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Properties of Solvent Cast Polycaprolactone Films Containing Pomegranate Seed Oil Stabilized with Nanocellulose[#]

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ABSTRACT

The increase of consumer demand for using natural products and reducing the use of non-compostable packaging materials have encouraged research on biodegradable polymers including natural components such as essential oils. Pomegranate seed oil (PSO) has active properties such as antimicrobial and antioxidant activities. The aim of this study was to prepare active polycaprolactone (PCL) films by using PSO. PCL films including PSO emulsions (5-30%), which were stabilized with nanocellulose (NC) particles, were prepared by casting method. The physical and active properties of PCL films were determined by means of water vapor permeability (WVP), mechanical properties, optical properties, release behaviour, and potential antimicrobial activity. The WVP values of PCL films was lower when incorporated with NC-stabilized PSO emulsions. The incorporation of PSO into PCL films in the form of NC-stabilized emulsions significantly reduced the transmittance and lightness values, which resulted in an increase in opacity. In the release tests, the slower release of PSO was observed for NC-stabilized films. The stabilization of PSO with NC showed to be less effective when high concentrations of oil (30%) were used. Film samples showed potential antimicrobial activity against *Escherichia coli*, and *Listeria monocytogenes*, however, a clear zone of inhibition around the film samples was not detected. Results also suggested that the antimicrobial effect was dependent on two important factors: the release behaviour of PSO through the film samples and, the direct interaction between PSO and microorganisms. These results showed that the combination PCL films and PSO stabilized with NC could be an interesting approach in active packaging technologies.

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Introduction

Essential oils are natural volatile compounds, which are formed by aromatic plants as secondary metabolites (Bakkali et al., 2008). These compounds are known for their active properties such as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and antioxidant properties. Among the various natural active agents, pomegranate seed oil (PSO) has gained attention due to its promising properties. PSO is extracted from pomegranate seeds and mainly includes punicic acid with oleic acid, linoleic acid and α-eleostearic acid (Kiralan et al., 2009). PSO has been studied for its beneficial effects like antioxidant and antimicrobial properties (Syed et al., 2007; Medjakovic and Jungbauer, 2013; Melo et al., 2014). PSO is a potentially valuable compound for food industry. However, the use of oils alone such as PSO has some drawbacks due to their sensitive structure to light, oxygen, heat and moisture, volatility, having strong odor and weak processability. Besides, the poor solubility of PSO in water limits its use for food applications. To overcome these limitations, PSO can be entrapped with a stabilizing agent into an efficient delivery system such as biodegradable polymers to promote easier handling and appropriate release.

Inorganic particles have been mostly used as stabilizers, but recently it has been shown that nanocellulose (NC) could also stabilize various systems. Different NC types have been stated as a potent stabilizing agent in solid-stabilized emulsions due to the amphiphilic character at the nanocrystals surface (Ougiya et al., 1997). Bacterial cellulose, nanofibrillated cellulose or cellulose nanowhiskers have been successfully used in water emulsions based on peppermint oil (Kasiri and Fathi, 2017), maize germ oil (Zhu et al., 2018), L-ascorbic acid (Khalid et al., 2013). In those mentioned systems, nano-sized particles form a rigid structure by adsorbing at the liquid−liquid interfaces and then provide inhibition of droplet coalescence. However, it requires an optimum balance between the solid−liquid and liquid−liquid interfacial tensions, which can be achieved when particles exhibit an amphiphilic character (Sebe et al., 2013).

In recent years, a tremendous attention has been attracted using bio-based or biodegradable polymers to face the environmental problems arising from excessive use of fossil based polymers (Gross and Kalra, 2002). Polycaprolactone (PCL) is a synthetic biodegradable polymer with its enhanced mechanical and barrier properties (Sharmin et al., 2012). Fabrication of active PCL films by incorporating active agents like PSO is a promising strategy. However, the direct incorporation of PSO into PCL films may result in poor adhesion, incomplete dispersion and phase separation. Therefore, the aim of this study was to combine NC-stabilized PSO with PCL to form an active film suitable for food packaging applications.

Material and Methods

Materials

Polycaprolactone (PCL, CAPA 6800) was purchased from Perstorp Holding AB (Sweden). Nanocellulose (NC), which has a crystallinity index of 80% and crystal length of 100 nm, was supplied by Blue Goose Biorefineries Inc. (BGB ULTRA^{TM}, Canada) in 8.0% (w/w) suspension. Ethyl acetate was used as an effective alternate solvent of chloroform, because chloroform is more toxic. Ethyl acetate (anhydrous, 99.8%) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals were analytical grade and purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Pomegranate seed oil (PSO) (including 71.93% punicic acid) was kindly obtained from the Western Mediterranean Agricultural Research Institute (Antalya, Turkey).

Preparation and Characterization of NC-stabilized PSO

The NC-stabilized emulsions were prepared using PSO and a NC aqueous suspension with 50mM NaCl at the required concentration. The NC particle and PSO ratio is one of the most important parameters on the performance of emulsion. In the preliminary studies, it was observed that the oil droplets started to coalescence when concentrations of NC lower than 0.5 mg/mL suspension were used. Besides, higher concentrations caused NC to accumulate and to be less effective. Thus, the concentration of NC in the aqueous suspension containing 50 mM NaCl was maintained at 2 mg/ml. It was observed that selected NC concentration is sufficient to cover the interfacial area. The oil/aqueous phase ratio was maintained as 20/80. The mixture of PSO and NC suspension was then homogenized (5000 rpm) for 5 min (DAIHAN HG-15A, Korea) to prepare the emulsion.

The average diameter (nm), zeta potential (mV), and polydispersity index (pdi) of prepared PSO-nanoemulsion were measured with a nanoparticle analyzer (Horiba Scientifica, Nanopartica, SZ-100V2) to ensure the success of the nanoemulsion production. The PSO included NC-based emulsion was observed by a Carl Zeiss inverted microscope (Primo Vert, Germany) using a digital camera (Primo Vert HDcam, Germany). The image of diluted PSO-nanoemulsion was taken with Labscope 2.0 (Carl Zeiss, Germany) video acquisition software.

Subsequently, the prepared emulsion was freeze-dried (BW-100F, Bluewave Industry Co., Ltd. Shanghai, China) to obtain NC-stabilized PSO.

Film Preparation

A PCL film solution was obtained by dissolving the PCL in ethyl acetate (5%, w/w). NC-stabilized PSO at 5, 10, 20, and 30% w/w in a dry PCL-based film were then added to the PCL film solution. 50 g of each film solution was cast onto a Teflon® coated plate (Ø=150 mm) and dried in ambient conditions. All film samples were conditioned at 25°C and 50% RH for one week before analysis. The thickness of conditioned films was measured at six random positions with a digital micrometer (Digimatic Micrometer Quantu-Mike IP65, Mitutoyo, Japan). PCL films containing NC-stabilized PSO at 5, 10, 20 and 30% w/w were coded as PCL-5, PCL-10, PCL-20 and PCL-30, respectively.

Optical Properties

The opacity of film samples was determined by placing 1×4 cm rectangular film strips into a UV-visible spectrophotometer (Shimadzu, UV-1601, Japan) test cell to take the absorption spectrum of the sample from 400 nm to 800 nm. The results were measured by the area under the curve divided by the film thickness and expressed in absorbance units (AU nm/mm). The transparency of the films was determined by measuring the percent transmittance at a wavelength of 450 nm using a UV-visible spectrophotometer (Shimadzu, UV-1601, Japan). The color of the films was measured with a Minolta Chroma Meter (CR-400, Konica Minolta, Inc., Japan) by using a white standard calibration plate (Y = 92.7, x = 0.3160, y = 0.3321) as a background for color measurement of the films. Results were expressed as CIE L^* (lightness), a^* (red–green) and b^* (yellow–blue) coordinates in the color space.

Mechanical Properties and Water Vapor Permeability (WVP)

The mechanical properties, namely tensile strength (TS) and elongation-at-break (E, %) were determined by the ASTM standard method D882 (ASTM, 2018). Films were mounted in the film-extension grips of the universal testing machine (Lloyd LR5, AMETEK, Inc, UK) and stretched at 50 mm/min.

The WVP of films was determined according to the E96/E96M-16 gravimetric method (ASTM, 2016). Film samples were exposed to 100% RH and the permeability measurements were performed by weighing the cups periodically (every 1.5 h for 48 h) at 25°C.

Release Studies

The release of PSO from the film samples into food simulant D1 (Ethanol 50% v/v) was tested. 100 mg film sample was immersed in 10 mL of the simulant in amber glass and continuously stirred throughout the experiment. After various exposure times, the concentration profile of each simulant was determined by absorbance measurements using the corresponding calibration curve. The most abundant active compound in PSO is punicic acid. The Korsmeyer Peppas (Korsmeyer et al., 1983) model was applied to the data to estimate the release kinetics.

$$M_t/M_\infty = kt^n$$

Where M_t/M_∞ is the fraction of active compound released at time (*t*), *k* is the rate constant (a characteristic of the matrix related to the diffusion process) and *n* is the diffusion exponent (characteristic of the release mechanism).

Antimicrobial Activity

The antimicrobial effects of film samples including PSO were tested against *Escherichia coli* (ATCC 26922) and *Listeria monocytogenes* (ATCC 19115) with a zone of inhibition assay on solid media. All microorganisms were incubated at suitable temperatures before the tests and the brain heart infusion (BHI) solid media was inoculated with these cultures (with colony counts from 10^6 to 10^8 CFU/mL). Film samples (Ø =15 mm) were placed on Petri dishes inoculated with bacterial strains. The plates were incubated at 37°C for 24 h and were then examined for antimicrobial activity.

Statistical Analysis

An analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to compare the different treatments at a 95% confidence level. The statistical analysis was performed using Minitab 17 software (Minitab Inc., Brandon, UK). Three observations were performed for each sample and each experiment was replicated three times.

Results and Discussion

Characterization of Prepared PSO-emulsion

The images of PSO emulsion were taken with an optical microscopy. As can be seen in Figure 1, PSO was dispersed in NC aqueous suspension in 50 mM NaCl without aggregation. This behavior might be due to that NC particles effectively adsorbed at the interface, resulting in a resistance against coalescence (Kalashnikova et al., 2012). The average diameter (nm), zeta potential (mV), and polydispersity index (pdi) of prepared PSO-emulsion were found as 151.3±14.6, -37.9±5.1, and 0.6±0.1, respectively. The zeta potential lower than -30 mV and higher than 30 mV are indication of stable systems to be sufficient for ensuring physical stability of nanoemulsion (Gurpreet and Singh, 2018). The zeta potential of prepared emulsion is within acceptable limits (±30 mV), which can be accepted as stable emulsion. The average diameter and pdi were used to understand the success of the nanoemulsion production. Typical nanoemulsions present pdi values between 0.3 and 0.5 and higher pdi values than 0.3 are indication of destabilized emulsions (Zdrali et al., 2019). The average diameter of prepared emulsion is between 20-200 nm that can be accepted as nanoemulsion (Gurpreet & Singh, 2018).

Optical Properties

Table 1 shows the optical properties of film samples. Visually, oil droplets were not detected on the surface of film samples by the help of high resistance to coalescence for the film solution including NC stabilized PSO. PCL films showed 30.07% transmittance whereas PSO including films presented significantly lower transmittance due to the existing of NC-stabilized oil layer, which could be used as an effective light barrier. These types of films could meet the requirements needed to prevent food products from oxidation induced by light. In general, the increasing amount of PSO resulted in lower transmittance and higher opacity values (P<0.05), which might be due to the impenetrable matrix created by oil droplets promoting light scattering throughout the film (Tongnuanchan et al., 2013). PCL films showed the highest L^* values consistent with the transparency values (P<0.05). The L^* values did not change significantly, however, the a^* and b^* values were found significantly higher than those of PCL films (P<0.05).

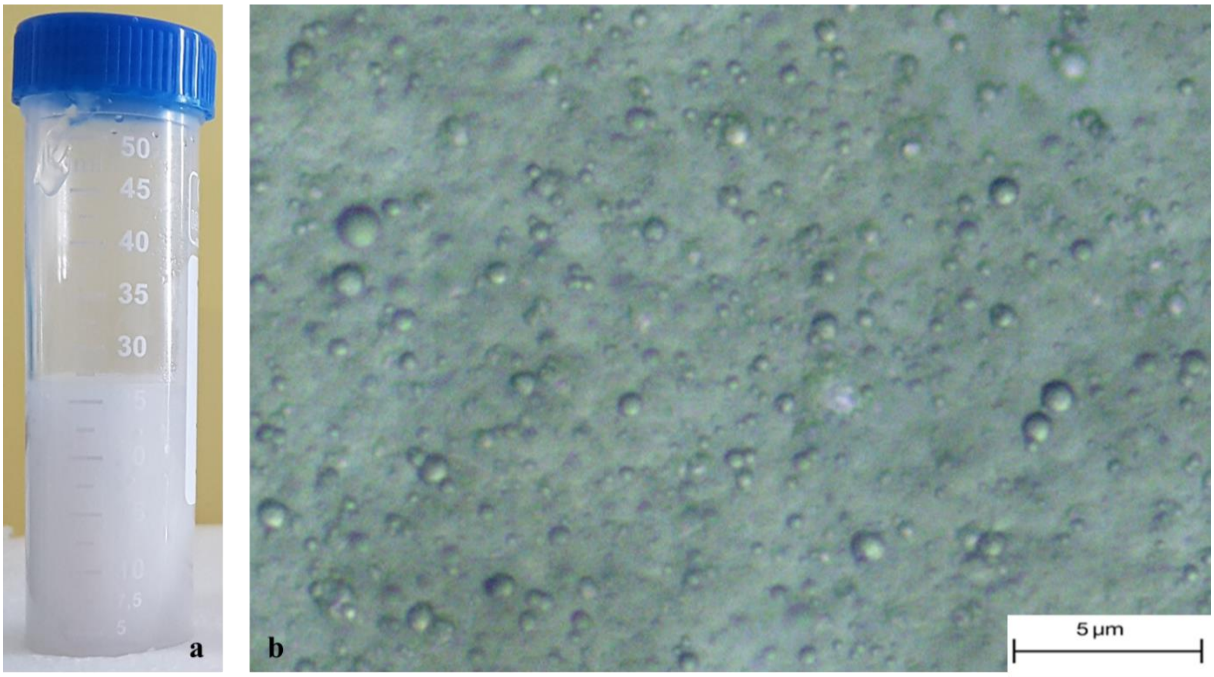

Figure 1 The image (a) and optical micrograph of prepared PSO-emulsion (b)

Table 1 Optical properties

Film sample	Opacity (AU nm/mm) ×10^3	T (%)	*L**	*a**	*b**
PCL	1.76±0.04^{c}	30.07±0.12^{a}	97.28±0.09^{a}	-0.13±0.03^{a}	2.13±0.02^{d}
PCL-5	2.71±0.15^{b}	3.93±0.50^{b}	96.52±0.07^{c}	-0.48±0.01^{c}	3.60±0.15^{a}
PCL-10	3.40±0.29^{ab}	3.63±0.35^{b}	96.60±0.04^{bc}	-0.46±0.04^{c}	3.30±0.12^{b}
PCL-20	3.26±0.15^{ab}	2.53±0.46^{b}	96.91±0.06^{b}	-0.37±0.01^{b}	2.87±0.10^{c}
PCL-30	3.92±0.35^{a}	2.40±0.26^{b}	96.84±0.24^{bc}	-0.36±0.05^{b}	2.85±0.07^{c}

$^{a\text{-}d}$ Means in the same column with different superscripts differ significantly (P<0.05) according to Tukey test

Table 2 Mechanical properties and water vapor permeability values

Film sample	Thickness (mm)	WVP ($10^{-6}gh^{-1}m^{-1}Pa^{-1}$)	TS (MPa)	E (%)
PCL	0.11±0.01^{b}	0.95±0.68^{b}	*	*
PCL-5	0.16±0.02^{ab}	3.10±0.62^{a}	*	*
PCL-10	0.19±0.02^{a}	3.16±0.78^{a}	31±2^{a}	40±1^{a}
PCL-20	0.19±0.01^{a}	1.59±0.33^{ab}	18±3^{b}	68±2^{a}
PCL-30	0.20±0.04^{a}	2.35±0.85^{a}	17±2^{b}	6±1^{b}

*PCL and PCL-5 films did not break at determined conditions
$^{a\text{-}b}$ Means in the same column with different superscripts differ significantly (p<0.05) according to Tukey test.

Table 3 Korsmeyer-Peppas model parameters

Film sample	*n*	*k*	R^2
PCL-5	0.2035^{c}	0.8934^{b}	0.89
PCL-10	0.2376^{b}	0.7644^{c}	0.87
PCL-20	0.3066^{a}	0.7688^{c}	0.78
PCL-30	0.2412^{b}	1.0194^{a}	0.88

$^{a\text{-}c}$ Means in the same column with different superscripts differ significantly (P<0.05) according to Tukey test

Mechanical Properties and Water Vapor Permeability (WVP)

Table 2 shows the mechanical properties and WVP values of film samples. The thickness of films increased when NC stabilized PSO was added to PCL films, whereas the thickness of PCL-30 films were the highest (P<0.05). The differences in the thickness of the film samples can be related to the interactions between PCL and other ingredients inside the film matrix (Talon et al., 2017).

The tensile strength (TS) of film samples decreased with the increase in PSO content (P<0.05). PCL-10 films exhibited the highest TS values while high oil fraction made films fragile. This behavior can be explained by the interfacial interactions between stabilized oil droplets and polymer matrix. Similarly, Zhu et al. (2018) reported that the high oil concentration resulted in more fragile films. The addition of higher PSO to the film samples tended to increase the chain mobility and decrease the TS. The decrease in TS at higher PSO levels than 20% might be also associated with an incompatibility between polymer and NC stabilized PSO. Besides, up to 20% PSO content the elongation of PCL films significantly increased and above 20% of PSO, it tended to decrease (P<0.05). These results are in agreement with Chen et al. (2016) who studied chitosan films including cinnamaldehyde-loaded nanoemulsions.

PCL films showed the lowest WVP values, and the increase in PSO content caused a decrease in water barrier resistance. The addition of a more hydrophobic compound did not result in a strong decrease in WVP. Similarly, it was reported that the addition of thymol nanoemulsions to protein/chitosan films (Robledo et al., 2018) and the addition of carvacrol and cinnamaldehyde nanoemulsions (Otoni et al., 2016) to soy protein increased the overall permeability.

Release Studies

The Korsmeyer-Peppas model parameters for PSO release in D1 simulant are shown in Table 3. For thin films, the *n* values equal to 0.5 is explained by Fickian diffusion and *n* equals to 1.0 is explained by relaxational transport, whereas anomalous transport behavior is defined by *n* values between 0.5 and 1.0 (Siepmann and Peppas, 2011). All film samples had lower *n* values than 0.5 resulted in non-Fickian behavior. These small values of *n* might be attributed to the partial film solubility in D1 simulant. The highest *k* value was observed in PCL-30 films and there was no significant difference between other film samples for *k* values.

The release behavior of PSO from film samples as a function of time is shown in Figure 2. The release of an agent from a polymeric matrix occurs firstly with the diffusion of solvent through the polymer matrix followed by the relaxation and then finally finished by the diffusion of agent through the polymer matrix (Tampau et al., 2018). The increase in PSO content resulted in faster release. The interaction between PCL and 5% of NC stabilized PSO might be favored thus leading a slower release of PSO with a more close structure.

Antimicrobial Activity

The agar diffusion method was performed to evaluate the inhibition capacity based on the clear zone surrounding the hole. The PCL films without NC-stabilized PSO were chosen as control and there was also a microbial growth under PCL films in solid media. Even though there was no growth with PSO-added film samples in the solid media inoculated with microorganisms, films did not show a clear zone. Since there was no microbial growth under PCL films including NC-stabilized PSO, it was considered that these films had a potential antimicrobial activity. This behavior can be explained by the decreased accessibility of NC-stabilized PSO compounds due to the effectiveness of NC adsorption at the oil interface. The strong interaction between PSO and NC inside PCL matrix might also prevent PSO from rapid release into solid media (Froiio et al., 2019). Recently, Xylia et al. (2017) reported that pomegranate seed extracts and oils had the potential to inhibit various microorganisms. Other researchers who have studied PCL films including different antimicrobial agents like pine resin (Chang et al., 2019), *Thymus capitatus* and *Origanum vulgare* essential oils (Granata et al., 2018) and pomegranate rind (Khalid et al., 2018) reported that the minimum inhibitory concentration of active agents was greater than the acceptable levels included in the films. The authors also stated that the effectiveness of antimicrobial activity of the film samples was strongly related to the retention and diffusivity mechanism of active compounds in the matrix.

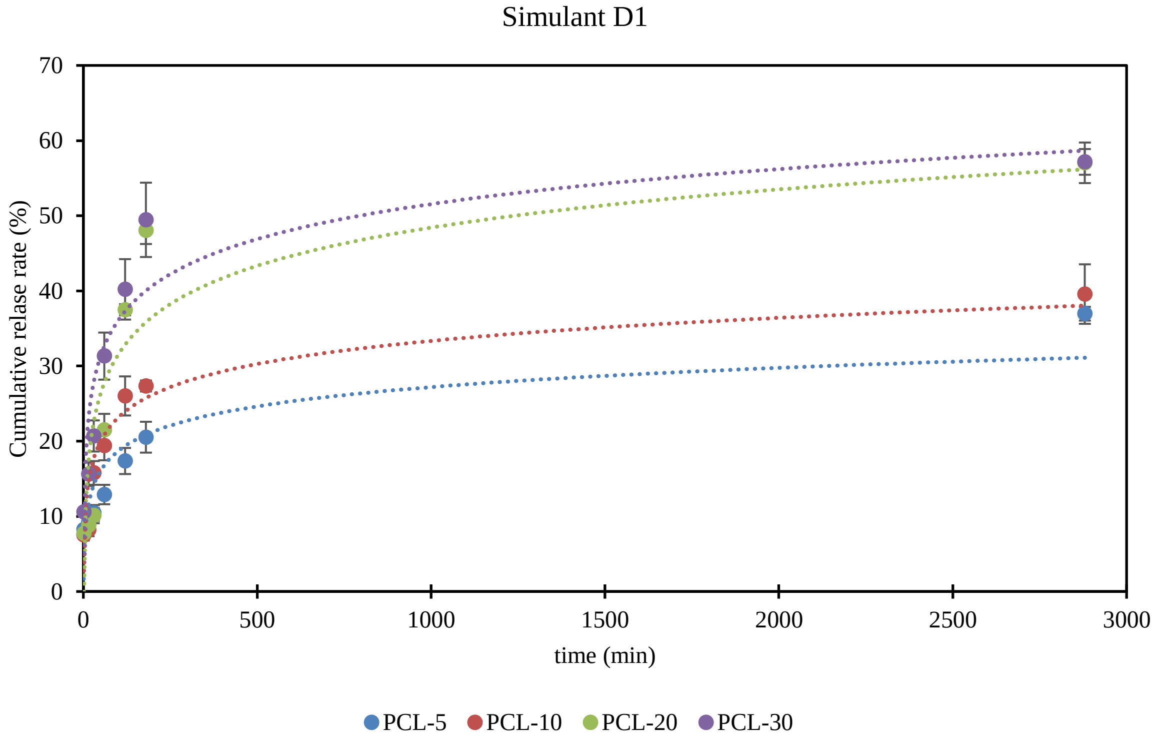

Figure 2 Cumulative release of PSO from film samples

Conclusion

In this study, the active PCL films were prepared by using PSO emulsions (5-30%), which were stabilized with NC particles. The barrier properties against water and the tensile strength of films including NC-stabilized PSO slightly decreased however, the release of PSO from films showed slower rates when stabilized with NC. The incorporation of PSO into PCL films in the form of NC-stabilized emulsions reduced the transmittance and lightness values whereas film samples showed a potential antimicrobial activity against *Escherichia coli* and *Listeria monocytogenes*. The stabilization of PSO with NC showed to be less effective when high concentrations of oil (30%) were used. These results suggest that the combination of PCL films and PSO stabilized with NC could be an interesting approach in active packaging technologies.

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