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Investigation of biodegradable coatings produced from three different protein sources for white cheese packaging

Üç farklı protein kaynağından beyaz peynir ambalajı olarak üretilen biyobozunur kaplamaların incelenmesi

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ABSTRACT

Biodegradable films and coatings; attract an increasing interest recently being natural and environmentally friendly alternatives to synthetic food packaging materials. Some terms such as "renewable, food preservative, thin layers, food life extender" might be used for defining these materials. Proteins have a high potential to be used in food packaging. This study aimed to compare the performances of three different protein sources (whey protein, soy protein and gluten) as biodegradable coatings for cheese samples. Effect of red grape juice addition into coating solutions was also investigated. In this study, 15 g coating solution is used to coat 3-3.5 g cheese slice in each treatment. The results of the study revealed that the coatings produced were elastic, adhesive, transparent and durable. Among the different coatings, gluten had a more stretch and adhesive structure. Soy protein and whey protein coatings inhibited the moisture loss in cheese samples slightly more than gluten films. Grape juice addition to soy and whey protein coatings made the cheese samples more resistant to microbial growth. The whey protein coating was the thickest among them. The results depicted that, whey protein coating with grape juice is the most promising application, by providing lower microbiological load and moisture loss in cheese samples, and depicting lower color and moderate solubility. In future studies, combined usage of fruit sources with protein coatings might be helpful to improve the cheese quality in biodegradable packages.

Key Words: Biodegradable, Coating, Gluten, Soy protein, Whey protein

ÖZ

Biyobozunur filmler ve kaplamalar sentetik gıda ambalaj malzemelerine alternatif, doğal ve çevre dostu malzemeler olarak son yıllarda giderek artan bir ilgi görmektedir. Bu malzemelerin tanımlanmasında "yenilenebilir, gıda koruyucu, ince tabaka, gıdanın raf ömrünü uzatıcı" gibi terimler kullanmak mümkündür. Proteinlerin gıda ambalajlamada kullanılmak üzere önemli bir potansiyele sahiptir. Bu çalışmada üç farklı protein kaynağının (peynir altı suyu proteini, soya proteini ve gluten) peynir örnekleri için biyobozunur kaplama olarak davranışlarının karşılaştırılması amaçlanmıştır. Çalışmada ayrıca kaplamalara eklenen üzüm suyunun etkileri de incelenmiştir. Bu çalışmada, her bir tür protein çözeltisi (15 g), 3-3.5 g peynir diliminin kaplanmasında kullanılmıştır. Çalışma tüm kaplamaların esnek, yapışkan, transparan ve dayanıklı olduklarını göstermiştir. Farklı kaplamalar arasında, glutenin daha esnek ve daha yapışkan bir yapıya sahip olduğu gözlemlenmiştir. Soya proteini ve peynir altı suyu proteini içeren kaplamalar peynir örneklerindeki nem kaybını gluten kaplamalara göre biraz daha fazla engelleyebilmişlerdir. Kaplama çözeltilerine üzüm suyu eklenmesi peynir örneklerini mikrobiyal gelişmeye karşı daha dayanıklı hale getirmiştir. Bütün materyaller arasında peynir altı suyu proteini içeren kaplamaların kalın olanlardır. Çalışmada elde edilen sonuçlara göre, üzüm suyu eklenen peynir altı suyu proteini kaplamaları, peynir örneklerinde daha düşük mikrobiyal yük ve nem kaybı sağlamaları, daha az renklilik ve kabul edilebilir düzeydeki çözünürlük düzeyleri ile en öne çıkan uygulama olmuştur. Gelecekte yapılacak çalışmalarda, meyve kaynaklarının protein kaplamaları ile birlikte kullanımının biyobozunur ambalaja sahip peynirlerin kalitesinin geliştirilmesinde daha faydalı olacağı düşünülmektedir.

Anahtar Kelimeler: Biyobozunur, Kaplama, Gluten, Soya proteini, Peynir altı suyu proteini

Introduction

Current increase in environmental consciousness of consumers has directed research more on packaging as the highest amount of solid waste produced by human is package based. Nowadays, biodegradable films packaging concept has raised due to their advantages to nature and wide range of potential application for food preservation and food protection.

Films and coatings are terms that are sometimes used as synonyms, although they represent different concepts. Film may be defined as “a thin layer of material that can be used as a cover or wrap” forming a stand-alone material; while coatings, being available in liquid form, are directly formed on the surface of the food product they are intended to be used with (Gennadios, 2002; Guimaraes et al., 2018; Costa et al., 2018).

Biodegradable films and coatings are most commonly obtained from polysaccharide or protein sources. Coatings from proteins can be obtained inexpensively and simply from either animals (such as keratin, collagen, gelatin, casein and whey proteins etc.) or plants (maize, wheat, soy, peanut etc.) are widely used (Akbari et al., 2007). Their particular mechanical and oxygen properties have been found to be superior in comparison to some polysaccharide and oil based counterparts (Khwaldia et al., 2004). Moreover, their high water vapor permeability also seems as an advantage to delay the drying of food materials, although this hydrophilic properties make them bad barriers to water at the same time (Guilbert, 1986).

Whey protein is one of the by-products of cheese production process and is made up of lactose, minerals and some of the vitamins, milk proteins and milk in small quantities. Whey proteins consist of 20% of milk proteins such as α -lactalbumin, β -lactoglobulin, bovine serum albumin, immunoglobulins and proteose-peptones, which are soluble at pH 4.6 (Kinsella and Whitehead, 1989). However, whey protein-

based coatings, although having a high biological value, seem to have a low moisture barrier function and high water vapor permeability in comparison to carbohydrate and lipid-based films. Therefore, it is generally recommended to use plasticizer materials such as glycerine, sorbitol, polyethylene glycol etc. to increase moisture barrier properties (McHugh et al., 1994; Gouna et al., 2007)). These plasticizers are also significant for decreasing the brittleness and increasing the flexibility of the whey protein films (Ozdemir and Floros, 2008).

Soy proteins are significant alternatives of plant derived protein films/coatings. They have a high content of protein (38% -44%), also isoflavones, omega-3-fatty acids and dietary fiber (Riaz, 2001; Liu, 2004). Soy protein isolates having at least 90% protein content are often used as raw materials in the biodegradable films. Their biodegradability rates are high, beside their functional properties such as stickiness, water and oil retention, texturing ability and fibrous structure make them as favorable alternatives for biodegradable packaging. Although they exhibit water retention properties; soy protein coatings are required to be enriched with auxiliary materials particularly in very humid conditions (Varzokas and Tzia, 2016). Soy protein sources have been used together with other materials such as stearic acid and pullulan (α -glucan) to increase the storage process of the kiwis (Temiz and Yeşilsu, 2006); apple pectin (Mariniello et al., 2003) and mozzarella cheese (Zhong et al., 2018).

Wheat gluten is a significant component of wheat. Moreover, it is also an important side product during the production of wheat starch. Wheat gluten coatings have strong mechanical properties and they are transparent, homogeneous and hydrophobic. They are commonly used in composite films by adding oil or cellulose to their structures, although they still need plasticizers for increasing their plasticity and surface area (Gontard and Guillaume, 2010; Pochat-Bohatier et al., 2005). A study on the glycerol-plasticized gluten films, highlighted the importance of different temperatures and relative

humidity values on mechanical and physical properties of the films produced (Kayserilioğlu et al., 2003). Generally, drying temperature has been reported to affect the mechanical and physical properties of wheat gluten-based films more than relative humidity changes (Kayserilioğlu et al., 2003).

Cheese may be defined as a food matrix consisting mainly of casein, fat, and water having a relatively short shelf life. It is widely consumed around the world for ages and represents an ideal food model system to study the coating process having a smooth surface, uniform texture, and regular shape (Zhong et al., 2014). The flavor formation in cheese, in parallel manner with other dairy products, is related to the activity of lactic acid bacteria synthesizing the intracellular enzymes during fermentation, to a lesser extent molds and yeasts (Karagozlu and Karagozlu, 2016). Studies on shelf-life extension of cheese have been achieved by using some chemical preservatives such as synthetic antioxidants and antibacterial agents. However, increasing consumer awareness toward the negative health consequences of synthetic additives also commonly arises as a barrier for acceptance of these products nowadays (Gurdian et al., 2017). Therefore, investigations about plant extracts, as potential natural food additives, have attracted increasing attention (Gutierrez et al. 2009; Akcan et al., 2017). To our knowledge, on the other hand, only a few or no previous attempts in literature have been found to evaluate the effects of fruit juices addition in protein based coating solutions.

Ezine cheese is a unique type of traditional Turkish white cheese that is produced by using milk from goat, sheep and/or cow. It is brined (pickled), and has a characteristic taste and aroma (Karagul-Yuceer et al., 2007). Very recent studies in literature focused on different biodegradable coatings to increase the shelf lives of various cheese varieties such as Prato cheese (de Lima Marques et al., 2017); queso blanco cheese (Gurdian et al., 2017).

This study aimed to compare the performances

of three different protein sources as biodegradable food packaging alternatives for Ezine cheese samples. Furthermore, effect of red grape juice addition into the coating solutions was also investigated.

Materials and Methods

Materials

Commercial protein powders of whey protein (Hardline, Turkey) (~95% w/w protein), soy protein (Alfasol, Turkey) (~70% w/w protein) and wheat gluten protein (Alfasol, Turkey) (~75% w/w protein) were utilized. Cardinal variety red table grapes (*Vitis vinifera* L.) (Istanbul, Turkey) were supplied from a local market and blended with a blender (Waring, USA). The grape puree was filtered with filter paper and grape juice was obtained as filtrate. White cheese samples were Ezine type (Tahsildaroğlu, Turkey).

Chemicals

Main chemicals used in the study, such as sodium hydroxide (NaOH), ammonium hydroxide (NH₄OH), ethanol (C₂H₆O), sodium azide (NaN₃), hydrochloric acid (HCl) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were all analytical reagent grade and purchased from Sigma-Aldrich (Germany). Glycerol (DuzeyLab, Turkey) was the used for elasticity.

Preparation of coating solutions

Films composed of different portions of protein sources, glycerol, water and bases necessary and were formed by casting the final solution (15 g) into 9-cm diameter Petri dishes on 3 g cheese slices. The coating solutions on cheese samples were dried for 24 hours at room temperature. Details for production methods of different types of coatings were given below.

Whey protein coating

The whey protein film was prepared by modifying a previous method (Kaya and Kaya, 2000). Distilled water (200 ml) was added into whey protein (16 g) and heated to 90°C. Finally,

the glycerol (8 ml) was added into mixture as the plasticizer and final mixture was centrifuged at 3000 *g* for 10 minutes (Heitich-Rotofix, Germany). The separated supernatant was used as the coating solution.

Soy protein coating

Soy protein based film forming method described by Nie et al. (2015) was modified. Soy protein of 14 g was weighed and 200 ml distilled water was added and homogenized. The pH of the mixture was adjusted to 11 by NaOH (2M). The mixture was heated to 70 °C. When the temperature was cooled to room temperature, 6 ml of glycerol was added into mixture as the plasticizer and the mixture was centrifuged at 3000 *g* for 10 minutes. Separated clear supernatant was used as the coating solution.

Wheat gluten coating

Wheat gluten based films were prepared by slightly modified method of Gontard et al. (1993) and Tanada-Palmu et al. (2004). 15 g of wheat gluten protein was weighed and mixed rapidly with 90 ml of ethanol. Then 200 ml of distilled water was added. To adjust the pH value to 10; 10 ml of NH₄OH (6 N) was added into mixture and then heated to 75°C. Glycerol (4.5 ml) was added. The mixture was centrifuged at 3000 *g* for 10 minutes and clear supernatant was collected to be used as coating solution.

Preparation of red grape juice and addition into coating solutions

For the treatments with red grape juice, red grapes were squeezed and filtered through filter paper. In grape juice added films, the same procedures explained above were applied until heating steps; and then squeezed red grape filtrates having dry matter content of 22% (1.5 ml), were added into all three different films before the glycerol addition steps.

Microbiological growth on coated cheese samples

Total yeast and mold populations and total coliform bacteria were evaluated on coated

cheese samples on days 1, 3 and 5 after coating applications. Yeast Extract Glucose Chloramphenicol (YGC) (Merck, Germany) was used for yeast and mold count and Violet Red Bile Agar (VRBA) (Merck, Germany) for coliform bacterial count. Biodegradable film coated cheese samples were homogenized for 1 minute in a stomacher by adding 9 ml sterile peptone water to 1 g sample. Serial dilutions of (10^{-1} - 10^{-5}) were plated (0.1 ml) in triplicates using spread plate method. For yeast and mold count, about 15-20 ml of YGC agar solution (at 45-50°C) was poured onto sterile petri plates kept until the solution cooled down to room temperature (about 25 °C) (in sterile conditions) (ISO 7954, 1988). For coliform bacterial count, serial dilutions of the samples (0.1 ml) were plated onto sterilized VRBA agar. Colony counting were made after 24-48 hours of incubation at 37°C.

All microbiological results were given as average colony forming unit g^{-1} film \pm standard deviations.

Total moisture contents of coatings

Moisture contents of the films were measured using halogen moisture analyzer (AND MX-50, Japan). Samples weighed (1 g) 1 and heated using the standard mode for gelatin (200°C, 15 minutes). Measurements were made in parallel, as % moisture.

Total protein content of coatings

Total protein contents were measured according to AOAC 920.87 method (AOAC International, 2002). The amount of protein was calculated by using the correction factor for gelatin (5.3). Analyzes were performed in duplicate.

Coating thickness

A digital caliper was used to determine the thickness of coatings. Average of measurements taken at 5 different points were calculated.

Moisture loss % during storage

To determine the moisture loss due to storage,

coated cheese samples were weighed on 1st, 3rd and 5th days of storage using the following formula.

$$\text{Moisture loss (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100 \quad (1)$$

Coating solubility

The modified method of Hoque et al., (2011), was used to determine the coating solubility. Samples were cut in sizes 1 x 1.5 cm and weighed, then they were taken up into centrifuge tubes and added to 10 ml of 0.1% (w / v) sodium azide (NaN_3) solution. The prepared liquid and samples were stirred at room temperature for 24 hours at 150 rpm on a shaker (Stuart-Orbital Shaker, China). Mixtures were then centrifuged at 4200 g for 10 minutes and vortexed for 1 min. Centrifugation was repeated once more. The mixtures were poured into tared petri dishes and allowed to stand at 105° C for 24 hours to obtain amount of the dry insolubilized film. Finally, they were weighed and film solubility was calculated for each sample by subtracting the amount of dry insolubilized film from the total weight.

Color determination

Color measurements for coatings were measured with a Chromameter (CR-400, Konica Minolta Holdings Inc., Tokyo, Japan). Hunter L,a,b values were used as: L* value was for measuring darkness/lightness; while a* value for redness; and b* value for yellowness in films. Color parameters for the standard white plane were as follows: $L_0 = 95.9$, $a_0 = 0.2$, $b_0 = 2.3$. The total color change (ΔE) in L, a, b values is calculated according to the following formula.

$$\Delta E^* = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (2)$$

Statistical analysis

Statistical analysis was performed using statistical software SPSS 20.0 (SPSS, Inc., Chicago, IL, USA) by one-way analysis of variance (ANOVA) ($p < 0.05$). Significance of differences between samples was tested by Duncan's New Multiple Range Test.

Results and Discussion

Microbiological growth on film coated cheese samples

Microbial growth during the storage of cheese is significant as it may cause off-flavors and decreased overall product quality, thus consequently may end up with serious product losses.

Total yeast count in coated white cheese samples after 3rd day of coating treatment were depicted in Table 1, as no microbial growth was evident on 1st day, whereas the number of colonies were too many to be counted on 5th day.

Table 1. Total yeast counts (log cfu ml⁻¹) on 3rd day¹
Çizelge 1. Toplam maya miktarları (log cfu ml⁻¹) (3.gün)¹

Sample Örnek	Total yeast Toplam maya
Soy protein with grape juice	5.65 ± 0.27 ^a
Soy protein no grape juice	5.69 ± 0.22 ^a
Whey protein with grape juice	5.42 ± 0.15 ^a
Whey protein with no grape juice	5.53 ± 0.16 ^a
Gluten protein with grape juice	< 2 ^b
Gluten protein with no grape juice	< 2 ^b

¹ Data given represent the mean value ± standard deviation of triplicate analyses. Values with different letters within the same column differ significantly ($p < 0.05$).

On the 3rd day, soy protein coating with no grape juice addition had the highest yeast content among all samples (5.69 log cfu ml⁻¹). According to the results no significant differences were detected between soy protein and whey protein coated cheese samples ($p > 0.05$). Gluten film coated cheeses had no yeast content at all, probable cause of this observation was the use of ethanol in the production of gluten coatings. These coatings showed an inhibition effect on microbial growth. Grape juice addition into soy and whey protein coatings, had a slight decreasing effect on the amount of total yeast, although this effect was not statistically significant ($p > 0.05$). In contrast, some previous studies have shown more profound effects on the microbial load decrease with grape juice or grape seed extract additions (Shah et al., 2014). Similarly, total bacteria in the chicken was 4.77

log cfu g⁻¹ in the sample with grape extract added and 6.49 log cfu g⁻¹ in the control group (Abu Hafsa and Ibrahim, 2018). No mold or coliform bacteria growth were detected in coated cheese samples of after each of 1, 3 and 5 days after treatment.

Moisture contents of coatings

Moisture contents of different films were depicted in Table 2. Whey protein film with grape juice had the highest moisture content (29.6%) and average moisture content was measured as 25.6±2.1 % in different types of films. Moisture content of whey protein film with grape juice was significantly higher ($p<0.05$) than all samples except gluten protein film with grape juice. Grape juice addition increased the moisture contents of all samples. Increased moisture content due to fruit sources incorporation into biodegradable films/coatings was common to previous literature (Hashemi *et al.*, 2016). The moisture levels of the films were in parallel to the findings of previous studies (Ramos *et al.*, 2013). Moreover, although difference in glycerol contents of the films in other studies has been proposed as a factor in different moisture contents measured (according to Ramos *et al.* (2013), increase in glycerol content from 40 to 60% (w/w) in whey protein films caused around 3.60% increase in moisture content, extent of glycerol differences has not been a distinct factor for the moisture contents measured in present study. Glycerol addition increase the soluble solids and its hygroscopic structure increases the moisture absorption by attracting water molecules (Galus and Kadzińska, 2016).

Total protein contents of coatings

Total protein contents of coatings are given in Table 2. The highest amount of protein was measured in whey protein with grape juice (59.7 %). Average amount of protein content was determined as 52.4±5.4 % for all films. Soy protein films had significantly lower amount of proteins than that of whey protein films ($p<0.05$). Both of whey protein isolates and whey protein

concentrates have been reported to contain comparatively higher amount of proteins (30-80% (w/w) for concentrates and above 90% (w/w) for isolates) (Mulvihill and Ennis, 2003); therefore the higher amount of protein in those films was as expected.

No differences detected in film protein contents after grape juice addition, and this was expected since grapes are poor in total protein content.

Coating thickness

Thickness measurements were displayed in Table 2. In soy protein and gluten protein coatings with grape and without grapes, thickness values were very close to each other; (0.23 – 0.24 mm) and (0.19 – 0.20 mm), respectively. The width values of whey protein films changed between 0.32 and 0.44 mm, respectively for samples with and without grapes, being slightly higher than the rest of the film samples. Differences were statistically significant ($p<0.05$). In coatings with grape juice, the thicknesses were slightly lower in comparison to those ones without grape juice. In literature, the increase in the moisture contents of films, has been related with the increased time for evaporation of water and consequent formation of thinner films (Anker *et al.*, 1999).

Average thickness of all films was measured as 0.27±0.09 mm. Thickness values measure in present study were higher than other studies in literature. In a previous study, thickness of films samples having whey protein increased due to the sorbic acid content addition as antimicrobial agent. The content of sorbic acid varied between 0.5% and 1.5% (w/w), while the thickness values changed between 0.12 mm and 0.14 mm (Cagrı *et al.*, 2001). Thickness differences between coatings are thought to be due to the ratio of plasticizer quantities as well as the proportions of protein powders in film formation (Ramos *et al.*, 2013). Increase in the protein concentration, may be related with the increase in thickness of the coating as high protein concentrations the enhanced protein gelling in coating materials produced (Anker *et al.*, 1999). Moreover, main

characteristics of the coating materials including solubility, density, viscosity, and surface tension are also effective on barrier, mechanical and optical properties of the coatings (Costa et al., 2018). Lower thickness of films has been

proposed as a positive factor for films to improve the over-all desirability of final packaged products, specifically for edible films (Khanzadi et al., 2015).

Table 2. Some properties of protein coatings¹

Çizelge 2. Protein kaplamaların bazı özellikleri

Sample	Moisture content, % % Nem içeriği	Protein content, % % Protein içeriği	Thickness (mm) Kalınlık (mm)
<i>Örnek</i>			
Soy protein with grape juice	25.3 ± 0.0 ^b	47.6 ± 0.8 ^b	0.23 ± 0.03 ^c
Soy protein with no grape juice	24.6 ± 0.2 ^b	44.3 ± 2.5 ^b	0.24 ± 0.06 ^c
Whey protein with grape juice	29.6 ± 0.1 ^a	59.7 ± 3.9 ^a	0.32 ± 0.06 ^b
Whey protein with no grape juice	24.1 ± 0.2 ^b	57.9 ± 0.1 ^a	0.44 ± 0.08 ^a
Gluten protein with grape juice	26.0 ± 0.0 ^{ab}	52.5 ± 2.9 ^{ab}	0.19 ± 0.01 ^c
Gluten protein with no grape juice	24.1 ± 0.1 ^b	52.5 ± 2.2 ^{ab}	0.20 ± 0.03 ^c
Average	25.6±2.1	52.4±5.4	0.27±0.09

¹ Data given represent the mean value ± standard deviation of duplicate analyses. Values with different letters within the same column differ significantly (p < 0.05).

Moisture loss % during storage

Moisture loss in cheese might be considered as a problem increasing the cheese hardness and leading to undesired organoleptic properties (Costa et al., 2018). During storage, changes in sample moisture contents was measured by monitoring their weight losses. According to the findings, the greatest part of moisture loss was measured in the first day, right after packaging for all samples. Values are given in Table 3. The changes in sample weights were statistically insignificant after the 1st day of storage (p>0.05). However, in samples with no grape juice, weight loss was measured between the 1st and 3rd days was slightly higher in samples with soy protein, than the other two samples. For the samples containing grape juice, on the other hand, the moisture loss from the 1st to 3rd day was higher than the no grape juice added counterparts, for all types of protein coatings. The highest decrease was detected in gluten coated samples.

In quite a recent study, it has been stated that in fruit samples such as coated apples, moisture loss % levels were around 20 and 40 %, respectively for whey protein or soy protein isolate coatings after 10 days of storage (Marquez et al., 2017). In the same study, maximum weight loss was reached only after 2 days of storage in

soy protein film coated carrot samples and has been related with the intrinsic hydrophilic nature of soy protein films (Rhim, 2004) and therefore they suggested using enzymatically reticulated films as having better protection properties (Marquez et al., 2017). Protein coatings have some similarities to protein gels, being viscoelastic materials those depicting characteristics of both solids and liquids. Therefore, interaction of different protein sources and small molecules, including water, plasticizers, and other additives existing in the coating material matrix, affect the mechanical properties of the protein coatings (Chen, 1995). Moreover, pH, pressure and heat also have been reported to have an impact of the gel strength. Value of coating solution pH, influences both the mechanical and barrier properties. The isoelectric point of the protein (that is pH 4.5 for soy protein and 7.5 for wheat gluten) has an important role on water vapor permeation and the oxygen permeation of the films as they have their maxima at the isoelectric point. In present study, average pH values were measured as 11.2 in soy protein, 11.1 in whey protein and as 10.0 in gluten coating solutions. Therefore, alkaline conditions over their isoelectric points might also have been effective the higher moisture loss values measured (Zink et al, 2016).

Table 3. % Moisture loss in coatings during storage ^{1,2}

Çizelge 3. Depolama sırasında kaplamalardaki % nem kaybı

Coatings without grape juice Üzüm suyu eklenmemiş kaplamalar			
Days of storage Depolama günü	Soy protein Soya proteini	Whey protein Peynir altı suyu proteini	Gluten Gluten
Day 0	100.0 % ^a	100.0 % ^a	100.0 % ^a
Day 1	45.6 % ^b (-54.4 %)	44.2 % ^b (-55.8 %)	44.3 % ^b (-55.7 %)
Day 3	40.6 % ^b (-5.0 %)	43.6 % ^b (-0.6 %)	42.9 % ^b (-0.4 %)
Day 5	39.8 % ^b (-0.8 %)	43.4 % ^b (-0.2 %)	42.5 % ^b (-0.4 %)
Coatings with grape juice Üzüm suyu eklenmiş kaplamalar			
Days of storage Depolama günü	Soy protein Soya proteini	Whey protein film Peynir altı suyu proteini	Gluten film Gluten
Day 0	100.0 % ^a	100.0 % ^a	100.0 % ^a
Day 1	46.1 % ^b (-53.9 %)	47.8 % ^b (-52.2 %)	45.1 % ^b (-54.9 %)
Day 3	42.6 % ^b (-3.5 %)	44.0 % ^b (-3.8 %)	43.5 % ^b (-11.4 %)
Day 5	42.3 % ^b (-0.3 %)	43.6 % ^b (-0.4 %)	43.3 % ^b (-0.2 %)

¹ Data given represent the mean value± standard deviation of triplicate analyses. Values with different letters within the same column differ significantly (p < 0.05).

² Data in parenthesis indicate the change % in moisture loss, during successive days

In a previous study in literature, Sarıoğlu and Öner (2006) measured the weight loss during storage of kaşar cheese on days of 1, 7, 30, 60 and 90 that was coated with a sodium caseinate-based film including plasticizer as sorbitol. A significant part of the weight disappeared at 10% on the 7th day and 5% on the 30th day.

Coating solubility

Solubility of coatings for soy protein, whey protein and gluten protein-samples were depicted Table 4 as amount of solved coating (g) and % coating solubility. The solubility values of soy and whey protein coatings were lower in

comparison to gluten coatings. Grape juice seemed to increase the solubility values measured and more remarkable effects were notified particularly in soy protein (8.6 % increase) and whey protein based films (16.4 % increase), in comparison to gluten based films (1.5 % increase). Lower solubility values are proposed as desirable for biodegradable films therefore the reduction in solubility measured after addition of grape juices into the films was in parallel to the previous results provided after addition of walnut oils (Galus and Kadzińska, 2016).

Table 4. Amount of solved film (g) and film solubility %

Çizelge 4. Çözünmüş film miktarı (g) ve % film çözünürlüğü

Sample Örnek	Amount of solved coating (g) Çözünmüş kaplama miktarı	Coating solubility, % % Kaplama çözünürlüğü
Soy protein with grape juice	0.017	40.3
Soy protein with no grape juice	0.012	31.7
Whey protein with grape juice	0.017	48.9
Whey protein with no grape juice	0.005	32.5
Gluten protein with grape juice	0.025	60.4
Gluten protein with no grape juice	0.019	58.9

In previous studies conducted, solubility values were measured as 35.1% in films having 5% soy protein isolate (Kunte et al.; 1997) and as 30% in films having whey isolate (Perez-Gago et al., 1999). Yoshida and Antunes (2004) studied the solubility of whey protein films at different pHs

(at different pH values of 5, 6.7 and 9), when the glycerol was used as plasticizer. According to their results, it was found that solubility decreased as pH alkalinity approached. As another example, the pH range from 4.5 to 7 has been reported for the maximum soy protein solubility results (Zink

et al., 2016). In present study, average pH values were measured as 11.2 in soy protein based film solutions, 11.1 in whey protein based film solutions and as 10.0 in gluten protein based film solutions. Therefore in the similar manner with the previous literature (Yoshida and Antunes, 2004); present solubility results were lower because of the high alkalinity.

It is generally necessary to heat the proteins to certain temperatures for gel formation in films. For whey proteins, for example, when the temperature is between 70-90°C and between 5-60 minutes, the maximum performance is obtained (Barbut and Foegeding, 1993; Bryant and McClements, 1998). Isoelectric point is also another factor on protein gel strength (Zink et al., 2016).

With the heat treatment, the degree of protein denaturation and unfolding increases, and protein-protein cross-linking is affected. Moreover, high temperature application causes proteins to irreversibly aggregate and eventually form gel networks. This leads to different inter and intramolecular hydrophobic and electrostatic interactions as well as hydrogen and disulfide bonds due to the presence of cysteine residues. All these changes therefore affect the solubility of the protein films (Perez-Gago and Krochta, 2001). Generally, heat treatment during film formation procedure has been reported as a factor to decrease the solubility of the films (Rhim et al., 2000).

Color determination

For color measurements; L, a, b values are given together with ΔE values in Table 5. According to the results, there was no significant difference in the lightness of solid films ($p>0.05$) and all films can be considered as moderately light, with only one exception; soy protein with no grape juice was slightly lighter in comparison to other films. According to a previous study in literature, the higher amount of yellow pigments in wheat may be the possible cause of less lightness in wheat in comparison to other protein sources (Evlice and Özkaya, 2011).

However, there was significant differences among both of a and b values ($p<0.05$) of different film samples. Soy protein films had significantly higher values for both of redness (a) and yellowness (b) measurements ($p<0.05$). Therefore, yellowness and redness were significantly different in comparison to other films. Difference in soy protein film color was also detectable visually.

It may be seen that addition of grape juice into the films had no significant effects in comparison to their no grape added counterparts, so it might be said that the lightness stayed fairly constant after grape juice addition. One possible reason might be the relatively lower concentration of the juices because grape extracts are important contributors to color that affects the color measurements significantly (Tacer-Caba et al., 2016).

Table 5. Color (L, a, b) values and total color change (ΔE) for film samples¹

Çizelge 5. Film örneklerinde renk değerleri (L, a, b) ve toplam renk değişimi (ΔE)

	L L	a a	b b	ΔE for films Filmler için ΔE
Soy protein film with grape juice	52.2±1.3 ^a	2.3±0.2 ^a	18.9±4.1 ^a	47.0±2.6 ^{ab}
Soy protein with no grape juice	47.9±0.2 ^b	2.5±0.2 ^a	20.8±0.8 ^a	51.6±0.2 ^a
Whey protein film with grape juice	50.5±3.3 ^{ab}	0.6±0.0 ^b	2.6±0.6 ^b	45.4±3.4 ^{ab}
Whey protein film with no grape juice	54.9±1.2 ^a	0.2±0.1 ^c	2.5±0.4 ^b	41.0±1.2 ^b
Gluten protein film with grape juice	52.0±3.2 ^a	-0.8±0.0 ^c	6.5±0.8 ^b	44.1±3.2 ^b
Gluten protein film with no grape juice	53.5±1.1 ^a	-0.7±0.1 ^c	6.5±0.7 ^b	42.6±1.2 ^b

¹ Data given represent the mean value± standard deviation of duplicate analyses. Values with different letters within the same column differ significantly ($p < 0.05$).

According to the findings of ΔE values; soy protein film had slightly higher than the other two

protein sources. Although, grape juices addition into the films was an increasing factor for color

difference, one exception was soy protein films in which grape juice addition was found to lower the extent of color change. Changes in ΔE values are thought to be due to pH and film thickness. The thickness of the film is between 0.19-0.44 mm. As a general visual evaluation, all coatings formed in the present work had the transparent color and solid, firm and elastic structure.

Conclusion

In this study, different protein coatings were analyzed for their performance as white cheese packaging. According to the findings of the study, whey protein coating with grape juice seemed superior to the other treatments by sustaining the lowest microbiological load and moisture loss in cheese samples, in addition to showing the least color change and moderate solubility as a coating material. Grape juice addition into the protein coatings was beneficial, however higher concentrations might work better in future applications.

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