influence of thickness and moisture on permeability, the thickness and MC values of the films containing 0.5% psyllium were found to be consistent with the WVP results.

Optical properties of edible bioactive films

Optical properties are crucial for edible films and food packaging applications, as they not only affect consumer preferences but also the quality of products (Davachi et al., 2021). The measured opacity values of the films ranged from 2.011 to 2.416. The opacity values of the prepared bioactive films were found to be lower than low-density polyethylene (LDPE, 4.264) films commonly used as commercial packaging materials and higher than oriented polypropylene (OPP, 1.566) films (Guerrero et al., 2011). Halász et al. (2022) determined the opacity values of pure psyllium mucilage films containing different proportions of glycerol in the range of 2.45-3.95. In another study (Krystyjan et al., 2017), the transparency values of starch-based films supplemented with psyllium mucilage ranged from 0.81 to 2.42. There was no significant difference in opacity among the films with different concentrations of PMP ($p>0.05$). This can be interpreted as the homogeneous distribution of PMP within the film matrix and its compatibility with the components present in the formulation. The light transmittance of films is dependent on the microstructural properties of the film and the homogeneous distribution of the components comprising the film. Weak compatibility among the components in the film matrix increases opacity by causing scattering or reflection of light at the interfaces (Zhang et al., 2020). Contrary to our findings, Krystyjan et al. (2017) reported that the addition of psyllium mucilage to starch-based films resulted in a more transparent appearance. Zhang et al. (2020) found that the light transmittance of films prepared with different ratios of WPI/psyllium mucilage increases depending on the mucilage content.

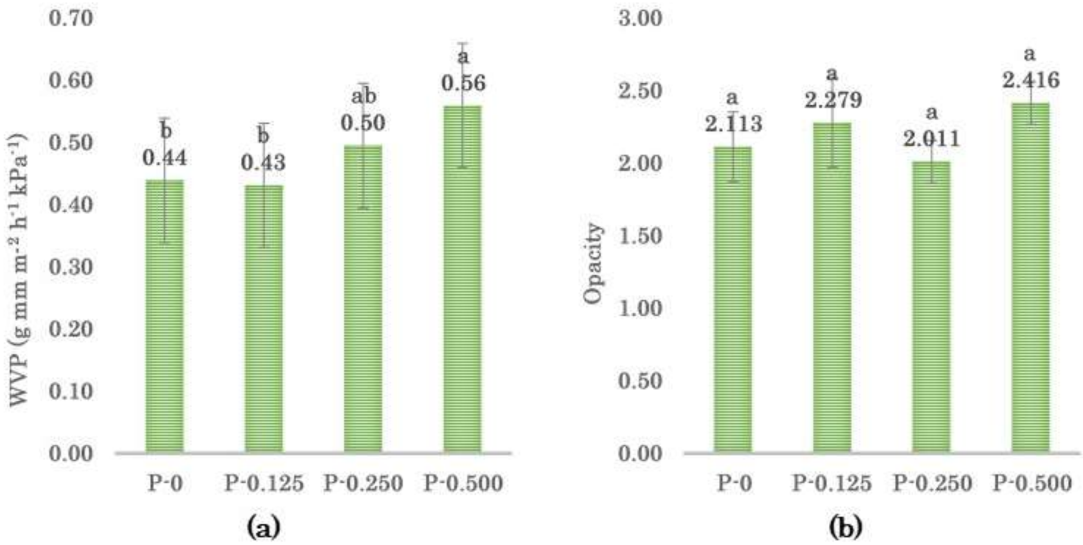

Figure 3. Water vapor permeability (a) and light barrier properties (b) of edible bioactive films
Şekil 3. Yenilebilir biyoaktif filmlerin su buharı geçirgenliği (a) ve ışık bariyer özellikleri (b)

Results are the same as mean ± standard deviation (n=3). Different lowercase letters indicate statistically significant differences (p<0.05) between samples. WVP, water vapor permeability, P-0: control, P-0.125: film containing 0.125% PMP, P-0.250: film containing 0.25% PMP, P-0.500: film containing 0.5% PMP.

The L*, a*, b*, and ΔE values of the bioactive films are shown in Table 1. The L*, a*, b*, and ΔE values were significantly (p<0.05) affected by the PMP concentration in the formulation. The film containing 0.5% PMP was found to have significantly (p<0.05) lower brightness compared to the control film. The a* values of the films decreased with increasing PMP content, while the b* values decreased. The highest ΔE values were observed in the films containing 0.25% and 0.500% PMP. The changes in the color values of the bioactive films indicate that the addition of PMP results in a shift toward darkness, greenness, and yellowness in the film. In contrast to our findings, Zhang et al. (2020) reported that the mucilage in WPI/psyllium mucilage-based films led to an increase in the *L* value and a decrease in the *a*, *b*, and ΔE values of the films. The reason for this discrepancy could be the variety and concentration of materials included in the formulation, as well as the film production technique.

Table 1. Colour characteristics of edible bioactive films
Çizelge 1. Yenilebilir biyoaktif filmlerin renk karakteristikleri

Film	L*	a*	b*	ΔE
P-0	88.10±0.20^{a}	0.40±0.09^{a}	2.01±0.52^{b}	3.88±0.56^{c}
P-0.125	87.62±0.54^{ab}	0.20±0.09^{b}	2.09±0.25^{b}	4.27±0.11^{cb}
P-0.250	87.70±0.06^{ab}	0.01±0.04^{c}	3.00±0.31^{a}	5.00±0.29^{a}
P-0.500	87.42±0.36^{b}	0.03±0.10^{c}	3.46±0.41^{a}	4.82±0.26^{ab}

Results are the same as mean ± standard deviation (n=3). Different lowercase letters in the same column indicate statistically significant differences (p<0.05) between samples. P-0: control, P-0.125: film containing 0.125% PMP, P-0.250: film containing 0.25% PMP, P-0.500: film containing 0.5% PMP.

Probiotic viability after drying of edible bioactive films

Figure 4 demonstrates the probiotic viability after the drying process in films containing different concentrations of PMP. There was a decrease in the viable cell count during the drying stage of the film-forming solutions. Khodaei et al. (2020) reported that the number of *S. boulardii* cells decreased during the drying of film-forming solutions in gelatin and low methoxyl pectin-based films. Similarly, de Oliveira

Filho et al. (2023) reported that drying of the filmogenic solution resulted in a reduction in the number of probiotics in films containing alginate, mangaba pulp, and *S. boulardii*.

The probiotic viability in the films after drying was found to be between 76.61% and 94.87%. The drying process did not have an acute toxic effect on the viability of probiotic cells. Semwal et al. (2022) reported that the drying process in films supplemented with chia mucilage based on sodium caseinate had no toxic effect on probiotic cells. This result may be associated with the moderate temperature application during drying, allowing the removal of water from probiotic cells without subjecting them to any significant thermal stress (Semwal et al., 2022). Additionally, the presence of sodium caseinate in bioactive film formulations may have contributed to the survival of probiotic cells under osmotic stress during drying. In protein-based films, proteins play a role in preserving cell membrane integrity by capturing free radicals and providing micronutrients (Semwal et al., 2022).

The effect of PMP on the drying stability of the probiotic was found to be significant ($p<0.05$). The film containing 0.25% PMP exhibited the highest probiotic viability, while the control film showed the lowest probiotic viability. The cell-protective effect of the mucilage during the drying process may be attributed to its chemical composition. Psyllium seeds are considered a notable source of prebiotics due to their composition, which includes various monosaccharides like arabinose and xylose (Martellet et al., 2022). These polysaccharides play a protective role in the survival of probiotics by interacting with cell membrane phospholipids (Semwal et al., 2022). Similarly, Semwal et al. (2022) discovered that incorporating chia mucilage based on sodium caseinate in probiotic films influenced the inactivation rates of *L. fermentum* and *L. brevis*.

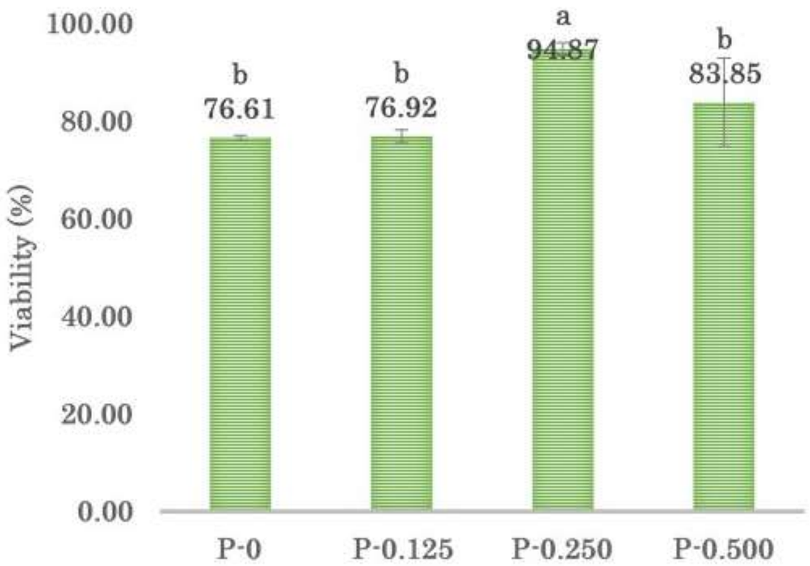

Figure 4. Viability of probiotics after the drying process
Şekil 4. Kuruma prosesi sonrası probiyotik canlılığı
Results are the same as mean ± standard deviation (n=3). Different lowercase letters indicate statistically significant differences ($p<0.05$) between samples. P-0: control, P-0.125: film containing 0.125% PMP, P-0.250: film containing 0.25% PMP, P-0.500: film containing 0.5% PMP.

CONCLUSION

In this study, bioactive edible films on sodium caseinate-based incorporating *S. boulardii* and different amounts of psyllium mucilage powder (PMP; 0%, 0.125%, 0.25%, and 0.5%) were developed. The physicochemical, barrier, and color properties of the obtained films, as well as the viability of probiotic cells after the drying process, were investigated. The effect of film formulations was significant on the tested properties of the films, except for opacity and water solubility. The highest thickness, moisture content, and water vapor permeability were observed in the samples containing 0.5% PMP. Furthermore, it was found that PMP led to the formation of darker, greener, and more yellowish films. During the drying process, the film formulations did not have a toxic effect on *S. boulardii*. Additionally, PMP exhibited a cell-protective effect during the drying process. The highest viability after drying was observed in the film containing 0.25%.

The obtained results demonstrate that the tested film formulations were suitable carriers for *S. boulardii* cells, and the addition of PMP to sodium caseinate films enhances the film properties and drying stability of the probiotic. Our plans regarding the development of bioactive films containing psyllium mucilage and probiotics include the utilization of other probiotic strains, examination of mechanical, SEM, and FTIR analyses, as well as the investigation of probiotic viability during storage. Furthermore, it is recommended that future studies systematically investigate the applicability of the developed film formulations for coating various food materials, thereby expanding our understanding of their potential in food preservation and packaging. The current findings demonstrate that the tested formulations serve as suitable carriers for *S. boulardii* and that PMP enhances the film properties and drying stability of the probiotic. Therefore, bioactive films incorporating psyllium mucilage and *S. boulardii* could present a novel alternative for the probiotic food market.

Author's Contributions

Dr. Ceylan designed the research plan, conducted laboratory experiments, performed statistical analyses, and wrote this article. Mrs. Aslan Kaya carried out the laboratory experiments. Prof. Dr. Atasoy conceptualized the study, provided financial support, and performed the writing-editing of the manuscript. All authors read and approved the final manuscript.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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