Short communication

Comparative evaluation on fatty acid and Matricaria recutita essential oil incorporated into casein-based film

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Sodium caseinate composite films containing lipids–oleic acid (OA), stearic acid (SA), or Matricaria recutita essential oil (MEO) – were prepared through emulsification and their physical, thermal, mechanical, and barrier properties were evaluated and compared. Furthermore, their antimicrobial effectiveness against Listeria monocytogenes, Staphylococcus aureus, and Escherichia coli was studied. Emulsified films were softer, less rigid, and more stretchable than pure films. The films’ water vapor barrier properties were found to decrease upon the addition of lipid content; this effect was greatly reduced when MEO was added. The presence of OA/SA and MEO decreased tensile strength and elastic modulus but increased the elongation at break. Thermal analysis of all emulsified films showed two endothermic peaks; these results confirmed those obtained by SEM studies, where a partial separation of the two phases occurred. The films’ antimicrobial activities were increased by incorporating lipids, particularly those containing MEO, which were more effective against the studied bacteria. This work showed that when taking all the studied variables into account, films formulated with MEO were found most suitable for various food applications.

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1. Introduction

Packaging is a crucial part of the food supply chain: it must protect food from environmental conditions like oxygen, light, moisture, microbes, mechanical stresses, and dust. The use of synthetic polymers and plastics for packaging has grown tremendously in the last century; however, this increase has created serious environmental problems due to the materials’ inability to biodegrade [1]. Moreover, the insecurity of oil and petroleum resources — the raw materials from which such packaging is derived — encourages the food industry to explore the use of natural biobased materials and polymers in packaging [2]. Recent decades have seen extensive investigation into biodegradable coatings or films prepared from biopolymers, including proteins, polysaccharides, and lipids, or their combinations. Edible coating and films comprise a new category of packaging materials. They can act as barriers to control the transfer of moisture, oxygen, carbon dioxide, lipids, and flavor components, and thus maintain the quality and increase the shelf life of food products. Moreover, biodegradable films can be used to carry active ingredients, such as antioxidant and antimicrobial agents that can control spoilage and pathogen proliferation in food during storage and distribution. Antimicrobial agents can be added by coating onto the food surface or can be incorporated into packaging materials with controlled migration to foodstuffs [3].

Sodium caseinate (SC) is the water-soluble form of casein (the main protein in cows’ milk), obtained by acid precipitation of casein. Casein’s random-coil nature means it can easily form thermoplastic films [4]. Owing to their excellent nutritional value and their numerous functional properties, such as their solubility in water and their ability to act as emulsifiers, the use of milk proteins such as casein to form edible films has been extensively studied [5–7]. However, as with other protein-based films, the highly hydrophilic nature of SC films limits their moisture-barrier ability when compared to commonly used synthetic-plastic films. One way of improving the moisture-barrier properties of SC films is to include lipidic materials in their formulation, such as fatty acids or essential oils [8]. In addition, due to the inherent brittleness of many natural packaging biopolymers including caseinates, plasticizers must be used to improve their ductile properties and to get the flexibility required for their manufacture [9]. For these purposes, the use of glycerol has been proposed, since it contributes to a reduction in material brittleness by the limitation of crosslinking and elimination of intermolecular forces [10].

Antimicrobial packaging is a form of active packaging that can extend the shelf life of a product [11]. These films could extend...
the shelf life and microbial safety of food products. They act to reduce, inhibit, or retard the growth of pathogen microorganisms in packed foods and packaging material. This method is one of the most effective means of preserving food quality.

*Matricaria recutita* (also known as *Matricaria chamomilla*) is a popular herbal plant, used as a remedy for thousands of years. It mainly grows indigenously in Europe, northwest Asia, and northern Africa, and is cultivated in North America and elsewhere around the world. The main constituents of the flowers include several phenolic compounds, primarily the flavonoids, apigenin, quercetin, patuletin, and luteolin, and their glucosides. The principal components of the essential oil extracted from the flowers are the terpenoids α-bisabolol and its oxides and azulenes, including chamazulene, which is responsible for the strong blue color of the extract. The components of chamomile thought to have antimicrobial properties include α-bisabolol, luteolin, quercetin, and apigenin [12]. Chamomile oil, at a concentration of 25 mg/mL, demonstrates antibacterial activity against such gram-positive bacteria as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus salivarius*, as well as some fungicidal activity against *Candida albicans* [13]. In addition, chamomile extracts block aggregation of *Helicobacter pylori* and various strains of *Escherichia coli*. Chamomile extract has also been shown to inhibit the growth of the polio and herpes viruses.

Much research effort has been made to improve mechanical and barrier properties of SC-based films using fatty acids [6,7,14], but to our knowledge few studies are available on the effects of essential-oil incorporation on the antimicrobial effectiveness of SC-based films. In addition, few studies have been conducted to compare various characteristics of SC films containing fatty acids and essential oils. Thus, this paper seeks to compare the antimicrobial effectiveness, as well as the water vapor permeability, thermal and mechanical properties, of SC-incorporated films with two lipids – stearic acid (SA) and oleic acid (OA) – and *Matricaria recutita* essential oil (MEO), which was chosen as antimicrobial agent in the formulation for its positive characteristics.

2. Materials and methods

2.1. Materials

Sodium caseinate (SC, purchased from Merck, Co., Darmstadt, Germany), essential oil (MEO, supplied by Zardband Company, Tehran, Iran), Tween 80, Span 80, Span 85, OA, SA and glycerol (Fluka, Sigma–Aldrich, St. Louis, MO, USA), were used to prepare film-forming dispersions. SC composition was 85.3% protein, 1.2% fat, and 1.8% ash. OA and SA were used as lipid part. All other reagents used were of analytical grade.

2.2. Bacterial strains

The bacterial strains used in this study were *Listeria monocytogenes* PTCC 1298, *S. aureus* PTCC 1431, and *E. coli* 0157:H7 PTCC 1533; these were provided by the Iranian Research Organization for Science and Technology (Tehran, Iran). The bacterial cultures were grown on the nutrient agar slant (Merck Co., Darmstadt, Germany) and kept at 4 °C. Subculturing was carried out every 14 days to maintain bacterial viability. Overnight cultures of bacterial strains were grown and agitated at 140–150 rpm in an incubator shaker for 24h in brain–heart infusion (Merck Co., Darmstadt, Germany) at 37 °C. A dilution series was carried out to meet the required bacterial population for seeding by using sterile distilled water.

2.3. Preparation of films

Films were prepared as described in our previous study with slight modification [15]. Emulsion solutions were prepared by mixing sodium caseinate (5% w/w based on liquid weight) with glycerol and distilled water; glycerol was added as plasticizer to the distilled water before adding SC. The SC powder was slowly added to the mixture (at 60–65 °C) with constant agitation (550 rpm). Then, the mixture was stirred and heated by a heater-stirrer (IKA® RCT basic, Germany) for 1 h at 80 ± 3 °C to form di-sulphide bonds in the casein structure; these bonds may improve the mechanical properties of produced film. Film without lipids or fatty acids was cast from this solution.

To produce films containing SA and OA, the fatty acids were weighed and then added to the mixture. The ratio of fatty acid was 1%; 17% of this amount was the SA portion, and the remainder was OA. This ratio was chosen because a higher percentage of SA would cause a phase separation before film formation. Before blending with the solution, the SA was melted by heating it to 50 °C. Next, 1.6 g of emulsifiers (span 80 = 8%, span 85 = 92% for achieving HLB = 2), were added to the mixture, disregarding the effect of casein on HLB. The dispersions were homogenized at 20,000 rpm for 3 min in an Ultra-Turrax T-25 homogenizer (IKA T25 Digital Ultra-Turrax, Staufen, Germany) with an S25N-25F probe.

Essential oil incorporated SC films were produced based on the emulsion-film-producing method using MEO (1% v/v) while no fatty acids were added. Tween 80 was added by 30% (w/w) of the MEO to decrease its hydrophobicity. The film-forming solutions were degassed under vacuum for 5 min to remove bubbles that could become pinholes after drying. All prepared film solutions, including the SA/OA and MEO incorporated films, were cast in a silicon mold (85 mm × 230 mm) and were dried in a vacuum oven at 50 °C until reaching constant weight. Dried films were peeled and stored in desiccators at 25 °C and 51% relative humidity (RH) until evaluation. Saturated magnesium nitrate (Merck, Darmstadt, Germany) solution was used to meet required relative humidity.

2.4. Determination of physical properties of films

2.4.1. Film thickness

Film thickness was measured using a micrometer (Mitutoyo No. 293-766, Tokyo, Japan). Measurements were made in at least five different places on each film and an average value was calculated.

2.4.2. Moisture content

The films’ moisture content (approximately 1 cm × 3 cm) was determined by measuring the weight loss of films before and after drying in a laboratory oven (Blue M Electric Co., Blue Island, IL) at 103 ± 2 °C until constant weight was reached (dry sample weight). Three replications of each film treatment were used for calculating the moisture content.

2.4.3. Film solubility in water

For this study, solubility in water was defined as the ratio of the water-soluble dry matter of film that is dissolved after immersion in distilled water [16]. A circular film sample was cut from each film, dried at 103 ± 2 °C for 24 h in a laboratory oven, and weighed to determine the initial dry weight. The solubility in water of the different SC films was measured from immersion assays in 50 ml of distilled water with periodic stirring for 6 h at 25 °C. After that period, the remaining pieces of films were taken out and dried at 103 ± 2 °C until constant weight (final dry weight).
The percentage of the total soluble matter (% TSM) of the films was calculated using Eq. (1):

% TSM = \frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}} \times 100 \tag{1}

TSM tests for each type of film were carried out in three replicates.

2.4.4. Water vapor permeability

Standard method E 96 [17] was used to determine water-vapor transmission rate, with a 75% RH gradient at 25 °C. Diffusion cells containing anhydrous calcium chloride desiccant (0% RH, assay cup) were sealed by the test film (0.00287 m² film area). To maintain a 75% RH gradient across the film, a sodium-chloride-saturated solution (75% RH) was used in the desiccators. The RH inside the cell was always lower than outside, and water-vapor transport was determined from the weight gain of the diffusion cell at a steady state of transfer. Changes in the weight of the cell were recorded to the nearest 0.0001 g and plotted as a function of time. The slope of each line was calculated by linear regression ($r^2 > 0.99$), and the water-vapor transmission rate was calculated from the slope of the straight line (g/s) divided by the test area (m²). All values for water-vapor transmission rate (WVTR) were corrected for air-gap distance between the calcium chloride and the film surface according to the equations of Gennadios, Weller, and Gooding [18].

After the permeation tests, the film thickness was measured, and water-vapor permeability (WVP) (g Pa⁻¹ s⁻¹ m⁻¹) was calculated as

\[ \text{WVP} = \frac{\Delta m}{A \Delta t \Delta p} \tag{2} \]

where $\Delta m/\Delta t$ is the weight of moisture gain per unit of time (g/s), $X$ is the average film thickness (mm), $A$ is the area of the exposed film surface (m²), and $\Delta p$ is the water vapor pressure difference between the two sides of the film (Pa). WVP was measured for three replicated samples for each type of film.

2.5. Mechanical properties

According to ASTM standard method D882 [19] a Testometric Machine M350-10CT (Testometric Co. Ltd, Rochdale, Lancs., England) was used to measure tensile strength (TS), elongation at break (EB) and elastic modulus (EM) of the SC composite films. All film strips were equilibrated at 51% RH for 48 h into a desiccator using saturated magnesium nitrate solution. The films were fixed with an initial grip separation of 50 mm and stretched at a crosshead speed of 50 mm/min. A microcomputer was used to record the stress–strain curves. At least 8 replicates of each test sample were run to achieve dependable data.

2.6. Thermal characteristics

The thermal properties of the films were carried out using DSC equipment (TA Instrument, New Castle, Del., USA). All samples of 2–4 mg were sealed in standard aluminum dishes, using a sealed empty aluminum dish as the reference sample. All samples were scanned at a heating rate of 10 °C/min between temperature ranges of −50 and 250 °C. Nitrogen was used as the purge gas at a flow rate of 20 ml/min. The melting point ($T_m$) was calculated as the temperature where the peak of the endotherm occurs.

2.7. Film microstructure

Microstructural analysis of the surface and cross-sections of the dried films was conducted by scanning electron microscopy (Philips-XL30, Eindhoven, Netherlands). All films were fractured in liquid nitrogen, mounted on aluminum stubs using a double-sided adhesive tape, and sputtered with a thin layer of gold using a BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein). All samples were examined using an accelerating voltage of 10.0 kV. Samples were photographed at an angle of 90° to the surface to allow observation of the films' cross-section.

2.8. Evaluation of antimicrobial activity of films

The disk-diffusion method was used to examine the antimicrobial characteristics of the films. Disks with a diameter of ~6 mm were cut out from the all sample films using a sterile punch. These disks were then placed on plates that contained a medium (blood agar and nutrient agar, solid-state medium). The medium had been previously inoculated by 100 µl of an overnight broth culture containing approximately $10^8$ CFU/ml of the test bacteria. The plates were incubated at 37 °C for 24 h. The diameter of the growth inhibition zones was measured using a caliper to the nearest 0.02 mm. The whole zone area was calculated then subtracted from the film disc area and this difference in area was reported as the “zone of inhibition” [20]. The tests were carried out in triplicate for each formulation.

2.9. Statistical analysis

Microsoft Windows Excel 2007 and SAS software (Version 9.1, Statistical Analysis System Institute Inc., Cary, NC, USA) were used to analyze the resulting data. Data were initially evaluated by analysis of variance (ANOVA), and then a Duncan’s multiple range tests were used to compare the difference among mean values of films’ properties at the level of 0.05.

3. Results and discussion

Table 1 shows the impact of incorporating MEO and fatty acids on the physical properties of SC films. Film thickness varied from 0.15 to 0.26 mm.

Films with SA/OA and MEO had lower moisture content – 19.76 and 14.16%, respectively – compared with the control films (21.73%). These results were similar to those of Ghasemlou, Khodaiyan, Oromiehie, and Yarmand [21], whose kefiran films

| Table 1 Comparison of physical properties of SC films obtained in this study (SC, sodium caseinate; OA, oleic acid; SA, stearic acid; MEO, Matricaria recutita essential oil; WVP, water vapor permeability).\(^a\)\(^b\). |
|---|---|---|---|---|
| Film type | Thickness (mm) | Moisture content (% d.b.) | Solubility in water (%) | WVP ($\times 10^{-10}$ g m⁻¹ s⁻¹ Pa⁻¹) |
| SC | 0.15 ± 0.03b | 21.73 ± 0.92a | 85.28 ± 5.33a | 1.09 ± 0.14a |
| SC+SA/OA | 0.21 ± 0.02a | 19.76 ± 0.83ab | 67.30 ± 7.64b | 0.95 ± 0.11b |
| SC + MEO | 0.26 ± 0.04a | 14.16 ± 0.26c | 43.91 ± 4.11c | 0.49 ± 0.05c |

\(a\) Means within each column with same letters are not significantly different ($P < 0.05$).

\(b\) Data are means ± SD.
tended to become more hydrophilic with an increase in oleic-acid content.

The solubility of the emulsified films decreased similarly. Lower solubility of MEO-containing films, compared to those containing fatty acids, may be explained by an increase in interaction between the hydroxyl groups of SC chains and MEO components, leading to a decrease in the availability of hydroxyl groups and consequent reduction in protein–water interactions. There is a possibility that polyphenolic compounds may be able to fit into the SC matrix and established interactions such as hydrogen or covalent bonding with active groups of SC; this would reduce availability of hydroxyl groups for interaction with water molecules, consequently leading to a more water-resistant film [22]. Table 1 shows that the average values of WVP decreased with incorporation of fatty acids and MEO. This was expected, as an increase in the hydrophobic compound fraction of a film usually leads to an improvement in its water-barrier properties. Our data showed that films prepared from SC and MEO exhibited lower transmission rates than those films with fatty acids. The effectiveness of MEO in reducing WVP can be partially explained by the more hydrophobic nature of the main components of this essential oil (apigenin, quercetin, patuletin, and luteolin), which are less polar than those of fatty acids. These results are in accordance with SEM micrographs of cross-sections of prepared films.

3.2. Mechanical properties

Mechanical properties reflect the durability of films and their ability to enhance the mechanical integrity of foods.

Fig. 1 shows the effects of incorporating fatty acids and MEO on the mechanical properties of SC films. The TS of the control film was 10.9 MPa; this decreased to 3.4 MPa in the films with fatty acids, and to 2.5 MPa in the films with MEO. On the other hand, the EB for the control casein film increased from 65.4% to 23.2% and 29.2% for the films with SA/OA and MEO, respectively. The addition of fatty acids caused a significant decrease ($P<0.05$) in TS due to the introduction of some discontinuities in the polymer matrix of the dried films, especially for the film with MEO. The poor mechanical properties obtained by the addition of MEO may be related to the structural arrangement of the lipid phase in the SC matrix. Thus, the structural discontinuities provoked by the incorporation of the MEO could explain the composite films’ lowest resistance to fracture. Some of these results are in line with those reported by Fabra et al. [7], who showed that plain films are stronger than films supplemented by oil compounds. However, the higher molecular weight of individual compounds such as phenolic constituents present in the MEO, as well as more hydrophobic properties of MEO compared with SA/OA, might have contributed to the lower tensile strength than that shown by fatty acid-formulated films by contributing to a decrease in interchain interactions.

3.3. Thermal characteristics

The thermal behavior of the pure and lipid-incorporated SC films was investigated using DSC.

Fig. 2 shows typical DSC curves for SC films and films with added SA/OA or MEO. The control films had a melting point ($T_m$) between 130 and 140 °C, although Chick and Hernandez [14] had reported a $T_m$ of 112.87 °C. This difference in the thermal property is reasonable due to the different method for film preparation, source of SC and effect of plasticizer. Thermal analysis of films containing OA/SA showed two endothermic peaks: at 46 and 146 °C. The incorporation of fatty acids into the film structure increased the total specific thermal capacity. Therefore, the DSC curve of these films was lowered relative to the DSC curve of the film without lipids. The DSC curve of film containing MEO showed also two endothermic peaks at 51 and 116 °C; these were very close to those of films with fatty acids. This decrease may be explained by the molecular structure of MEO, which has an effect on the overall chain mobility in the SC film [14]. These results coincide closely with those from the SEM studies, where a partial separation of the two phases may have occurred.

3.4. Film microstructure

In an attempt to study microstructural changes in the films, scanning electron microscopy (SEM) was conducted to visualize the surface and cross-section topography of all emulsified SC films.
Fig. 2. Representative examples of DSC curves of SC (a), SC-OA-SA (b) and SC-MEO (c) films.

Table 2
Antimicrobial activities of various emulsified SC films (SC, sodium caseinate; OA, oleic acid; SA, stearic acid; MEO, Matricaria recutita essential oil).\(^a,b\).

<table>
<thead>
<tr>
<th>Film type</th>
<th>Inhibition zone (mm(^2))</th>
<th>S. aureus</th>
<th>L. monocytogenes</th>
<th>E. coli (O157:H7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>0.00b</td>
<td>0.00c</td>
<td>0.00b</td>
<td></td>
</tr>
<tr>
<td>SC + (SA/OA)</td>
<td>10.88 ± 2.18a</td>
<td>11.55 ± 2.27b</td>
<td>0.00b</td>
<td></td>
</tr>
<tr>
<td>SC + MEO</td>
<td>15.91 ± 2.13a</td>
<td>30.46 ± 9.25a</td>
<td>10.22 ± 14.43a</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Data are means ± SD.
\(^b\) Values within each column with different letters are significantly different (\(P<0.05\)).

Fig. 3 shows SEM micrographs of the outer surface (left) and cross-section (right) for the SC films with fatty acids or MEO. The pure SC film displayed a compact, smooth, and continuous microstructure with no irregularities. However, adding lipids, either fatty acids or MEO, created a heterogeneous structure in which oil droplets were entrapped in the continuous polymer network. The microstructure of the films containing SA/OA showed the appearance of lipid droplets dispersed throughout the surface, which could possibly lead to the formation of an uneven surface. However, MEO-containing films showed more extensive discontinuities compared to those containing fatty acids. This difference is probably due to the different behavior of oils during homogenization and drying, which is determined by the oil type and the complex interactions between the oil and the protein, resulting in different dried-film structures [23]. The existence of holes in these films might be related to the volatility of the essential oil.

3.5. Antimicrobial properties

Table 2 shows the inhibitory effect of emulsified SC films against L. monocytogenes, S. aureus, and E. coli O157:H7. There was no inhibition zone around the pure SC films, indicating that the SC films alone had no antimicrobial activity.

This observation was in agreement with that reported by Kristo et al. [3], who showed that additive-free SC films were not effective by themselves in inhibiting the growth of bacteria. The results showed that all composite films containing fatty acids and MEO inhibited the growth of the three test bacteria, with the exception of E. coli, which was not inhibited by the incorporation of fatty acids. As expected, antimicrobial activity was stronger when MEO was incorporated into films. L. monocytogenes was found to be the most sensitive bacterium to MEO, followed by S. aureus and E. coli.

An inhibition zone of 30.46 mm\(^2\) was observed for L. monocytogenes around the disc with MEO. The total inhibitory zone was 15.91 mm\(^2\) for S. aureus and 10.22 mm\(^2\) for E. coli. The inhibitory effect of MEO-added films on these bacteria indicated a diffusion of MEO from these films to the solid medium, which consequently inhibited their growth. S. aureus and L. monocytogenes are Gram-positive microorganisms that are more sensitive to essential oil [24]. It may be that the cause of higher resistance of Gram-negative bacteria to plant essential oils is the more complex double membrane enveloping these organisms, as compared with the single-membrane glycoprotein/teichoic acid of Gram-positive bacteria. Resistance also seems to be related to the rate of antimicrobial dissolution in the lipid phase of the membrane. The constituents of MEO thought to have antimicrobial properties include α-bisabolol, luteolin, quercetin, and apigenin. In a recent study by Tolouee, et al. [25], α-bisabolol was recognized as the main constituent of MEO, comprising 56.86% of the total oil. Therefore, the antibacterial activity of MEO may be attributed to the presence of α-bisabolol in the oil as the main component, but the mechanism of the action is still unclear.
4. Conclusion

MEO has potential for incorporation into the design of new film-forming dispersions based on SC. Incorporation of OA/SA and MEO improved the barrier properties of casein-based films; WVP was reduced the most by the incorporation of MEO. The presence of fatty acids and MEO decreased TS while increasing EB values. The TS value decreased when fatty acids were added; however, it reached its lowest value when MEO was added. Although a visual analysis showed the films to be homogeneous with no porosity, when observed at microscopic level they showed roughness, which can be attributed to the formation of greater lipid aggregates in both the internal and surface parts of the film. Thermal analysis of all emulsified films showed two endothermic peaks; these results confirmed those obtained by SEM studies, where a partial separation of the two phases occurred. Films containing MEO exhibited a high inhibitory effect on *L. monocytogenes*, *S. aureus*, and *E. coli*. This work showed the possibility of obtaining advanced edible films having flexibility and good antimicrobial and barrier properties when MEO is incorporated into SC films. The films developed in this study will have applications in packaging a wide range of food products, particularly those that are highly oxidative and microbial-sensitive. Further investigations are needed to test the effectiveness of these films on selected food systems.
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References