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CHARACTERIZATION AND APPLICATION OF WHEY PROTEIN ISOLATE BASED EDIBLE FILMS CONTAINING CLOVE AND APRICOT ESSENTIAL OILS

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ABSTRACT
The aim of this research is to investigate the effects of essential oils on the mechanical and antimicrobial properties of protein-based edible films. Clove and apricot oils were added to whey protein isolate (WPI) solutions and were named C-WPI and A-WPI, respectively. Kaşar cheese was chosen as a food sample to investigate the behavior of modified films on a food product. It was observed that the oil-protein interaction increased the elasticity but decreased the strength of the emulsified films. The incorporation of oil into the film matrix resulted in a more opaque appearance but also increased thermal stability. It was detected that both types of essential oils impart antimicrobial properties of the films, but clove oil is more effective than apricot oil. In conclusion, the successful antimicrobial properties of the modified WPI film make it a potential packaging material, especially for food products prone to microbiological spoilage.
Keywords: Whey protein isolate, clove essential oil, apricot essential oil, active film, texture profile analysis, Kaşar cheese

KARANFİL VE KAYISI ESANSİYEL YAĞLARI İLE AKTİF HALE GETİRİLMİŞ PEYNİR ALTI SUYU PROTEİNİ İZOLAT BAZLI YENİLEBİLİR FİLMLER

ÖZ
Bu araştırmanın amacı, uçucu yağların protein bazlı yenilebilir filmlerin mekanik ve antimikrobiyal özelliklerine etkilerini araştırmaktır. Peynir altı suyu protein izolatı (WPI) solüsyonlarına karanfil ve kayısı yağları eklenerek sırasıyla C-WPI ve A-WPI olarak adlandırıldı. Modifiye filmlerin bir gıda ürünü üzerindeki davranışını araştırmak amacıyla gıda örneği olarak Kaşar peyniri seçilmiştir. Yağ-protein etkileşiminin emülsifiye filmlerin elastikiyetini arttırdığı ancak mukavemetini azalttığı gözlendi. Yağın film matrisine dahil edilmesi, daha opak bir görünüme neden oldu, fakat aynı zamanda termal stabiliteyi de arttırdı. Her iki uçucu yağ türünün de filmlere antimikrobiyal özellikler kazandırdığı ancak karanfil yağının kayısı yağından daha etkili olduğu belirlendi. Sonuç olarak, değiştirilmiş WPI filminin başarılı antimikrobiyal özellikleri, onu özellikle mikrobiyolojik bozulmaya yatkın gıda ürünleri için potansiyel bir ambalaj malzemesi haline getirmektedir.
Anahtar kelimeler: Peynir altı suyu proteini izolatı, karanfil esansiyel yağı, kayısı esansiyel yağı, aktif film, mekanik özellik, tekstür profil analizi, Kaşar peyniri

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INTRODUCTION

Kaşar cheese, pasta-filata type of cheese, is one of the most preferred cheese type in Turkey (Sefa et al., 2020). The fresh Kaşar cheese is produced from pasteurized milk (sheep or cow's milk) and it is sold as un-ripened (Eroglu et al., 2016). Although mold growth is not so expected in fermented milk products, it has been the main problem in Kaşar cheese. The having color, structural and aroma defects caused by mold growth lead to economic loss (Erdoğan et al., 2001). There have been different techniques to prevent losing cheese due to especially microbial growth, however some needs direct incorporation of antimicrobials into cheese or dipping cheese into antimicrobials solutions, etc. In order to ensure microbial food control, it is essential to use preservatives in the industry. Essential oils are natural, non-synthetic bioactive substances. The antimicrobial and antioxidant activities of these environmentally friendly substances are pretty high. Hence, essential oils can be utilized to save perishable foods from microbial spoilage (Feyzioglu and Tornuk, 2016). The most essential oils are classified as food flavorings, however, they have antimicrobial properties also (Bączek et al., 2017). The researches in food packaging mainly focus on developing packaging materials safe to contact to foods itself and including some natural agents like essential oils especially with anti-microbial properties which enhance food safety in terms of human health and to extend their shelf life (Mahcene et al., 2020; Mahcene et al., 2021). Essential oils obtained from plants and spices are widely used in the development of antimicrobial biodegradable films and coatings (Lee et al., 2019). They contain active antimicrobial compounds such as phenols and terpenes (G. Al-Hashimi et al., 2020). Eugenol, carvacrol, and thymol are phenol compounds found naturally in cinnamon, cloves, sage, and thyme (G. Al-Hashimi et al., 2020). They can be utilized safely as food additives, since these compounds are classified as GRAS by the FDA (Food and Drug Administration) (Haro-González et al., 2021). In this study, oil of clove -an herb based extract- which is very rich in especially eugenol was utilized as an antimicrobial agent. In some previous studies, clove essential oil was tested on different medias and proved to have a very good inhibitory effect on *L. monocytogenes*, *Campylobacter jejuni, Salmonella Enteritidis, Escherichia coli* and *Staphylococcus aureus* (Radünz et al., 2019). Eugenol is used as a food additive and classified to be a safe substance according to the United States (FDA) (Haro-González et al., 2021). It was reported that according to chemical composition analysis, clove has different 36 components. The highest component is eugenyl with the value of 88.58%; eugenyl acetate (5.62%) and β-caryophyllene (1.39%) are the other two highest ones, respectively. Some of the other components, which are less than one percent in proportion, are 2-heptanone, ethyl hexanoate, humulenol (0.27%) and calacorene (0.11%). The other type of essential oil preferred in this research is apricot oil. Apricot *(Prunus Armeniaca L. (Rosaceae))* is a valuable fruit plant also used in the pharmaceutical/medicine industry. *Prunus Armeniaca L. (Rosaceae)* is a good source of natural sugar. The plant is a good source of polyphenols, fatty acids, sterols, carotenoids and volatile substances. Apricot has been the subject of many studies due to its important active properties such as anti-microbial, anti-oxidant, anti-mutagenic, anti-inflammatory and reducing effect on several enzymes (Wang et al., 2020). Active and green packaging approach can be a good alternative for food industry. Since it is possible to release the antimicrobial compound from the packaging material over a long period of time, as in the active packaging system, this activity can also continue throughout the transportation and storage period of the food system. Packaging material supplemented with antimicrobial agents can prevent microbial contamination of food by minimizing both the growth rate and the maximum growth population. Additionally, prolonging the lag-phase of the main (target) microorganism or inactivating it by contact are other possible pathways that active films provide for food preservations (Yemiş et al., 2017). It is a fact that active packaging technology is a rapidly developing system, but more research is needed on the rheological and microbiological effects of these approaches on packaged foods, especially considering nutritional quality and human safety (Firouz et al., 2021).

This work attempts to investigate the effect of incorporating two different essential oils (clove oil and apricot oil) into whey protein isolate films. Mechanical, physical and structural properties of the modified films were evaluated and the films were applied to fresh Kaşar cheese samples by dipping methods to test their success on a food material. Microbial and structural changes of uncoated (control) and coated cheese samples stored at 4°C were monitored periodically for up to one month. Although there have been some studies in the literature on the characterization of edible films, there has been very limited research on food applications. This study will contribute to the literature by adding apricot and clove oils to the whey protein isolate film system for the first time and providing a solution for Kaşar cheese, where mold growth is a problem.

MATERIAL AND METHOD

Whey protein isolate (98% protein content) was purchased from Davisco International Inc. (Le Sueur, MN). Water was double distilled and the plasticizer glycerol (88% purity) was from Sigma-Aldrich (St. Louis, USA). Essential oils of clove and apricot were obtained from a local market in Gaziantep. Fresh Kaşar cheese samples were kindly provided from a local firm immediately after its production. All cheeses had the same production date and composition.

Experimental Studies

The study was performed within two sections. In the first section, WPI based edible films with essential oils (active films) and without essential oils (control and pure WPI films) were prepared, after that their physical properties were detected. The amount to be used was determined according to our preliminary studies, and since the use of extra emulsifying agents was not preferred, it was decided to use the minimum percentage of essential oil that gives good antimicrobial results. In the second section, the application of WPI films on a model system; Kaşar cheese was investigated.

Whey Protein isolate-based film preparation

Aqueous solution of 4% (w/w) WPI was produced by blending WPI powder in distilled water. Prepared mixtures were put in a 90°C water bath and kept for 30 minutes. Then, the mixtures were cooled to 24°C (room temperature), after that 7% (v/v) amount of glycerol was added. Clove and apricot essential oils were added with 4% (v/v) ratios as essential oil concentration per film solution (100 mL of solution contains 4 gr WPI, 7 mL glycerol and 4 mL essential oil). It was homogenized vigorously for 2 min at 20,000 rpm using a homogenizator (IKA T18 Ultra Turrax, Staufen, Germany). 15 mL of solutions were spread on 15 cm^2 glass plates and waited to dry under ambient conditions (23 ± 2°C and 40 ± 5% relative humidity) for 24 hours then dried film samples were peeled. Conditioning for the samples was done at 23 ± 2°C and 40 ± 5% relative humidity (Kaya and Kaya, 2000).

Film characterization

Texture Analyzer (Stable Microsystems, TA-XT2i model, Godalming, UK) was utilized to measure the strength and elasticity characterization of edible films (Erdem, 2023). Brightness were tested with the device of BYK Gardner Micro-gloss 45 and opacity was determined by Diffusion Systems (M57D) (Kaya and Kaya, 2000). Transmission infrared spectra of the films were evaluated by Perkin Elmer Spectrum 100 (Perkin Elmer Ltd, Beaconsfield, UK) (Rai and Poonia, 2019). Thermogravimetric analysis (TGA) was made with Perkin Elmer Precisely, TGA 4000. Samples for TGA measurements were prepared from several pieces cut from the films to a mass of about 6 mg and placed in capsules of the analyzer. Data were taken at temperatures between 20°C and 800°C. The heating rate was set at 10°C/min (Sukyai et al., 2018).

Application of WPI edible film on Kaşar cheese

To provide full covering of cheese samples with the edible films, dipping techniques were performed using WPI edible coating solutions which have the same composition of the films (Reyes-Avalos et al., 2016). WPI based coating solutions were used to coat Kaşar cheese samples. Fresh Kaşar cheese samples were sliced to the same width and length (2.5 cm x 2.5 cm) but 1.3 cm in height. The prepared cheeses were stuck on toothpicks and then coated by dipping method

(Figure 1). They are dipped into film solutions two times, each lasting 1 min. Ten set of samples were prepared, each set was formed with five uncoated, five WPI film coated, five A- WPI (WPI based film containing apricot essential oil) coated, five C-WPI (WPI based film containing clove essential oil) coated cheese samples were placed on each set. Then two sets were used for microbiological analysis for first day and last day as initial and final measurements. Weight loss of uncoated and coated samples was determined using one set of samples. Others even sets used for texture and rheological analysis after 1, 2, 3, 7, 14, 21 and 28 days of storage. The samples stored at 4°C during experiment.

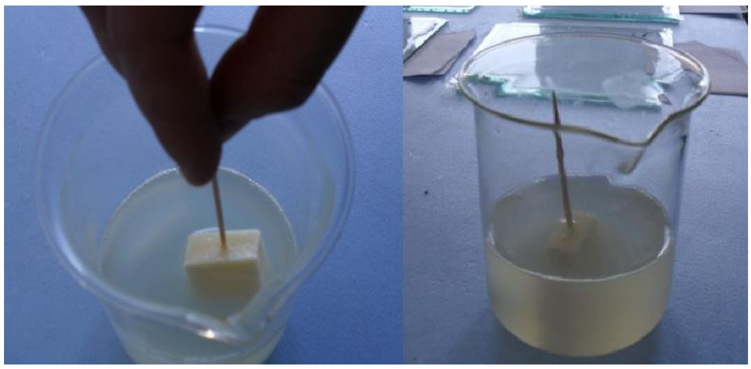

Figure 1. Application of dipping method for coating of cheese samples.

Microbiological analysis of uncoated and coated Kaşar cheese samples

The total microbial count of cheeses was tested by the way of standard plate count using spread technique (Nazim et al., 2013). PC agar was prepared for 20 petri dishes for four sample groups; uncoated cheeses, WPI coated cheeses, A-WPI and C-WPI coated cheeses. 25 g of each kind of cheese samples were mixed with 250 ml sterilized (120 ± 1°C for 20 min) 0.1% peptone water and homogenized for 20 minutes. 10^{-1} dilutions of samples were used during experiment. Petri dishes were incubated at 37°C during 2 days and observations were given as colony forming unit per gram of sample (cfu/g sample). This procedure was applied for initial and final measurements. Coated samples were waited one day to make them stable and initial measurement was made. Final measurement was made at 28th day.

Texture profile analysis (TPA) of Kaşar cheese samples

A texture analyzer model TA-XT2i (Stable Microsystems, UK) was used for measuring texture profiles of all of the cheese samples, uncoated (control) and coated (Erdem and Kaya, 2022). Measurements of coated samples were made on days 1, 2, 3, 7, 14, 21 and 28 of storage. Measurement conditions were adjusted as test speed 1 mm/s and 10% strain. Rectangular shaped probe was used with dimension 4 x 5cm. Temperature of texture analyzer was adjusted as 10°C. Temperature control was made by using Peltier apparatus XT/PP. This apparatus provides acceptable surface temperature to measure small and weak products like adhesive which sensitive to compression.

Determination of weight loss of uncoated and coated Kaşar cheese samples

After film solution dried on cheese samples the initial weights were obtained. Five samples of each C-WPI, A-WPI, and WPI coated and uncoated cheese were numbered and placed on refrigerator (4°C). Cheese samples were weighted periodically using a sensitive scale with a sensitivity of 0.0001g (XB220A, Precisa) (Reyes-Avalos et al., 2016).

Statistical Analysis

Statistical analysis were performed using analysis of variance (ANOVA) to examine the effect of essential oils on edible films and effect of these film types on cheese structure and microbial growth by using SPSS software (v.16.0.0). One way ANOVA was used to compare means of parameters and Duncan test was applied as post-Hoc test. The results were tabulated as the mean ± standard deviation (n = 5).

RESULTS AND DISCUSSION

Mechanical Properties

The maximum stress in an edible film can be determined by mechanical tensile strength testing. Tensile strength (TS) refers to the extent of film integration as well as the potential for heavy-duty-use. The percentage of elongation at break (EB) is an indicator of the stretching ability of the film (Erdem and Kaya, 2022). Mechanical property observations of the studied samples are given in Table 1. Neat WPI film showed the highest (1.87 MPa) and A-WPI had the lowest (1.01 MPa) TS values. The observation of lower TS for modified films could be attributed to reduced protein-protein interactions between polymer molecules due to the presence of the essential oils. It was determined that addition of both clove and apricot essential oils into the edible film matrix was found to increase EB (Table 1). The elastic modulus values of C-WPI and A-WPI films were significantly lower than neat WPI, so it is a fact that oil incorporation into film matrix had a more pronounced effect on this property than the tensile strength. McHugh and Krochta (1994) have investigated the properties of whey protein based films made of high glycerol content and reported statistically low tensile strength and high elongation at break (McHugh and Krochta, 1994). In a similar way, higher elasticity was observed in some previous studies with lipid incorporation into film matrix system (Erdem et al., 2019; Rai and Poonia, 2019). Essential oil addition may have resulted in development of a heterogeneous film structure due to non-uniform oil dispersion on the film surface and offering some discontinuities of the film structure. Therefore, this may have changed stretching ability of the film. Also, since clove and apricot essential oils used in the study are liquids at room conditions, they might be placed in the matrix in the form of easily deformable oil droplets and this might increase the extensibility of the film (Galus, 2018). This means used essential oils were bound with WPI and this provided increase in percent elongation of the films.

Table 1. Mechanical and optical properties of edible films.

Film Type	Tensile Strength (MPa)	Elongation at Break (%)	Elongation Modulus (MPa)	Brightness	Opacity
WPI	1.87 ± 0.12[a]	84.85 ± 7.21[a]	30.94 ± 2.20[a]	90.67 ± 2.18[a]	15.67 ± 2.90[a]
A-WPI	1.01 ± 0.07[b]	99.73 ± 13.43[c]	1.17 ± 0.39[c]	51.67 ± 1.31[c]	34.67 ± 7.21[c]
C-WPI	1.15 ± 0.07[b]	164.34 ± 24.84[b]	7.17 ± 1.05[b]	71.00 ± 1.21[b]	26.50 ± 1.50[b]

Values were given as mean ± standard deviations. Different superscript letters in the same column indicates statistical differences (Duncan's test, $\alpha \leq 0.05$), (WPI: whey protein isolate based edible films, C-WPI: clove essential oil incorporated whey protein isolate based edible films, A-WPI: apricot essential oil incorporated whey protein isolate based edible films.)

Optical Properties

Optical properties of samples were tabulated in Table 1. According to the results, it was understood that there was an inverse relation between brightness and opacity. WPI film had the highest brightness and transparent structure; however opacity increased with essential oil addition. Table 1 presented that A-WPI was found to be more opaque (34.67) than C-WPI (26.50). It has been reported that the heterogeneity and/or accumulation of oil particles promoted poor transparency, resulting in reduced UV light transmission in the film matrix (Erdem, 2023). It is important to note here that the WPI films contain two types of plasticizers, glycerol and water, as well as the addition of essential oils, making these systems quite complex from a structural and physicochemical perspective. Additionally, some incompatibility between the different film forming resources such as polysaccharide-lipid or protein-lipid could affect the amount of light passing through the film specimen and decreases the transparency. Actually, higher opacity could be a desired property for emulsion based edible films to lessen the oxidative degradation reactions and preserve the packed food (Galus, 2018).

Fourier Transforms Infrared Spectroscopy (FTIR)

FTIR spectroscopy was used to study the interaction between WPI film matrix and essential oils. The spectra of the WPI films with two different essential oils (clove and apricot) are presented in Figure 2. The absorption peak at around 3300 cm^{-1} showed –OH stretching vibration and the C–H stretching band at around 2950 cm^{-1} for film specimens. In the present study, the bend of amide I, amide II and amide III were determined at about 1630 cm^{-1}, 1530 cm^{-1} and 1240 cm^{-1}, respectively. The main components of the clove oil is eugenol, which contains phenolic hydroxyl and benzene ring. Besides, it contains tartaric acid and other substances. The bigger obtained peak at around 1380 cm^{-1} for C-WPI and A-WPI in the FTIR spectrum might correspond with the presence of deformation vibration of eugenol methyl. That indicated the existence of hydrophilic and hydrophobic interactions between the polymers in the film matrix after essential oil incorporation into protein matrix. It was understood that the addition of essential oil did not cause a significant change in natural protein characteristics, but slightly increased the hydrophobic property.

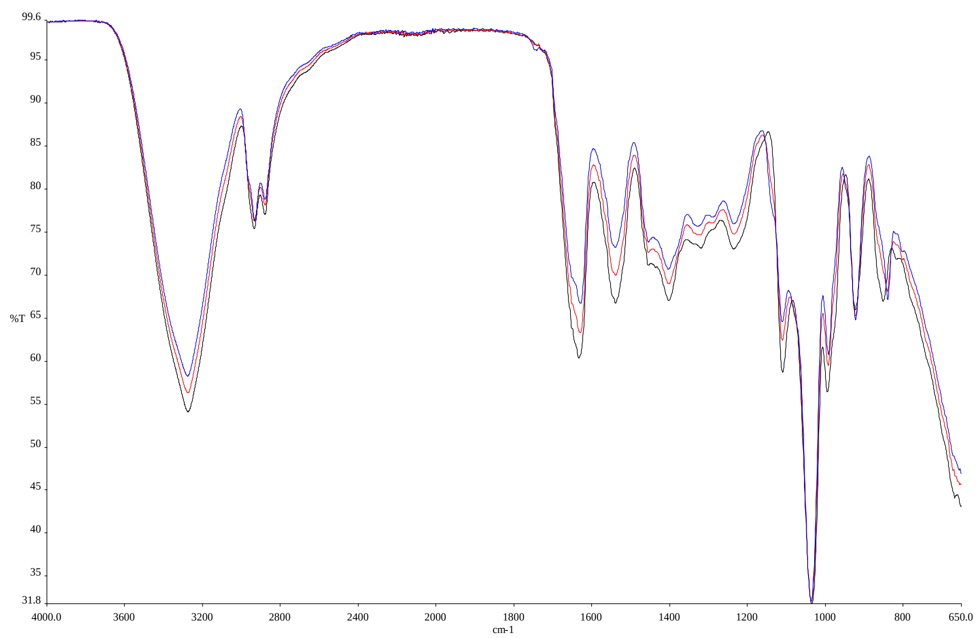

Figure 2. Fourier Transform Infrared (FTIR) spectra of films: WPI (black line), C-WPI (red line), A-WPI (blue line).

Thermogravimetric Analysis (TGA)

Thermogravimetric curves of the WPI, C-WPI and A-WPI films tested at a rate of 10°C min^{-1} were represented in Figure 3. All film types showed similar mass loss results, with two main phases. The 1st stage was observed up to 200°C and probably occurred due to loss of adsorbed and/or bound type water. The 2nd stage, which was related with degradation of protein, was investigated to observe behavior of the edible films with and without the presence of essential oils. Thermograms showed that the degradation temperature for all film types was between 150 and 200°C (Figure 3). According to results it was understood that essential oil addition slightly enhances the thermal stability of the films. According to the curve, while C-WPI had the highest decomposition onset temperature, A-WPI was found to be the second most thermally stable film. These thermogram results means that WPI polymer chain interaction positively affected from oil addition (Erdem and Kaya, 2021; Tavares and Noreña, 2019).

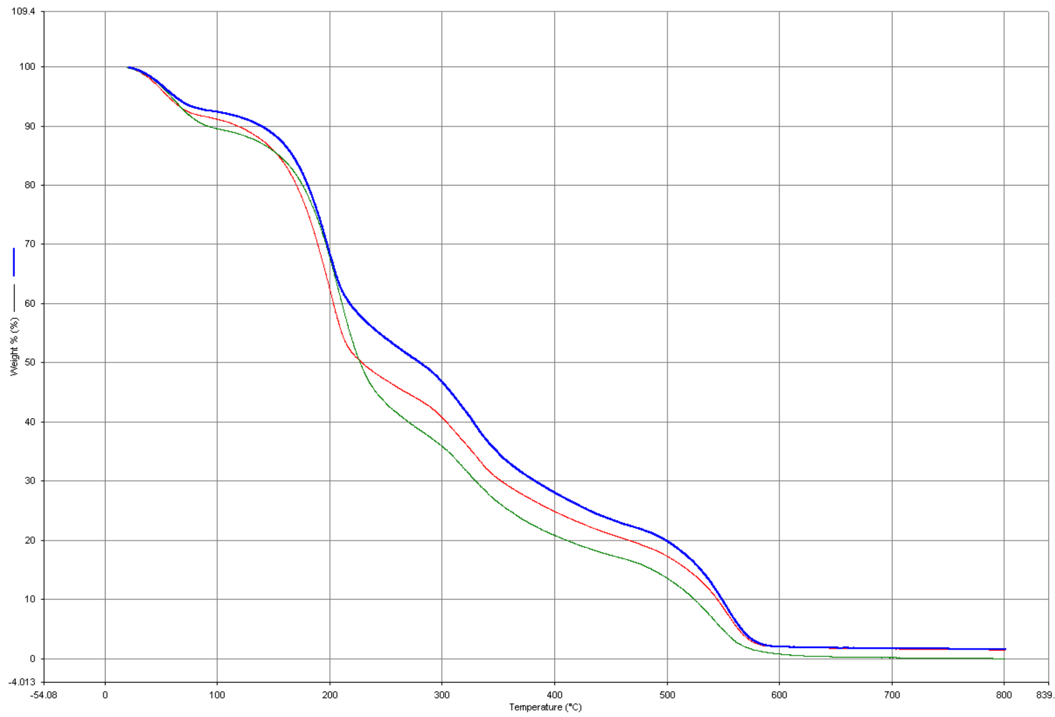

Figure 3. Thermogravimetic thermograms for WPI (red line), C-WPI (blue line) and A-WPI (green line) films.

Microbiological Analysis

The number of colonies of A-WPI, C-WPI, and WPI coated and control (uncoated) cheese specimens were counted and represented in Figure 4. The some of the treated and untreated samples were used to measure the initial microbial loads and the rest of the films were stored at refrigerator temperature (4°C) for five weeks to determine the effect of possible antimicrobial activity of the essential oils on total microorganisms. It is possible to state that using clove oil was very effective on eliminating microbial growth of the cheese samples. With the comparison of uncoated cheese microbial growth counts, C-WPI and A-WPI coating caused 98% and 37% reduction in growth of microorganisms. By the way, it is possible to say that each of the essential oils of apricot and clove oil can be used as an antimicrobial agent because it was determined that films containing clove and apricot essential oils has a significant effect to prevent growth of microorganisms ($p < 0.05$). It was understood that the best in preventing microbial growth in cheese was clove oil. There was smaller increasing in colony count of C-WPI and A-WPI coated cheese samples with respect to their initial count. However microbial growth rate of uncoated and WPI film coated cheese were closer to each other. The effectiveness of clove essential oil against microbial growth when in direct contact with the inoculated culture had already been reported by Goñi (2009) (Goñi et al., 2009). This inhibition ability of clove was due to high amount of euganol (G. Al-Hashimi et al., 2020). Erdoğan Orhan and Kartal (2011) were stated that the plant of *P. armeniaca* was a valuable inhibitory effect on tested microorganisms which was proved with the MIC values (31.25 and 500 µg/mL). In their study, the extracted material from the plant was butanol and Gram (+) bacteria (especially *Micrococcusluteus* (MIC 31.25 µg/mL) were more affected and inhibited from butanol extract (MIC values 31.25–250 µg/mL) (Erdoğan Orhan and Kartal, 2011).

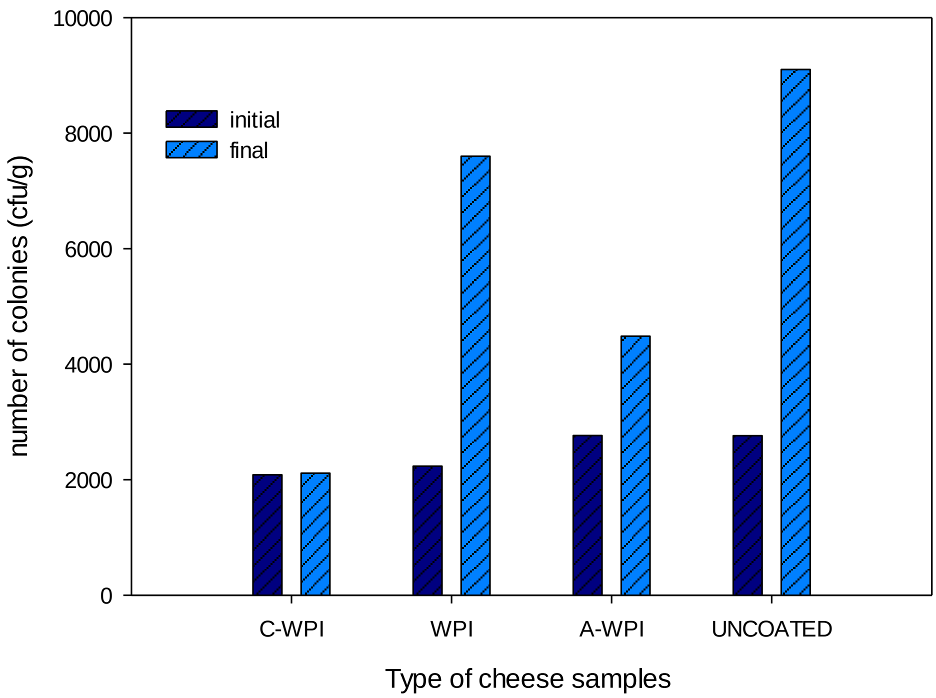

Figure 4. Comparison of colony count between initial and final measurements for all type of cheese samples.

Texture Profile Analysis

Texture Profile Analysis (TPA) test consists of compressing bite size pieces of food two times in a motion that simulates the action of jaw, and extracting from the resulting force time curve a number of textural parameters. The primary TPA parameters are hardness, cohesiveness, springiness, adhesiveness and gumminess of were tabulated in Tables 2-7. For all cheese samples studied, hardness values obtained from texture profile analysis were increased (Table 2). It has been known that hardness value of a cheese is increased with decreasing moisture contents (Demirhan and Cihangir, 2021). Moisture in the cheeses acts as a plasticizer and is another important factor determining the texture. Due to the hydrophilic nature of whey protein based edible films, they lost moisture and became harder than initial they are less effective moisture barriers. The adhesiveness or 'adhesive force' is defined as the work necessary to overcome the attractive forces between the surface of the food and the surface of other materials. It is measured as the negative area of work between the two compressions. The adhesiveness of the coated and uncoated Kaşar cheese samples were given in Table 3. As it can be seen, C-WPI and A-WPI film coated cheese samples show similar change. This result dedicates that addition of essential oils make film different from WPI film. Springiness can be measured using several ways, but most typically, by the distance of the detected height of the product on the second compression as divided by the original compression distance. There was an increasing trend in springiness for coated cheese samples (Table 4) containing especially essential oil of clove oil at the beginning of storage period, however after 14 days springiness declined. Uncoated sample did not show an important change during storage period. This result was in agreement with the texture profile analysis data as all the cheeses exhibited similar springiness values WPI and A-WPI coated cheese samples. Cohesiveness was evaluated by placing a sample between the molar teeth and estimating the amount of deformation before rupture. Cohesiveness, chewiness and gumminess values of all cheese samples at all studying time period were closer to each other (Table 5-7). Changes in cohesiveness, anything that changes the ability of the proteins to interact with water or other proteins can also influence cheese adhesiveness. According to the results obtained, it was understood that the texture parameters were affected by time, not the film types.

Table 2. Change in hardness (N) values for uncoated and WPI, C-WPI, and A-WPI coated cheese samples during different storage days.

Film Type	1st day	2nd day	3rd day	7th day	14th day	21th day	28th day
Uncoated	35.80±6.69^{a}	70.36±6.52^{a}	59.62±2.72^{a}	92.65±4.47^{a}	197.86±20.60^{a}	177.47±2.94^{a}	204.42±7.34^{a}
WPI	49.96±17.55^{b}	82.71±4.87^{b}	89.01±3.98^{b}	153.27±9.71^{b}	225.28±10.12^{b}	203.30±7.03^{ab}	182.72±8.74^{ab}
A-WPI	30.25±7.82^{a}	78.93±2.55^{ab}	95.44±3.96^{b}	134.97±6.78^{c}	221.44±21.13^{b}	214.91±19.27^{b}	169.35±9.07^{b}
C-WPI	57.26±12.65^{c}	77.98±4.42^{ab}	62.09±7.96^{a}	127.66±2.04^{c}	223.66±23.19^{b}	228.25±21.85^{b}	247.08±8.45^{c}

Different superscript letters in the same column indicates statistical differences (Duncan's test, $\alpha \leq 0.05$), (WPI: whey protein isolate based edible films, C-WPI: clove essential oil incorporated whey protein isolate based edible films, A-WPI: apricot essential oil incorporated whey protein isolate based edible films.)

Table 3. Change in adhesiveness values for uncoated and WPI, C-WPI, and A-WPI coated cheese samples during different storage days.

Film Type	1st day	2nd day	3rd day	7th day	14th day	21th day	28th day
Uncoated	-3.48±0.14^{a}	-0.58±0.09^{a}	-0.56±0.09^{a}	-1.86±0.41^{a}	-3.38±0.16^{a}	-1.04±0.25^{a}	-3.39±0.63^{a}
WPI	-0.98±0.06^{b}	-0.24±0.07^{b}	-0.25±0.11^{b}	-2.27±0.29^{b}	-3.28±0.45^{a}	-3.69±0.11^{b}	-1.04±0.17^{b}
A-WPI	-2.12±0.09^{c}	-0.12±0.01^{c}	-0.17±0.01^{c}	-1.04±0.28^{c}	-1.55±0.07^{b}	-2.28±0.37^{c}	-1.67±0.24^{c}
C-WPI	-2.43±0.18^{c}	-0.31±0.07^{d}	-0.34±0.05^{d}	-0.99±0.15^{c}	-3.74±0.27^{c}	-3.27±0.83^{d}	-4.13±0.54^{d}

Different superscript letters in the same column indicates statistical differences (Duncan's test, $\alpha \leq 0.05$), SE: Standard error, (WPI: whey protein isolate based edible films, C-WPI: clove essential oil incorporated whey protein isolate based edible films, A-WPI: apricot essential oil incorporated whey protein isolate based edible films.)

Table 4. Change in springiness values for uncoated and WPI, C-WPI, and A-WPI coated cheese samples during different storage days.

Film Type	1st day	2nd day	3rd day	7th day	14th day	21th day	28th day
Uncoated	0.90±0.02^{a}	0.92±0.01^{a}	0.88±0.02^{a}	0.84±0.02^{a}	0.91±0.09^{a}	0.84±0.03^{a}	0.89±0.02^{a}
WPI	0.90±0.01^{a}	0.92±0.01^{a}	0.88±0.02^{a}	0.90±0.01^{b}	1.09±0.06^{b}	0.89±0.02^{a}	0.89±0.03^{a}
A-WPI	0.92±0.02^{a}	0.91±0.02^{a}	0.91±0.01^{a}	0.89±0.05^{b}	1.05±0.03^{b}	0.93±0.02^{ab}	0.87±0.02^{a}
C-WPI	1.26±0.04^{b}	0.93±0.02^{a}	0.92±0.01^{a}	0.90±0.02^{b}	1.14±0.01^{b}	1.04±0.02^{b}	0.95±0.01^{b}

Different superscript letters in the same column indicates statistical differences (Duncan's test, $\alpha \leq 0.05$), SE: Standard error, (WPI: whey protein isolate based edible films, C-WPI: clove essential oil incorporated whey protein isolate based edible films, A-WPI: apricot essential oil incorporated whey protein isolate based edible films.)

Table 5. Change in cohesiveness values for uncoated and WPI, C-WPI, and A-WPI coated cheese samples during different storage days.

Film Type	1st day	2nd day	3rd day	7th day	14th day	21th day	28th day
Uncoated	0.85±0.02[a]	0.84±0.01[a]	0.83±0.01[a]	0.82±0.01[a]	0.89±0.02[a]	0.88±0.01[a]	0.89±0.02[a]
WPI	0.85±0.01[a]	0.82±0.03[a]	0.85±0.01[a]	0.87±0.01[a]	0.94±0.02[a]	0.90±0.01[a]	0.91±0.01[a]
A-WPI	0.85±0.01[a]	0.82±0.01[a]	0.84±0.01[a]	0.86±0.01[a]	0.92±0.03[a]	0.90±0.02[a]	0.88±0.01[a]
C-WPI	0.86±0.02[a]	0.83±0.25[a]	0.84±0.01[a]	0.86±0.01[a]	0.91±0.04[a]	0.90±0.02[a]	0.94±0.29[a]

Different superscript letters in the same column indicates statistical differences (Duncan's test, $\alpha \leq 0.05$), SE: Standard error, (WPI: whey protein isolate based edible films, C-WPI: clove essential oil incorporated whey protein isolate based edible films, A-WPI: apricot essential oil incorporated whey protein isolate based edible films.)

Table 6. Change in chewiness (N) values for uncoated and WPI, C-WPI, and A-WPI coated cheese samples during different storage days.

Film Type	1st day	2nd day	3rd day	7th day	14th day	21th day	28th day
Uncoated	31.51±5.75[a]	53.93±5.01[a]	45.70±2.16[a]	61.69±4.42[a]	174.64±21.17[a]	135.06±5.24[a]	161.61±9.46[a]
WPI	38.78±13.30[b]	62.63±4.03[b]	67.13±4.47[bc]	120.80±10.18[b]	265.21±44.01[b]	156.16±8.04[ab]	147.79±11.29[b]
A-WPI	24.75±7.05[c]	58.91±2.48[ab]	71.89±3.06[b]	122.62±10.16[b]	431.33±98.11[c]	166.50±8.42[b]	129.17±6.27[c]
C-WPI	68.59±26.11[d]	60.26±2.31[ab]	60.19±6.55[c]	102.81±3.41[c]	256.28±37.91[b]	283.80±91.02[c]	221.32±10.83[d]

Different superscript letters in the same column indicates statistical differences (Duncan's test, $\alpha \leq 0.05$), SE: Standard error, (WPI: whey protein isolate based edible films, C-WPI: clove essential oil incorporated whey protein isolate based edible films, A-WPI: apricot essential oil incorporated whey protein isolate based edible films.)

Table 7. Change in gumminess (N) values for uncoated and WPI, C-WPI, and A-WPI coated cheese samples during different storage days.

Film Type	1st day	2nd day	3rd day	7th day	14th day	21th day	28th day
Uncoated	30.09±6.03[a]	58.79±5.47[a]	49.96±2.32[a]	75.38±4.85[a]	179.40±22.55[a]	152.24±5.45[a]	181.52±9.07[a]
WPI	42.43±14.50[b]	68.19±4.29[b]	75.95±4.08[b]	133.97±9.75[b]	221.82±11.86[b]	176.97±5.66[b]	165.96±9.49[b]
A-WPI	26.23±7.07[a]	64.86±2.69[ab]	79.33±3.36[b]	102.94±7.79[c]	211.37±23.04[b]	207.90±21.76[c]	148.75±7.89[c]
C-WPI	49.61±10.56[c]	64.86±3.54[ab]	66.23±7.76[c]	114.50±4.16[bc]	234.57±11.48[b]	177.27±19.19[b]	232.45±8.69[d]

Different superscript letters in the same column indicates statistical differences (Duncan's test, $\alpha \leq 0.05$), SE: Standard error, (WPI: whey protein isolate based edible films, C-WPI: clove essential oil incorporated whey protein isolate based edible films, A-WPI: apricot essential oil incorporated whey protein isolate based edible films.)

Weight Change

Figure 5 shows the weight loss of coated and uncoated cheese samples measured during the storage periods of samples stored at 4°C. Weight of samples was decreased sharply within a week no matter they were uncoated or coated with WPI, C-WPI and A-WPI films. Then, losing moisture of the samples was continuous slowly up to 28 days storage without any important difference by applying coating types to the cheese samples. Similar results were found by Perez-Gago et al. (2005) who coated fresh apple pieces with the emulsion coatings from whey protein isolate, whey protein concentrate or hydroxypropyl methylcellulose as the hydrophilic phase, and beeswax or carnauba wax as the lipid phase, and weight loss were measured during storage. Their results showed that coating application did not reduce weight loss in fresh-cut apples (Perez-Gago et al., 2005). In another study, protein-sunflower oil based coating application showed that effective protection on textural structure of cake by high moisture preservation ability. The authors reported that after three day storage while uncoated cake sample had the highest firmness value (115.02 N), the cake coated with SPI-oil had the lowest (41.15 N) and this value was the closest one to the control cake (21.57 N) (Erdem and Kaya, 2021). The results obtained in this previous study proved that the coating application, especially with the increase of sunflower oil in the coating formulation, helped prevent moisture loss in the cake samples.

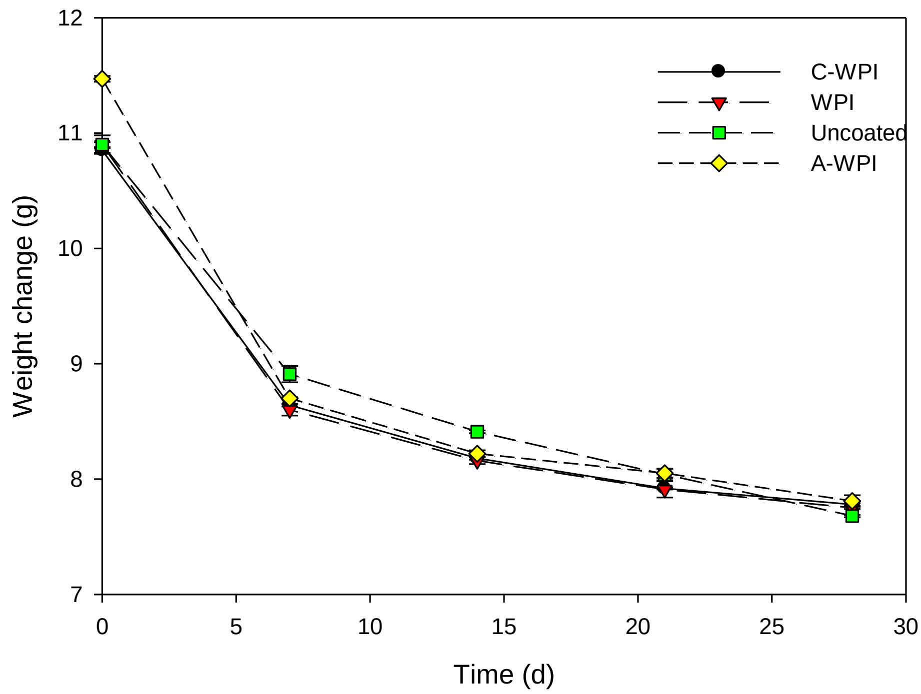

Figure 5. Weight change of WPI, C-WPI, A-WPI and uncoated cheese samples during storage.

CONCLUSION

In the present study, the WPI film solutions were enriched with different essential oils was used to produce bioactive edible films and its application on Kaşar cheese was investigated. Film textural properties revealed that more elastic films were obtained with the oils addition and FTIR spectrum and optical results showed that hydroscopicity of the WPI film was increased. With the incorporation of clove oil thermal stability was improved but a negligible change occurred in the TPA of the Kaşar specimens. In this study, good antimicrobial activities were detected for both essential oils in the WPI film matrix system, but clove oil had by far higher microbial count reduction results than apricot. Weight change and/or moisture loss showed the same trend in all coated and uncoated cheese samples. In conclusion, both of the C-WPI and A-WPI film samples produced in this study can be used as active packaging systems to delay the antimicrobial reactions in the food products. However, future studies were needed to ensure that the packaged cheese also had better textural properties.

DECLERATION OF INTEREST

The authors declared that there is no conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Yeşil Işık ERDEM: Formal analysis, Investigation, Validation. Sevim KAYA (Corresponding author)**:** Project administration, Conceptualization, Writing-Reviewing and Editing. Burcu GÖKKAYA ERDEM: Writing-Original draft preparation, Writing-Reviewing and Editing, Statistical Analysis.

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