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Use of egg white protein powder based films fortified with sage and lemon balm essential oils in the storage of lor cheese

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Abstract

Edible film was produced by adding 3 % sorbitol (w/v) to egg white protein powder (EWPP). The first group of *lor* cheese samples was coated with a film fortified by sage essential oil (SEO) and the second group of samples was coated with films enriched by adding lemon balm essential oil (BEO) at various concentrations [0.5 %, 1 %, 2 % (v/v)]. The films were labeled as $EWPP_{SEO(0.5)}$, $EWPP_{SEO(1)}$, $EWPP_{SEO(2)}$, $EWPP_{BEO(0.5)}$, $EWPP_{BEO(1)}$, $EWPP_{BEO(2)}$ to indicate the type and the concentration of the additive. The third batch of the lor cheese samples was coated exclusively with non-fortified EWPP and the fourth batch was uncoated. All of the cheese samples were artificially contaminated with *Escherichia coli* O157:H7 (*E. coli* O157:H7), *Listeria monocytogenes* (*L. monocytogenes*) and *Staphylococcus aureus* (*S. auerus*). Viable cell counts of these species, yeasts and moulds were determined after the cheese production. All the samples were stored at +4 °C. Their physicochemical and microbiological properties were examined on the 1st, 7th, 15th and 30th day of the storage. Thereat significant ($P<0.05$) relationships between the increase in the essential oil concentrations and the increase in film thickness, water vapor permeability, inner and outer hardness, decrease in the weight loss, improvement in fat barrier property, and microbial counts during storage were found. These properties were found to be significantly affected in the 2 % (v/v) SEO and BEO samples, while the effects of other additive concentrations were insignificant ($P>0.05$). Physicochemical and antibacterial properties were more significant in SEO at all concentrations compared to BEO. However, the antifungal effect of BEO was higher than that of SEO. The antifungal effect of BEO was the same at 1 % (v/v) and 2 % (v/v) concentrations. *E. coli* O157:H7 was the most resistant microorganism to the essential oils while *L. monocytogenes* was the most sensitive. EWPP showed a bacteriostatic effect on the microorganisms and bactericidal effects were determined on the 30th day of the storage against *L. monocytogenes* and yeast-moulds.

Key words: egg white protein, sage, lemon balm, essential oils, lor cheese, storage

Introduction

In Turkey, whey is used in the production of lor cheese, which has several different local names such as basket lor, herbed lor, şor lor, Tire mud cheese, and sırvatka lor (Kırdar, 2009). In lor cheese production, serum proteins are boiled for precipitation, kept for 24 hours and filtered through fine-mesh cloth for 1-4 days. When the desired consistency is attained, 2-3 % salt is added and the product is consumed fresh without the fermentation and ripening process. The natural microbiological flora is destroyed by the heating process applied during production why post-production contaminations represent a risk for the product. Consequently, shelf-life

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of lor cheese is short. Lioliou et al. (2001) reported that microbiological load increased and shelf-life decreased during storage in cheeses produced from whey using traditional methods. The microflora of lor cheese changes even at cold storage and microbiological load increases in terms of *Enterobacteria*, yeasts-moulds, *E. coli* and *Staphylococcus* species. Therefore, in order to avoid deterioration of cheeses made from whey, various methods were employed including irradiation (Tsiotsias et al., 2002), coating with edible films (Kavas and Kavas, 2014), etc.

Egg white protein (EWP) mainly consists of the proteins ovalbumin (54 %), ovotransferrin (12 %), ovomucoid (11 %), lysozyme (3.5 %) and ovomucin (3.5 %), and the minor proteins avidin (0.05 %), cystatin (0.05 %), ovomacroglobulin (0.5 %), ovoflavoprotein (0.8 %), ovoglycoprotein (1.0 %) and ovoinhibitor (1.5 %) (Kovacs-Nolan et al., 2005). EWP has functional properties including nutritional functions (Abeyrathne et al., 2013), health (Omana et al., 2010) and antimicrobial and antiviral effects. The antimicrobial and antiviral effects are associated to lysozyme (Wan et al., 2006), ovomucin (Omana et al., 2010), ovomucoid which is a good trypsin inhibitor (Oliveira et al., 2009), ovoinhibitors, ficinpapain (enzyme inhibitors), antioxidant ovotransferrin (OTf) (Wu and Acero-Lopez, 2012), bioactive peptides (Zhang et al., 2011), avitin (biotin-binding), and an alkaline pH which limits the bacterial growth (approximately between pH 7.5-8; Banwart, 1983). In previous studies on EWP, it has been reported that edible films produced by using EW ovalbumin and lysozyme together provided a packaging material with high antimicrobial properties. Different edible film formulations were developed by using EWP with different biopolymers, mechanical and water vapor permeability properties of the various produced films have been examined (Gennadios et al., 1996).

Edible films are generally produced with biologically hydrophilic materials including protein, starch, pectin, cellulose, alginates and carrageenan (Taqi et al., 2011). Edible films obtained exclusively from biopolymers (carbohydrate and protein based) have weak mechanical properties, are brittle and can crack during the drying stage (McHugh and Krochta 1994). These problems could be solved by adding plasticizers to the film composition such as glycerol, propylene glycol, sorbitol or polyethylene glycol (Coupland et al., 2000; Dutta et al., 2009). Sorbitol (S) is an important plasticizer due to its lower moisture absorption and 100 % dissolution capabilities (Krochta and De Mulder-Johnston, 1997; Ressouany et al. 1998). With the addition of different essential oils to protein based films, composite films with good water vapor barrier properties could be produced (Gennadios et al., 1998; Hanani et al., 2013; McHugh et al., 1994; Shellhammer and Krochta, 1997). Essential oils, which have gained attention in recent years due to their antimicrobial activities, are frequently used to control the growth of pathogenic bacteria and degradation in foods (Joerger, 2007; Zivanovic et al., 2005).

Sage (*Salvia officinalis* L.) and lemon balm (*Melissa officinalis* L.) are aromatic plants belonging to the Lamiaceae (L.) family. Sage (*Salvia officinalis* L.) is a plant known for its antioxidant and antimicrobial properties (Bozin et al., 2007). Essential oil (SEO) obtained from the leaves of *Salvia officinalis* L. has antibacterial and antifungal effects due to its α-thujon, β-thujon, borneol, 1,8- sineol and α-pinen contents (Bozin et al., 2007; Tajkarimi et al., 2010). *Melissa officinalis* L. is rich in flavonoids (cynaroside, kosmoziin, ramnocytrin, isoquercetin, ursolic and oleanolic acid, caffeic and rosmarinic acid) and essential oils (α-pinene, β-pinene, linalool, citronellal, borneol, neral, geraniol and geranial) (Herodez et al., 2003). *Melissa officinalis* L. essential oil (BEO) is known for its antibacterial (Dukic et al., 2004), strong antifungal and antiviral (Allahverdiyer et al., 2004) properties.

There are few studies on coating lor cheese with protein based edible films and extending its shelf-life. Additionally, there are no studies found in the literature on coating lor cheese with EWPP based film and the determination of the effects of this film on some properties of the cheese. To address this deficiency, this study aimed to investigate the effect of coating lor cheese with edible films obtained by the fortification of S+EWPP based film with sage and lemon balm essential oils at different concentrations [0.5 % (v/v); 1 % (v/v); 2 % (v/v)] on the extension of shelf-life. Also, it was aimed to determine the effects of the film coatings on the physicochemical properties of lor cheese and specifically the effects of using two antimicrobial essential oils incorporated into the film against *Escherichia coli* O157:H7 *(E. coli* O157:H7*)*, *Listeria monocytogenes (L. monocytogenes)*, *Staphylococcus aureus (S. aureus)* and yeasts-moulds.

Materials and methods

Egg white protein powder (EWPP) and D-sorbitol

For the preparation of coating material, Alfasol® egg white protein powder (EWPP) (pH 7.00; total microorganisms <100 cfu/g; Coliform <10 cfu/g; *S. aureus* and Salmonella content: none and humidity ratio 7.10 %) was obtained from Kimbiotek Chemical Agents Inc. (İstanbul-Turkey) while D-sorbitol (S1876) was obtained from Sigma-Aldrich.

Essential oils

Essential oils were obtained from sage (*Salvia officinalis* L.) leaves (SEO) which were purchased from flora Yenişarbademli plateau (Isparta-Turkey). Lemon balm (*Melissa officinalis* L.) fresh leaves were used to obtain the essential oils (BEO) and were purchased from flora Aegean region (Turkey). Depending on the amount of the plant material available, SEO and BEO were obtained by a 3 hours long hydro-destillation using a Clevenger-type apparatus (Bounatirou et al., 2007). SEO (3.83 pH) containing 1,8 Cineole 40.6 %, α-thujon 22.56 %, β-thujon 12.37 %, borneol (α-terpinol) 9.6 %, α-pinen 3.8 %, β-pinen 1.8 %, camphene 1.3 %, limonen 1.2 %, carvacrol 0.7 %, and linalool 0.3 % was obtained from *Salvia officinalis* L. species. BEO (4.12 pH) containing gerenial 38.20 %, neral 30.59 %, citronellal 16.14 %, geranyl acetate 6.15 %, 3-Octanone 1.9 %, methyl geranat 0.4 %, linalool 0.3 %, menthol 0.14 %, α-pinen 0.11 %, β-pinen 0.08 % and limonen 0.05 % and was obtained from *Melissa officinalis* L. species. The oils used were those of *Salvia officinalis* L. and *Melissa officinalis* L., and the active components were obtained from Sigma-Aldrich (Steinheim, Germany). The active substances of the essential oils were determined by a Shimadzu GC-9A Model gas chromatograph equipped with Thermon-600T.

GC analysis of essential oils and volatile compounds

GC analyses were carried out on a Shimadzu GC-9A gas chromatograph equipped with Thermon-600 T (30 m × 0.25 mm × 0.25 μm film thickness). The oven temperature was programmed at 15-200 °C/min for the total of 15 minutes. Other operating conditions were: carrier gas, nitrogen with a flow rate of 10.0 mL/min; injector and detector temperatures were 250 °C and 300 °C, respectively; split ratio 1:20; column pressure 56.8 h Pa.

Lor cheese

Whey obtained during kashar cheese production was taken into a stainless steel tank and heated to 85 °C. During heating 2 % salt was added. The process was continued until the formation of curd. The product formed on the surface of the tank after the heating process was taken to a filtration tank and filtered in cold temperature (+4 °C) through mesh cloth for 2 days. Pressure was applied to the product to remove the excess whey content in the curd. The lor cheese was divided into 8 batches. The first batch was the control sample (K) (Figure 1B) and the 2nd batch was coated with EWPP based film (Figure 1C). Other batches were coated with SEO fortified ($EWPP_{SEO(0.5);}$ $EWPP_{SEO(1);}$ $EWPP_{SEO(2)}$) (Figure 1D) and BEO fortified ($EWPP_{BEO(0.5);}$ $EWPP_{BEO(1);}$ $EWPP_{BEO(2)}$) films (Figure 1E).

Preparation of edible film solution

Edible films were prepared according to Pintado et al. (2010) and Mchugh and Krochta (1994), with some modifications. Accordingly, 5 % w/v egg white protein powder (EWPP) was dissolved in pure water, 3 % w/v sorbitol (S) was added to the solution and the solution was centrifuged at 20,000 rpm for 1 min (3-16 K Type-Model, Sigma, Germany). pH of the homogenized mixture was adjusted to 8 and kept in a water bath at 45±2 °C for 30 min in order to improve the mechanical properties of the film solution and maintain the protein denaturation. The solution was then cooled to room temperature. The cooled solution was filtered and divided into six equal parts; sage essential oil (SEO) was added to the first three parts in different concentrations [0.5 % (v/v) $EWPP_{SEO(0.5)}$; 1 % (v/v) $EWPP_{SEO(1)}$ and 2 % (v/v) $EWPP_{SEO(2)}$]. Lemon balm essential oil (BEO) was added to the second parts in different concentrations [0.5 % (v/v) $EWPP_{BEO(0.5)}$; 1 % (v/v) $EWPP_{BEO(1)}$ and 2 % (v/v) $EWPP_{BEO(2)}$]. Following the sage and lemon balm essential oil addition, in order to maintain the homogeneous distribution of oil in the solution, Tween 20 (0.5 % (v/v)) was added (Zivanovic et al., 2005) and the solution was centrifuged once again at 20,000 rpm for 1 minute (3-16 K Type-Model, Sigma, Germany (Torlak and Nizamoglu, 2011; Table 2).

Preparation and storage of samples

E. coli O157:H7 (ATCC 43895), *L. monocytogenes* (ATCC 19118) and *S. aureus* (ATCC 6538) strains used for the artificial contamination of lor cheese samples were obtained from Hemakim Corporation (Turkey). Yeast-mold enumeration was carried out immediately after the cheese production. For the artificial contamination, 10^6 cfu/g (6 Log cfu/g) inoculum of *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* were used. In order to maintain the artificial contamination, lor cheese samples were divided into 50 g portions and immersed in *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* inoculum separately. Cheese samples were kept in each inoculum for 15 min for contamination and bacterial adhesion. Artificially contaminated cheese samples and the samples prepared for yeast and mold enumerations were coated with films by immersing in film solutions containing sage and lemon balm essential oils at different concentrations which were prepared as explained above. Accordingly, lor cheese samples were immersed in film solutions for 90 s, removed, hold 3 min, immersed again in a film solution for 60 s and removed. Following the immersion process, cheese samples which were coated with EWPP (Figure 1C), $EWPP_{SEO}$ (Figure 1D) and $EWPP_{BEO}$ (Figure 1E) based films were left to dry at 10 °C for 4-5 h. Control (K) samples which were not coated with films were stored at 4±1 °C following the artificial contamination. The prepared samples were stored at 4±1 °C for 30 days and *E. coli* O157: H7, *L. monocytogenes*, *S. aureus* and yeast-mold counts of samples were calculated as Log_{10} cfu/g on the 1st, 7th, 15th and 30th day of the storage.

Physical - chemical analysis

Weight loss percentages of lor cheese samples during storage were determined gravimetrically. pH values were examined with a SS-3 Zeromatic pH meter (Beckman Instruments Inc., California,

Table 1. Preparation of edible films solution

Egg White protein powder (% 5 w/v) solution
↓
Sorbitol addition (% 3 w/v)
↓
Centrifugation (20,000 rpm/1 min)
↓
pH adjustment (pH=8)
↓
Heating (45±2 °C/30 min)
↓
Cooling (room temperature)
↓
Filtering
↓
Essential sage and lemon balm oils addition (0.5 %; 1 %; % (v/v))
↓
Tween 20 (0.5 % (v/v)) addition
↓
Centrifugation (20,000 rpm/1 min)
↓
Sage and lemon balm essential oils fortified edible films

Table 2. Preparation and storage of samples

Lor Cheese (50 g)
↓
Artificial contamination (10^6 cfu/g)
↓
Immersion into inoculum (Hold for 15 minutes)
↓
First Immersion into film solution (90 seconds)
↓
Hold (for 3 minutes)
↓
Second Immersion into film solution (60 seconds)
↓
Drying (10 °C/4-5 hours)
↓
Storage (4±1 °C/30 day)

USA). Acidity (°SH) and fat content (%) were analysed according to AOAC (2000). The inner-outer hardness was determined at 3±1 ºC with a penetrometer (4500 CT3 texture analyser Brookfield Made in USA). Film thicknesses were measured with a micrometer at 0.005 precision (Digimatic Micrometer/Japan). Water vapor permeability of films was determined using ASTM E96-80 (1983) method gravimetrically at 25 °C. Vapor permeability was calculated by finding the slope of weight-time line and substituting it in the following formula:

$$\text{Slope} = \frac{P^*A^*\Delta p}{x}$$

Where: P: Permeability (g mm m^{-2} h^{-1} kPa^{-1}); A: Surface area (m^2); Δp: Partial pressure difference of the gases (kPa); x: Film thickness (mm)

Microbiological analysis

E. coli O157:H7 was enriched in selective modified EC Broth at 35-37 °C'de for 24-48 h. For enumeration of *E. coli* O157:H7 Sorbitol MacConkey Agar containing Cefixime-Tellurite Supplement was used and incubated at 35-37 °C for 24-48 h. After incubation, sorbitol negative colonies were counted. *L. monocytogenes* was enriched in Listeria selective Enrichment Broth at 30 °C'de for 24 hours. For enumeration of *L. monocytogenes*, Palcam Listeria Selective Agar (Base) was inoculated and incubated at 37 °C for 48 hours. *S. aureus* was enriched in Brain Hearth Infusion Broth at 37 °C'de for 48 hours. Five percentage of Egg Yolk Tellurite emulsion was added to Baird Parker Agar and incubating under aerobic conditions at 35-37 °C for 24-48 hours. Then, colonies were counted (Food and Drug Administration, 2001). For yeast and mold enumeration, Yeast-extract-glucose chloramphenicol agar (YGC) (Merck 1.16000) was used with incubation at 25 °C for 3-5 days (IDF Standard 94 B, 1990).

Statistical evaluation

Five different cheese samples were examined with 3 parallels and 2 repetitions. For this purpose, SPPS version 15 statistical analysis package software was used. Data significance as a result of analysis of variance (ANOVA) were tested according to the Duncan multiple comparison test at $p<0.05$ level.

Results and discussion

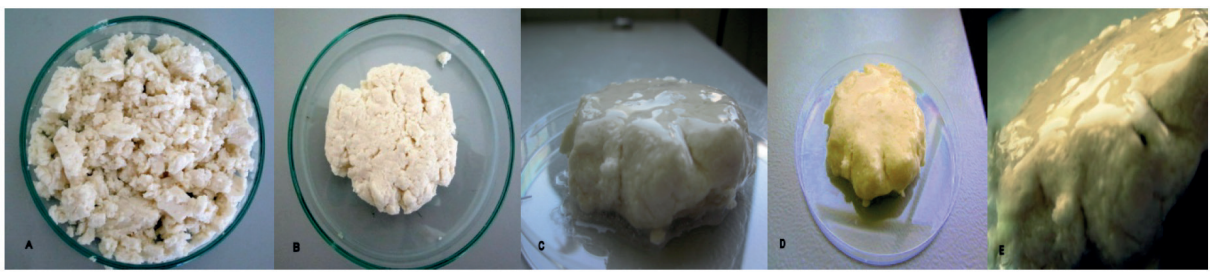
Physical-chemical properties

The composition of the lor cheese (Figure 1A) was pH 5.71, dry matter 41.2 %, fat 19.24 % and protein 18.11 %.

Thickness and water vapor permeability values of $EWPP_{SEO(0.5)}$; $EWPP_{SEO(1)}$; $EWPP_{SEO(2)}$; $EWPP_{BEO(0.5)}$; $EWPP_{BEO(1)}$ and $EWPP_{BEO(2)}$ obtained by adding SEO or BEO to EWPP are given in Table 3. Film thickness values obtained by fortification of EWPP based film with SEO or BEO at 0.5 % (v/v) and 1 % (v/v) were all similar, although the thickness of the film obtained by SEO addition was higher than those obtained by BEO addition. Taqi et al. (2011) reported that the film thickness values increased in white albumin based films with the addition of olive oil and oleic acid in a dose-dependent manner. Additionally, it was reported that film thickness increased and water vapor permeability decreased in edible films containing hydrophobic agents such as wax and vegetable oils (Greener and Fennema 1989; Kester and Fennema, 1986; Kamontip and Adisak, 2001).

Trends in the film thicknesses were similar to those of water vapour permeability. Accordingly, water vapour permeability of the samples obtained by coating with edible films fortified with SEO or BEO at different concentrations were lower than those coated with EWPP based film without fortification. Water vapour permeability of the samples

Figure 1. (A): Uncoated lor cheese (B): Uncoated formed lor cheese (K) (C): Coated lor cheese with EWPP (D): coated lor cheese with $EWPP_{SEO(2)}$; (E): coated lor cheese with $EWPP_{BEO(2)}$



obtained by coating with edible films prepared with the addition of SEO or BEO at 0.5 % (v/v) and 1 % (v/v) were close to each other. The relationship between the essential oil type and water vapor permeability in these concentrations was not significant (P>0.05). However, water vapor permeability of film obtained with 2 % (v/v) SEO addition to was significantly lower than that of BEO (P<0.05). Additionally, increasing the concentration of SEO and BEO to the film improved the water barrier property. This result was consistent with previous reports on edible films fortified with different fatty acids (konjac flour, fatty acid, stearic acid, oleic acid and olive oil) (Kamontip and Adisak, 2001; Kamper and Fennema, 1984; Park et al., 1994; Taqi et al., 2011). The results obtained in this study regarding film thickness and water vapour permeability were consistent with those reporting that EWP with the addition of various herbal ingredients can be used in the preservation of foods.

Values of inner and outer hardness of the samples coated with edible films fortified with different concentrations of SEO or BEO were lower for all concentrations compared to those coated with non-fortified EWPP (Table 4). Hardness values of K sample (uncoated) were higher than those determined for film coated samples. The relationship between essential oil concentration/type and inner-outer hardness values was significant (P<0.05). Accordingly, inner-outer hardness values were lowest in samples coated with films containing 2 % (v/v) SEO or BEO. Additionally, inner-outer hardness values of films with different concentrations of SEO were lower than those with BEO.

Regarding the storage period, differences between the weight losses in samples were not significant (P>0.05). Weight loss values of the samples coated with edible films fortified with different concentrations of SEO or BEO were lower compared to those coated with non-fortified EWPP (Table 4). Regarding weight losses, the difference between the film coated samples and non-coated control sample was significant (P<0.05). Water barrier properties increased with the addition of both essential oils in a dose-dependent manner. Overall, it could be observed that EWPP based film constituted a good water barrier and that this property increased along with the concentration of essential oil (SEO or BEO) added to the film. Sarıoglu and Oner (2006) reported that film coating caused a decrease in the inner and outer hardness of cheese samples. Additionally, many studies already reported that film coating prevented water vapour transmission and decreased weight losses (Krochta and De Mulder-Johnson, 1997; Sarıoglu and Oner, 2006). Further, although protein based films were highly permeable to water which usually leads to weight loss, using protein based film together with lipids was reported to be effective for the prevention of weight losses (Koyuncu and Savran, 2002).

Fat levels increased on the 1st day of the storage with the addition of SEO and BEO to EWPP based film at different concentrations. The relationship between 0.5 % (v/v) and 1 % (v/v) essential oil addition and the increase in fat content was not significant (P>0.05), although the relationship between 2 % (v/v) essential oil addition and the increase in fat content was significant (P<0.05).

Table 3. Film thicknesses and water vapor permeability of EWPP, $EWPP_{SEO(0.5)}$, $EWPP_{SEO(1)}$, $EWPP_{SEO(2)}$, $EWPP_{BEO(0.5)}$, $EWPP_{BEO(1)}$ and $EWPP_{BEO(2)}$ based films

Samples	Thickness/mm ±б	Water vapor permeability (g mm m^{-2} h^{-1} kPa^{-1})
EWPP	0.178 ± 0.004^{A}	7.66 g mm m^{-2} h^{-1} kPa^{-1A}
$EWPP_{SEO(0.5)}$	0.179 ± 0.009^{B}	7.64 g mm m^{-2} h^{-1} kPa^{-1B}
$EWPP_{SEO(1)}$	0.181 ± 0.002^{B}	7.63 g mm m^{-2} h^{-1} kPa^{-1B}
$EWPP_{SEO(2)}$	0.186 ± 0.006^{B}	7.55 g mm m^{-2} h^{-1} kPa^{-1B}
$EWPP_{BEO(0.5)}$	0.179 ± 0.008^{C}	7.65 g mm m^{-2} h^{-1} kPa^{-1C}
$EWPP_{BEO(1)}$	0.181 ± 0.009^{C}	7.64 g mm m^{-2} h^{-1} kPa^{-1C}
$EWPP_{BEO(2)}$	0.183 ± 0.010^{C}	7.58 g mm m^{-2} h^{-1} kPa^{-1C}

б: Standard deviation (n=3)
A,B,C, The differences between the values in the same column are statistically significant (p<0.05)

Table 4. Changes in weight loss (%) and inner-outer hardness values of samples coated with different films and non-coated samples (p<0.05), (n=3)

	Storage time (day)	K±6	EWPP±6	$EWPP_{SEO(0.5)}$ ±6	$EWPP_{SEO(1)}$ ±6	$EWPP_{SEO(2)}$ ±6	$EWPP_{BEO(0.5)}$ ±6	$EWPP_{BEO(1)}$ ±6	$EWPP_{BEO(2)}$ ±6
Outer hardness (g)	1st	38.14±0.19^{aA}	27.11±0.36^{bA}	25.42±0.21^{cA}	23.30±0.81^{dA}	21.14±0.35^{eA}	25.89±1.43^{cA}	24.11±1.28^{gA}	23.05±0.29^{dA}
	7th	73.21±1.15^{aA}	60.02±1.41^{bB}	56.33±0.39^{cA}	55.21±0.62^{dB}	51.32±0.48^{eA}	56.92±1.09^{cA}	57.41±1.61^{fB}	55.83±0.71^{dA}
	15th	86.21±0.35^{aA}	74.89±2.11^{bB}	70.17±0.15^{cA}	68.16±0.54^{dB}	64.71±1.43^{eA}	70.88±0.84^{cA}	69.23±0.94^{dB}	66.11±0.48^{eA}
	30th	163.84±0.43^{aB}	127.43±0.78^{bB}	122.73±0.09^{cB}	119.21±0.19^{dB}	114.56±2.13^{eB}	123.11±0.47^{fB}	121.32±0.51^{gB}	118.21±0.12^{hB}
Inner hardness (g)	1st	24.15±0.14^{aA}	22.21±0.34^{bA}	21.51±0.11^{cA}	21.09±0.41^{c}	19.15±0.83^{dA}	22.05±0.43^{e}	21.81±1.43^{c}	20.84±0.14^{fA}
	7th	36.56±0.56^{aA}	26.45±1.25^{bA}	25.32±0.45^{cA}	24.89±0.46^{d}	22.53±0.71^{eA}	25.88±0.33^{c}	25.23±0.28^{c}	23.41±0.62^{fA}
	15th	43.25±0.28^{aA}	36.53±1.38^{bA}	34.21±0.73^{cA}	33.78±0.25^{d}	31.79±1.18^{eB}	35.11±0.56^{f}	34.27±0.74^{c}	32.47±0.73^{gAB}
	30th	96.23±0.71^{aB}	48.52±0.51^{bB}	46.58±0.61^{cB}	44.25±0.16^{d}	41.57±1.13^{eB}	47.57±0.75^{f}	45.53±1.41^{g}	43.11±0.89^{hB}
Weight losses (%)	1st	-	-	-	-	-	-	-	-
	7th	9.24±0.21^{a}	8.54±0.11^{b}	7.32±0.42^{c}	7.25±0.87^{d}	6.73±0.11^{e}	7.34±0.51^{c}	7.26±0.41^{d}	6.85±0.52^{e}
	15th	7.86±0.12^{a}	6.15±0.59^{b}	6.08±.0.83^{c}	5.97±0.13^{d}	4.21±0.77^{e}	6.12±0.17^{b}	6.01±0.81^{c}	4.58±0.60^{f}
	30th	4.21±0.18^{a}	3.85±0.74^{b}	3.72±0.21^{c}	3.68±0.58^{d}	2.11±0.44^{e}	4.01±0.23^{f}	3.81±0.62^{b}	2.41±0.49^{g}

6: Standard deviation (n=3)
a,b,c: The differences between the values in the same line are statistically significant (P<0.05)
A,B: The differences between the values in the same column are statistically significant (P<0.05)
K = Control
EWPP = egg white protein powder; SEO = Sage essential oil (various concentrations 0.5%, 1%, 2% (v/v))
BEO = lemon balm essential oil (various concentrations 0.5%, 1%, 2% (v/v))

The average fat values of samples coated with $EWPP_{SEO(2)}$ (20.63 %) and $EWPP_{BEO(2)}$ (20.37 %) on the 1st day of the storage were higher than both the other concentrations, uncoated control sample (19.24.%) and non-supplemented EWPP coated (19.24 %) samples. This was associated with the dissolution tendency of the hydrophobic property of essential oil, hence the kashar cheese in lipid phase, depending on the increase in acidity (Holley and Patel, 2005), and the high water barrier properties of protein based films (Koyuncu and Savran, 2002). The fat barrier properties of samples coated with EWPP and edible films with the addition of SEO and BEO to EWPP were high, the highest value observed at 2 % (v/v) essential oil addition. During storage, the relationship between the average fat values of K and EWPP based film coated samples was not significant (P>0.05). Our results were compatible with previous which reported that composite films with good mechanical, fat, oxygen and water vapor barrier properties can be produced by the addition of different essential oils to protein based films (Gennadios et al., 1998; Hanani et al., 2013; McHugh et al., 1994; Shellhammer and Krochta, 1997), and also with studies reporting that EWP based films show similar properties to other protein based films (Ball, 1987; Gennadios et al., 1998).

Microbiological properties

Coating the cheese samples with EWPP based film and edible films obtained with the addition of SEO or BEO at different concentrations to EWPP had bacteriostatic effects from the 1st day of storage and bactericidal effect in the further days of storage (Table 5). The antimicrobial effects of all SEO and BEO supplemented films were higher than EWPP based film. Additionally, the antibacterial effect of SEO was higher than that of BEO at all concentrations, although the antifungal effects of BEO were higher. Overall, significant relationships were determined between coating the cheeses with EWPP based film and antimicrobial activity and also between the increase in the antimicrobial activity and the addition of essential oils at all concentrations (P<0.05). The relationship between the antimicrobial effect and the extension of storage period was also significant (P<0.05). This result was associated with slower transmission of antimicrobial agent from film layer to food in the edible film systems, with a higher concentration of antimicrobial agent remaining

Table 5. Average microorganism counts and standard deviation values in the samples coated with different films and non-coated samples (n=3); ($p<0.05$)

Microorganisms	Storage time (day)	K±σ	EWPP±σ	$EWPP_{SEO(0.5)}$ ±σ	$EWPP_{SEO(1)}$ ±σ	$EWPP_{SEO(2)}$ ±σ	$EWPP_{BEO(0.5)}$ ±σ	$EWPP_{BEO(1)}$ ±σ	$EWPP_{BEO(2)}$ ±σ
E. coli O157:H7	1st	7.27±0.33^{aA}	7.15±1.02^{aA}	6.85±0.11^{bA}	6.42±0.27^{cA}	4.14±0.08^{dA}	7.04±0.83^{aA}	6.94±1.42^{bA}	5.27±1.19^{eA}
	7th	7.83±0.41^{aB}	7.03±1.44^{bA}	5.78±0.92^{cB}	4.33±0.15^{dB}	2.84±0.02^{eB}	5.91±0.19^{fB}	5.24±1.10^{gB}	4.33±1.44^{hB}
	15th	7.95±0.12^{aC}	6.91±1.33^{bB}	5.68±0.54^{cC}	3.74±0.08^{dC}	AE	5.80±1.53^{eB}	4.16±0.19^{fC}	2.41±0.17^{gC}
	30th	7.98±0.21^{aC}	6.61±1.08^{bC}	5.46±0.82^{cD}	3.18±0.43^{dD}	AE	5.63±0.46^{eC}	3.87±0.57^{fD}	AE
S. aureus	1st	7.42±1.14^{aA}	7.29±0.04^{bA}	5.87±0.99^{cA}	4.51±1.28^{dA}	3.21±0.47^{e}	6.04±0.52^{fA}	5.82±1.46^{gA}	4.79±1.58^{hA}
	7th	7.71±1.36^{aB}	6.94±0.17^{bB}	3.92±0.14^{cB}	2.83±1.10^{dB}	AE	4.36±0.12^{eB}	4.17±0.08^{fB}	3.22±0.41^{gB}
	15th	7.83±1.62^{aC}	6.83±0.10^{bB}	3.02±0.03^{cC}	AE	AE	3.71±0.05^{dC}	3.29±0.18^{eC}	AE
	30th	7.92±1.27^{aD}	6.66±0.22^{bC}	AE	AE	AE	2.88±0.47^{cD}	2.66±0.27^{dD}	AE
L. monocytogenes	1st	7.16±1.34^{aaA}	6.14±0.25^{bA}	5.42±0.24^{cA}	4.02±0.19^{dA}	2.46±1.53^{e}	5.77±1.32^{fA}	4.76±0.23^{gA}	3.17±1.07^{hA}
	7th	7.44±0.18^{aB}	4.85±0.47^{bB}	3.14±0.35^{cB}	2.36±1.11^{dB}	AE	4.25±1.44^{eB}	3.34±0.14^{fB}	2.21±0.28^{gB}
	15th	7.61±0.11^{aC}	2.65±0.12^{bC}	AE	AE	AE	2.27±0.37^{cC}	2.01±0.76^{dC}	AE
	30th	7.73±0.12^{aD}	AE	AE	AE	AE	AE	AE	AE
Yeast and mold	1st	ND	ND	ND	ND	ND	ND	ND	ND
	7th	4.85±0.09^{aA}	3.77±0.04^{bA}	2.22±1.06^{cA}	1.12±1.38^{d}	AE	1.16±1.46^{d}	AE	AE
	15th	6.92±0.42^{aB}	2.83±0.54^{bB}	1.23±1.12^{cB}	AE	AE	AE	AE	AE
	30th	7.98±1.07^{aC}	AE	AE	AE	AE	AE	AE	AE

a,b,c: The differences between the values in the same line are statistically significant ($P < 0.05$)
A,B: The differences between the values in the same column are statistically significant ($P < 0.05$)
ND = Non detected
AE = Antimicrobial effect

in the film and at the surface of the food, thus providing a long-lasting effect against microorganisms (Coma et al., 2002; Cagri et al., 2002). Also, in relation with the pH decrease in cheese samples, the increase in hydrophobic properties of the essential oils and their consequent easier diffusion across cell membranes most likely influenced the increase in antimicrobial activity (Holley and Patel, 2005). Additionally, it is likely that the high 1,8 cineole (40.6 %), α-thujon (22.56 %) and β-thujon (12.37 %) contents of SEO, the high gerenial (38.20 %), neral (30.59 %) and citronellal (16.14 %) contents of BEO and the high acidity of both SEO (pH 3.83) and BEO (pH 4.12) significantly contributed to the increased antimicrobial effects.

SEO (Bozin et al., 2007; Tajkarimi et al., 2010) and BEO (Allahverdiyev et al., 2004; Araujo et al.2003; Dukic et al., 2004) are essential oils with strong antimicrobial effects. Our results are consistent with literature data. Additionally, the previously demonstrated antimicrobial properties of EWP are confirmed for EWPP based edible films in this study. This antimicrobial effect was increased with the addition of SEO and BEO to EWPP based film in a dose-dependent manner.

The bacteriostatic effect determined in samples coated with EWPP based films was lower than those coated with EWPP based films which contained SEO and BEO from the 1st day of the storage. A bactericidal effect was determined on the 30th day against *L. monocytogenes*, yeasts and moulds. The antimicrobial effect of EWPP was lower than those of SEO and BEO at all concentrations. Microorganism levels increased in the control sample; while the highest counts were determined for yeasts and moulds. Yeasts and moulds were not detected in any of the samples on the 1st day of the storage. However, their counts increased in coated samples from the 7th day of the storage onward. In film coated samples, different levels of antifungal effects were determined from the 7th day depending on the concentration of the essential oil concentrations. SEO and BEO addition to EWPP based film at 0.5 % (v/v) had a bacteriostatic effect against all microorganisms from the 1st day of the storage. SEO had bactericidal effects against *L. monocytogenes* on the 15th day and on yeast-moulds and *S. aureus* on the 30th day of the storage. BEO showed antifungal effects on the 15th day and a bactericidal effect against *L. monocytogenes* on the 30th day. The bactericidal effect of BEO against *L. monocytogenes* was higher than that of EWPP throughout the storage. The bacteriostatic effect of SEO against *S. aureus* increased until the end of the storage; this effect was lower than that of SEO and higher than that of EWPP. The antibacterial effect of SEO at 0.5 % (v/v) was higher than those of EWPP and BEO, and the antifungal effect was lower than that of BEO while higher than that of EWPP. Additionally, the bacteriostatic effect of SEO against *E. coli* O157:H7 was higher than those of EWPP and BEO. The antibacterial effect observed with 0.5 % (v/v) addition was higher only than EWPP.

SEO and BEO addition at 1 % (v/v) had a bacteriostatic effect against *E. coli* O157:H7, *S. aureus* and *L. monocytogenes* from the 1st day of the storage. This effect was stronger compared to that observed for 0.5 % (v/v) essential oil addition. The bacteriostatic effect of SEO was stronger than that of BEO; this effect continued for both of the essential oils throughout the storage. On the 15th day it was observed that SEO demonstrated bactericidal effects against *L. monocytogenes* and *S. aureus* as well as antifungal effects against yeasts and moulds. No yeasts and moulds were detected in 1 % (v/v) BEO samples and a bactericidal effect of BEO against *L. monocytogenes* was determined on the 30th day. The antimicrobial effect was the same as EWPP on the 30th day, although it was generally stronger in comparison to EWPP. The bacteriostatic effect against *S. aureus* and *E. coli* O157:H7 continued throughout the storage. The bacteriostatic effect of BEO against *S. aureus* and *E. coli* O157:H7 was stronger than that of EWPP and weaker than that of SEO. The antifungal effect of BEO was stronger than those of SEO and EWPP.

SEO and BEO addition to EWPP based film at 2 % (v/v) had a bacteriostatic effect against *E. coli* O157:H7, *S. aureus* and *L. monocytogenes* from the 1st day of the storage onward. With 2 % (v/v) SEO addition, a bactericidal effect was determined on 7th day against *L. monocytogenes* and *S. aureus* and an antifungal effect was determined against yeast and moulds. A bactericidal effect of BEO against *L. monocytogenes* and *S. aureus* was detected on the 15th day. BEO and SEO added at 2 % (v/v) to film had antifungal effects against yeasts and moulds, an effect was the same as BEO addition at 1 % (v/v) concentration. SEO demonstrated a bactericidal effect against *E. coli* O157:H7 on the 15th day, while this effect was not detected for BEO until the 30th day.

The most sensitive microorganism to all concentrations of essential oils was *L. monocytogenes*, followed by *S. aureus* and *E. coli* O157:H7. The high resistance to essential oil antibiotic activity of *E. coli* O157:H7 was possibly due to the ability of the species to thrive at low pH (pH<3.6) and its general resistance to acidity (Lake et al., 2002; Park et al., 1999). Regarding *L. monocytogenes* sensitivity it was most probably associated with the increase of acidity and the presence of antimicrobial agents (Doyle, 1988; Wilkins et al., 1972). The results regarding *S. aureus* could be associated with the sensitivity of the species to the components found in the essential oils. A bactericidal effect against *E. coli* O157:H7 was detected in samples coated with films prepared by the addition of 2 % (v/v) SEO and BEO. It was also observed that EWPP had a bactericidal effect against *L. monocytogenes*, an antifungal effect against yeasts and moulds and a bacteriostatic effect on other microorganisms on the 30th day. BEO showed a stronger antifungal effect compared to SEO at all concentrations. The presented results regarding the antifungal effects of BEO were in compliance to those of some previous studies (Araujo et al., 2003; Dukic et al., 2004). It was demonstrated that antibacterial and antifungal effects increased with the increase in essential oil concentrations.

Conclusion

It could be concluded that BEO might be used as an antifungal agent and SEO might act as an antimicrobial agent in lor cheese. In future studies, different concentrations of these two essential oils should be applied to different food products in order to further expand the usage and optimize the production methods. It could also be concluded that EWPP based films might be exclusively used in edible film systems for the preservation of foods. An effective film could be obtained in terms of both, physico-chemical and antimicrobial perspectives. EWPP based films could be exclusively used in edible film production and the appearance properties were similar to other protein based films. Addition of essential oils to EWPP based film improved both, physico-chemical and antimicrobial properties in a dose-dependent manner. The best essential oil concentration in terms of physico-chemical and antimicrobial properties was 2 % (v/v). SEO resulted in better physico-chemical and antimicrobial properties at all concentrations than BEO. It was also determined that BEO might be effective in the preservation of lor cheese. BEO demonstrated stronger antifungal effects than SEO.

Upotreba jestivih filmova na bazi proteina bjelanjka jajeta obogaćenih esencijalnim uljima kadulje i limunske trave u svrhu čuvanja sira lor

Sažetak

Jestivi film proizveden je dodatkom 3 % sorbitola (v/v) proteinima bjelanjka u prahu (EWPP). Prva skupina uzoraka sira lor presvučena je jestivim filmom obogaćenim esencijalnim uljem kadulje (SEO), dok je druga grupa uzoraka bila presvučena filmovima obogaćenim dodatkom esencijalnog ulja limunske trave u različitim koncentracijama [0.5 %, 1 %, 2 % (v/v)]. Filmovi su označeni nazivima EWPP-$_{SEO(0.5)}$, EWPP$_{SEO(1)}$, EWPP$_{SEO(2)}$, EWPP$_{BEO(0.5)}$, EWPP$_{BEO(1)}$, EWPP$_{BEO(2)}$ kako bi se lakše razlikovalo o kojoj je vrsti i koncentraciji dodatka riječ. Treća grupa uzoraka lor sira presvučena je čistim, neobogaćenim filmom proteina bjelanjka jajeta (EWPP) dok četvrta grupa uzoraka nije bila presvučena nikakvim filmovima. Svi testirani uzorci sira namjerno su kontaminirani sojevima *Escherichia coli* O157:H7, *Listeria monocytogenes* (*L. monocytogenes*) i *Staphylococcus aureus* (*S. auerus*). Nakon postupka proizvodnje, u svim testiranim uzorcima sira određen je broj živih stanica prethodno navedenih sojeva, kao i broj prisutnih kvasaca i plijesni. Nadalje, svi su uzorci čuvani pri +4 °C te im je tijekom razdoblja čuvanja (1., 7., 15. i 30. dan) određivan fizikalno-kemijski sastav i mikrobiološka kvaliteta. Pritom se utvrdilo da su povećanje koncentracije esencijalnog ulja i debljine jestivog filma značajno (p<0,05) povezani s propusnošću vlage, unutarnjom i vanjskom čvrstoćom sira, gubitkom na težini, sprječavanjem gubitka masti i mikrobiološkom kvalitetom tijekom skladištenja. Pokazalo se da su sva navedena svojstva značajno poboljšana u uzrocima presvučenim filmovima s dodatkom 2 % (v/v) esencijalnih ulja kadulje odnosno limunske trave, dok druge ispitivane koncentracije nisu bile statističke značajne (p>0,05).

Dodatak esencijalnih ulja kadulje u usporedbi s esencijalnim uljem limunske trave pri svim koncentracijama pokazao je značajniji utjecaj na fizikalno-kemijska i antibakterijska svojstva. Međutim, esencijalno ulje limunske trave pokazalo je jače antifungalno djelovanje u odnosu na esencijalno ulje kadulje. Nadalje, navedeni antifungalni učinak bio je jednak neovisno o koncentraciji (1 % (v/v) i 2 % (v/v)) esencijalnog ulja limunske trave. Soj *E. coli* O157:H7 bio je najotporniji prema djelovanju esencijalnih ulja, dok je soj *L. monocytogenes* bio najmanje otporan. EWPP je pokazao bakteriostatski učinak na mikroorganizme te je baktericidni učinak prema soju *L. monocytogenes* i kvasacima i plijesnima bio detektiran i nakon 30 dana čuvanja.

Ključne riječi: protein bjelanjka jajeta, kadulja, limunska trava, esencijalna ulja, lor sir, čuvanje

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