

Use of Turmeric (*Curcuma longa* L.) Essential Oil Added to an Egg White Protein Powder-Based Film in the Storage of Çökelek Cheese

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Abstract

In this study, edible film (EWPP) was produced by using 1.5% (w/v) sorbitol + 5% (w/v) egg white protein powder (EWPP) + 0.5% (w/v) alginate and 1% (v/v); 2% (v/v) turmeric essential oil (EO_T). Çökelek cheese samples were artificially contaminated with *Escherichia coli* O157:H7 and *Staphylococcus aureus* at a level of 10⁶ cfu^{-g}. Counts of these organisms and yeast-mould were determined after the cheese production. After coating some of the Çökelek samples with film, all samples were stored at 4 °C for 30 days. Antimicrobial activities and selected physical-chemical parameters were assessed on the 1st, 7th, 14th and 30th days of storage. EWPP was found to have good water barrier properties, which improved with the addition of 1% (v/v) and 2% (v/v) turmeric essential oil to the film, and EWPP was determined to have antimicrobial effects. During storage, *Escherichia coli* O157:H7 and *Staphylococcus aureus* levels increased in the control samples, while they decreased in the film-coated samples. Microbial growth was found to be highest in the control sample throughout the period of storage.

Keywords

Edible film, Çökelek cheese, Turmeric essential oil, Antimicrobial effect

Introduction

Çökelek cheese is obtained either by increasing the acidity of whole/skimmed cow, sheep or goat milk or by using yoghurt or ayran [1]. Its composition varies depending on the raw materials. Çökelek cheese, which, as a result of the heat treatment of milk, simultaneously contains casein and serum proteins, has high water activity, contains high levels of protein and calcium, and has low levels of oil [2, 3]. It has different names in different regions of Turkey, which include *Eksimik*, *Kes*, *Akçakatik*, *Kesmikör Urda*, *Minci-Minzi* and Çökelek [4]. Çökelek cheese is a suitable medium for microbial growth because of the lack of hygienic conditions in its production, improper storage conditions, being offered to the market without packaging, and its pH and high water activity [2].

Egg white protein (EWP) has antimicrobial and antiviral properties [5, 6]. Egg white protein powder (EWPP) is obtained by drying EWP with conventional drying methods. Protein-based films have poor and fragile mechanical properties as a result of the cohesive energy density of polymers [7]. These problems are eliminated by adding certain plasticizers (glycerol, propylene glycol, sorbitol and polyethylene glycol) to protein-based films [8]. Sorbitol (S) distinguishes itself by its ability to attract lower levels of moisture, when compared to the other plasticizers, and its ability to completely dissolve [9]. Although having similar properties to those of other protein-based films [10], EWP-based films

have higher water vapor permeability than whey protein isolates and soy protein-based films [11]. Edible films are used as carrier matrixes for antimicrobial substances. The antimicrobial properties of herbal essential oils (EOs), which have become prominent in recent years, are frequently used to control pathogenic bacterial growth and spoilage in food products [12].

High levels (5 to 6%) of hydrophobic [13] essential oil (EO_T) is obtained from the rhizomes of turmeric (*Curcuma longa* L.), which is a member of the Zingiberaceae family [14]. EO_T is especially rich in α - and β -turmerone and ar-turmerone [15]. It was reported that the EO_T from rhizomes had an antibacterial effect on *P. vulgaris*, *K. pneumoniae* [16, 17], and *B. cereus* and *B. subtilis* [18] but did not affect *S. typhimurium*, *S. aureus*, *E. coli* and *L. monocytogenes* [19, 20].

On the other hand, it was reported that EO_T obtained from leaves had a strong antibacterial effect on *B. cereus*, *S. aureus* [21] and *Cl. perfringens*, and a poor antibacterial effect on *E. coli* [22]. However, Rath et al. [23] reported that essential oil obtained from rhizomes did have an antibacterial effect on *E. coli*. The antibacterial and antifungal effects of EO_T are associated with ar-turmerone, which is one of its main components [24].

No reports were found on coating çökelek cheese with essential oil-containing protein-based edible film to prolong its shelf life. Moreover, there isn't a report on using EWPP-based film as the material in the edible film coating of çökelek cheese or its effect on certain properties of çökelek cheese. Hence, the present study aimed to coat çökelek cheese with different concentrations of essential oil (EO_T) obtained from the rhizomes of turmeric (*Curcuma longa* L.) with EWPP [1% (v/v) and 2% (v/v)], sorbitol and alginate (algae)-based film in order to prolong its shelf life.

Materials and Methods

EWPP, alginate and D-sorbitol

For the preparation of coating material, Alfasol® 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) and D-sorbitol (S1876) was obtained from Sigma-Aldrich and alginate were obtained from Fluka-Norway.

Essential oils

Which is obtained from turmeric (*Curcuma longa* L.) rhizome the essential oils EO_T investigated in this work were purchased from flora Umurlu (Aydın-Turkey). Depending on the amount of the plant material available, EO_T was obtained by hydro-distillation for 3 h using a Clevenger-type apparatus [25]. The oils used were those of *Curcuma longa* L. the active components were obtained from Sigma-Aldrich (Steinheim, Germany).

Analysis of essential oils and volatile compounds by GC/MS

GC/MS analyses were carried out using Agilent 6890

GC system with Agilent 5973 MS system. The column was use DB-5MS 30 m x 0.25 mm x 0.25 μ m (5% Diphenyl/95% Dimethylpolysiloxan) Agilent. The oven temperature was initially held at 50 °C/3 min, programmed to 160 °C at 1.5 °C/min, then to 315 °C at 3 °C/min and the final temperature was held for 30 min. the carrier gas helium with a flow rate of 1.0 ml/min. The split mode 1:20 was used. The injection volume was 1.0 μ l. The mass spectrometer was operated in the electron impact mode (70 eV). The ion source temperature was held at 230 °C. The transfer-line was maintained at 280 °C. The scanned mass range was from 30 to 500 u.

Çökelek cheese

Cheese samples were produced from cow milk. After adding water equal to half the volume of the milk and keeping it at room temperature for 24 to 48 hours, the milk was separated from its oil. The remainder of the milk was heated up to 95 °C for curdling and cooling (4 to 6 hours) and then filtered using a press cloth (2 to 3 hours). A weight of 50 kg/10 kg was applied to the filtered curd, and filtering at room temperature was continued overnight. The composition of the çökelek cheese was: pH 4.73; dry matter 35.1%; oil 3.6%, titration acidity (LA%) 0.315% and protein 26.12%. The çökelek cheese was divided into four batches: the first batch was coated with film after artificial contamination (Control); the second batch was coated with only EWPP-based film after artificial contamination; the third and fourth batches were coated with 1% (v/v) and 2% (v/v) EO_T -added EWPP-based film ($EWPP_{EO_T(1)}$; $EWPP_{EO_T(2)}$), respectively.

Preparation of edible film solution

Edible films were prepared according to Pintado et al. [26] and Mchugh and Krochta [7], with some modifications. Accordingly, 5% w/v EWPP was prepared, and after the addition of 1.5% w/v sorbitol to the solution, a homogenization process was carried out in a homogenizer (DAIHAN Premium Hotplate Stirrer, MSH-A, Ceramic-Coated Plate, up to 380 °C, 1,500 rpm). The mixture pH was adjusted to 8 and kept in a water bath at 45 ± 2 °C for 30 minutes in order to improve the mechanical properties of the film solution. Then, 0.5% w/v alginate was added in the standby stage. The solution was then cooled to room temperature and alginat-sorbitol-amended EWPP was obtained. The cooled solution was filtered (sterile filtering) and divided into three equal parts; first part was coated edible film only EWPP, second part was contain 1% (v/v) $EO_{T(1)}$ ($EWPP_{EO_T(1)}$), third part was contain 2% (v/v) $EO_{T(2)}$ ($EWPP_{EO_T(2)}$) and the fourth part (C) was applied to artificial contamination which was uncoated edible film. Following the turmeric essential oil addition, in order to maintain the homogeneous distribution of oil in the solution, Tween 20 (0.5% (v/v)) was added [27] and the solution was centrifuged again at 20,000 rpm for 1 minute (3-16 K Type-Model, Sigma, Germany [28]). As a result, edible film containing EWPP based 1% (v/v) and 2% (v/v) EO_T [$EWPP_{EO_T(1)}$; $EWPP_{EO_T(2)}$] were obtained. Cheese samples coated with these films were left to dry at room temperature for 24 hours (Figure 1).

Preparation and storage of samples

E. coli O157:H7 (ATCC® 25922) and *S. aureus* (ATCC® 25923) strains used for the artificial contamination of çökelek 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_{10} cfu^g) inocula of *E. coli* O157:H7 and *S. aureus* were used. In order to maintain the artificial contamination, çökelek cheese samples were divided into 50 g portions and immersed in *E. coli* O157:H7 and *S. aureus* inocula separately. Cheese samples were kept in each inoculum for 15 mins 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 turmeric essential oil at which were prepared as explained above. Accordingly, çökelek cheese samples were immersed in film solutions for 120 seconds, removed, hold 3 minutes, immersed again in film solution for 90 seconds and removed. Following the immersion process, cheese samples which were coated with EWPP, EWPP_{EOT(1)} and EWPP_{EOT(2)} based films were left to dry at 10 °C for 4-5 hours. Control (C) 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, *S. aureus* and yeast-mold counts of samples were calculated as Log₁₀ cfu^g on the 1st, 7th, 14th and 30th days of the storage (Figure 1).

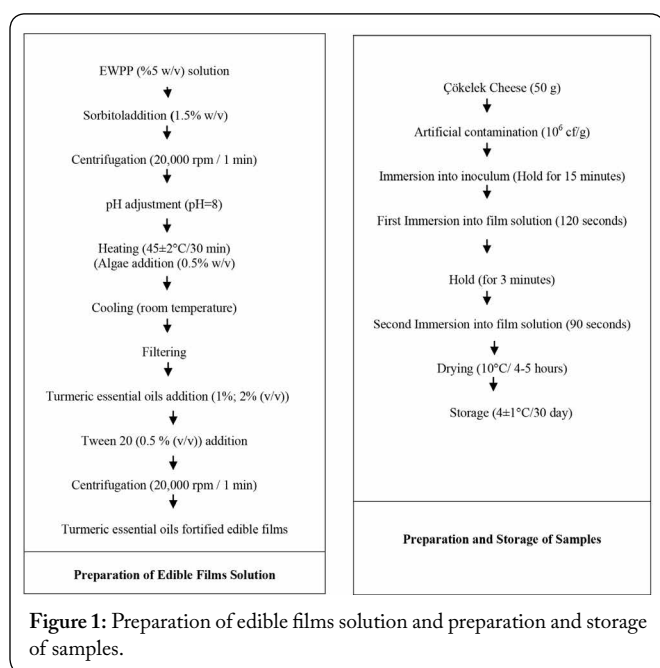


Figure 1: Preparation of edible films solution and preparation and storage of samples.

Physical - chemical analysis

Weight loss percentages of çökelek cheese samples during storage were determined gravimetrically. pH values were examined with a SS-3 Zeromatic pH meter (Beckman Instruments Inc., California, USA). Acidity (%SH) and fat content (%) were analyzed according to AOAC 2000 [29]. The inner-outer hardness was determined at 3 ± 1 °C with a penetrometer (4500 CT3 texture analyzer Brookfield made

in USA). Film thicknesses were measured with a micrometer at 0.005 precision (Digimatic Micrometer Japan). Water vapor permeability of films were determined using ASTM E96-80 1983 [30] method gravimetrically at 25 °C. WVP was calculated by finding the slope of weight-time line and substituting it in the following formula.

$$\text{Slope (C)} = \frac{WVP \times A \times \Delta p}{X} \quad WVP = C \frac{x}{A \times \Delta p}$$

A: Surface area (m²)

WVP: Water vapor permeability (g mm m⁻² h⁻¹ kPa⁻¹)

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 hours. For enumeration of *E. coli* O157:H7 was used Sorbitol MacConkey Agar containing Cefixime-Tellurite Supplement and incubating at 35-37 °C for 24-48 hours. After incubation, sorbitol negative colonies were counted. *S. aureus* was enriched in Brain Heart Infusion Broth at 37 °C/de for 48 hours. 5% 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 [31]. 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 [32].

Statistical evaluation

Four different cheese samples were examined with 3 parallels and 2 repetitions. For this purpose, SPSS 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

The active substances of the EO_T containing; ar-turmerone (33.42%), α-turmerone (22.35%), β-turmerone (20.14%), ar-curcumerene (3.09%), α-zingiberene (0.8%), α-pinene (0.3%) and 1.8-cineole (0.2%) was obtained from *Curcuma longa* L. species.

The thickness of EWPP based film was 0.174 mm and the thickness of 1% (v/v) and 2% (v/v) EO_T supplemented EWPP_{EOT(1)} and EWPP_{EOT(2)} respectively 0.179 mm and 0.182 mm (Table 1). The relationship between the increase in essential oil concentration and the increase in film thickness was statistically significant ($p < 0.05$). Water vapor permeability was higher in samples coated with EWPP based film than in those coated with EWPP_{EOT} based film; this difference was statistically significant ($p < 0.05$). The addition of EO_T to the film in increasing concentrations improved the water barrier property.

Inner and outer hardness values of the samples coated with edible films fortified with different concentrations of

EO_T were lower for all concentrations compared to those coated with non-fortified EWPP. Hardness values of K sample (uncoated) were higher than those determined for film coated samples. The relationship between essential oil concentration 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) EO_T. In regards to storage days, the differences between the weight losses in samples were significant ($p < 0.05$).

Table 1: Film thicknesses and water vapor permeability of EWPP, EWPP_{EO_T(1)} and EWPP_{EO_T(2)}, based films.

Samples	Thickness/mm \pm δ	Water vapor permeability (g mm m ⁻² h ⁻¹ kPa ⁻¹)
EWPP	0.174 \pm 0.005 ^A	6.66 g mm m ⁻² h ⁻¹ kPa ^{-1A}
EWPP _{EO_T(1)}	0.179 \pm 0.001 ^B	6.51 g mm m ⁻² h ⁻¹ kPa ^{-1B}
EWPP _{EO_T(2)}	0.182 \pm 0.003 ^B	6.45 g mm m ⁻² h ⁻¹ kPa ^{-1C}

δ : Standard deviation (n = 3)

A, B, C, The same column indicate significant differences

Weight loss values of the samples coated with edible films fortified with different concentrations of EO_T were lower compared to those coated with non-fortified EWPP. 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.

Fat levels increased on the 1st day of the storage with the addition of EO_T to EWPP based film at different concentrations. This was associated with the dissolution tendency of the hydrophobic property of essential oil, hence the çökelek cheese in lipid phase, depending on the increase in acidity [33], and the high water barrier properties of protein based films [36]. The increase in fat level in the samples due to the increase in concentration during storage was also related to the high lipophilic character of *Curcuma longa* L. essential oil [13]. During storage, the relationship between the average fat values of K and EWPP based film coated samples was not significant ($p > 0.05$). Our study results are compatible with previous studies 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 [34, 35], and also with studies reporting that EWP based films show similar properties to other protein based films [11].

Microbiological properties

In our study, cheese samples were artificially contaminated with pathogenic microorganisms at 10⁶ cfu^{-g}. The antimicrobial effects of all EO_T supplemented films were higher than EWPP based film. Additionally, the antibacterial effect of 2% (v/v) EO_T was higher than that of 1% (v/v) EO_T at during storage. However the antifungal effect of EO_T were higher than that of antibacterial effect. 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 high concentration of antimicrobial agent remaining in the film and at the surface of the food, thus providing a long-lasting effect against microorganisms [36]. 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 likely play a role in the increase in antimicrobial activity [33].

EO_T addition to EWPP based film at 1% (v/v) had a bacteriostatic effect against all microorganisms from the 1st day of the storage. EO_{T(1)} had bactericidal effects against yeast-moulds on the 14th day of the storage. The bactericidal effect of 2% (v/v) EO_T addition on yeast-mold was observed on day 7. The results of this study on yeast-mold were in agreement with the results obtained by studies reporting the strong antifungal activity of EO_T [37-39]. Only the EWPP-coating showed a bacteriostatic effect on yeast-mold until the end of the storage period. In the samples coated with EWPP, yeast-mold was decreased by 5 log₁₀ cfu^{-g} at day 7, by 4 log₁₀ cfu^{-g} at day 14 and by 3 log₁₀ cfu^{-g} at day 30. Our results for *S. aureus* and *E. coli* contradicted the results indicating that EO_T did not have an antibacterial effect, or a poor antibacterial effect [19, 20, 22] and that it had an antimicrobial effect on non-pathogenic bacteria [40]. However, they were in agreement with other results indicating that EO_T did have an antimicrobial effect [16-18, 23, 24].

In this study, EO_T at all concentrations showed a bacteriostatic effect on *S. aureus* and *E. coli* O157:H7 beginning from day 1. EO_T which was added to the EWPP-based film at 2% (v/v) concentration, showed a bactericidal effect on *S. aureus* at day 14, and 1% (v/v) of EO_T had a bacteriostatic effect on *S. aureus* until the end of storage.

In the samples containing 1% (v/v) EO_T, the *S. aureus* level was decreased by 5 log₁₀ cfu^{-g} at day 14 and by 3 log₁₀ cfu^{-g} at day 30. Coating with EWPP-based film had a bacteriostatic effect on *S. aureus*, but this effect was lower than the bacteriostatic effect obtained by adding 1% (v/v) EO_T. At day 7 of storage, in the samples coated with EWPP, the *S. aureus* level was decreased by 5 log₁₀ cfu^{-g}; it was decreased by 4 log₁₀ cfu^{-g} at day 30. During the storage, the effect of EO_T on *E. coli* O157:H7 was bacteriostatic at all concentrations, albeit the effect at 2% (v/v) level was higher than that the 1% (v/v) level. With the addition of 1% (v/v) EO_T, the number of *E. coli* O157:H7 was decreased by 5 log₁₀ cfu^{-g} at day 14, while, with the addition of 2% (v/v) EO_T, it was decreased by 4 log₁₀ cfu^{-g} at day 14, and the decrease in both concentrations remained at these levels until the end of the storage.

The EWPP-based film coating had a low bacteriostatic effect on *E. coli* O157:H7. In the EWPP-coated samples, the number of *E. coli* O157:H7 was decreased by 6 log₁₀ cfu^{-g} during storage. The low antimicrobial effect of coating with EWPP, and EWPP containing EO_T at different concentrations, on

E. coli O157:H7 was associated with the ability of *E. coli* O157:H7 to grow in mediums with a low pH (pH < 3.6) and its resistance to acidity [41]. Furthermore, *E. coli* O157:H7 contains an outer phospholipid membrane that acts as a barrier and renders the membrane impermeable to lipophilic compounds [42], and studies have shown that this membrane protected the bacteria against the essential oils [43].

Microbial growth was found to be highest in the C sample throughout the period of storage. *S. aureus* levels in the C sample were higher compared to *E. coli* O157:H7 levels in the same sample on the 7th day of the storage, although the case was the opposite on the 15th and 30th days of the storage. This was associated with an increase in acidity on the 15th and 30th days. In our study, the increase in acidity determined in C sample throughout the storage period was higher than that in film-coated samples.

Conclusion

The results obtained in this study regarding film thickness and water vapor permeability are consistent with those reporting that EWP with the addition of various herbal ingredients can be used in the preservation of foods [44, 45].

Overall, it was determined that EWPP based film constituted a good water barrier and that this property increased along with the concentration of essential oil added to the film. In many studies, it has been reported that film coating prevents water vapor transmission and decreases weight losses [46, 47].

The results have shown that the most susceptible microorganisms to the increasing concentrations of the essential oil were yeast-mold and *S. aureus*, respectively, and the most resistant microorganism to essential oil was *E. coli* O157:H7.

Our results regarding *S. aureus* and *E. coli* O157:H7 are in agreement with some of the studies in the relevant literature, albeit contradicting with some others. This was attributed to the antimicrobial activity of essential oils, its lipophilicity or hydrophobicity, its chemical structure, the existence of functional polar groups, the aromaticity of the essential oil [48], the concentration and the extraction method [49]. In addition, the collective microbial activity of multiple essential oil compounds is higher than that of a single oil compound [50].

The low levels of the bactericidal effect of the essential oil at selected concentrations on *E. coli* O157:H7, and the low levels of the bacteriostatic effect of EWPP-based film on all of the selected microorganisms, were attributed to the high protein ratio (26.12%) in cottage cheese. Burt 2004 [51] reported that high protein content decreased the activity of essential oils. On the other hand, as a result of simultaneously using EO_T and EWPP at different concentrations, a synergetic effect emerged in their antimicrobial properties, and it was concluded that the high lipophilicity of EO_T and the high levels of ar-turmerone in its composition (33.42%), contributed to this effect.

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