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Starch and whey protein isolate films including an aroma compound stabilized by nanocellulose

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Abstract: Bergamot essential oil (BO) shows biological activities and is widely used as a flavoring for food products; however, its application in foods is limited because of the low stability and solubility in water. This study aimed to prepare whey protein isolate (WPI) and starch (S) films, including nanocellulose based BO nanoemulsions, potentially enhancing BO functionality. BO nanoemulsion was obtained by using a nanocellulose dispersion (2 mg/mL) including 3 mM $CaCl_2$ with a ratio of 2/8 (v/v, BO/nanocellulose dispersion) and then suitably dispersed (2%, w/w) in the WPI and S film-forming solutions to obtain film samples. The water vapor permeability (WVP), mechanical and optical properties and BO's release from those obtained films were studied. The WVP ($p<0.05$) and tensile strength ($p>0.05$) of films were improved, whereas opacity increased with the addition of BO nanoemulsion ($p<0.05$). The release of BO from S films was faster than in WPI films. These results showed that nanocellulose could be used as carriers for essential oils such as BO to enhance its functionality within bio-polymeric matrices intended to be used as relevant carriers of aroma compounds.

Key words: Bergamot oil, nanomaterials, nanoemulsion, starch, whey protein isolate.

INTRODUCTION

Essential oils, as secondary metabolites of aromatic flowers and plants, show lipophilic, volatile, and unstable characters and have been widely used as flavors, fragrances, and active agents, mainly due to their active properties such as antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Vahedikia et al. 2019). Bergamot oil (BO) is one of those mentioned valuable oils, including linalool and linalyl acetate (Mannucci et al. 2017). Many studies have demonstrated that BO possessed antimicrobial, anti-inflammatory, antiviral, and anticancer properties (Bora et al. 2020, Marchese et al. 2020). Besides, the BO flavor components are used in many food products such as Earl Grey tea (Orth et al. 2013); however, these components are often dissipated during the preparation and/or storage of food products due to their highly volatile and unstable nature (He et al. 2018). Moreover, the use of essential oils in different applications is hindered due to their low bioavailability, poor water solubility, low stability, and rapid oxidation (Gromadzki et al. 2017). Researchers have proposed different approaches such as encapsulation to improve stability of hydrophobic active agents, to enhance the solubility of essential oils, to provide efficient resistance during processing, to control the release rate, as well as for better stability and hiding unwanted flavors or enhancing flavor properties (Assadpour & Mahdi Jafari 2019, Das et al. 2021, Özogul et al. 2022, Rehman et al. 2020, Riaz et al. 2019). Such novel systems include nanoparticles, micelles, nanoemulsions, nano-hydrogels, liposomes, chelation with

metals, and phospholipid complexes (Asabuwa Ngwabebhoh et al. 2018, Bahrami et al. 2020).

The nanoemulsions are composed of an aqueous phase and an oily phase in droplets with smaller diameters than 100 nm (Ashaolu 2021). These systems reduce the gravitational force due to the smaller size of the particles, thus avoiding coalescence or flocculation, interface deformation and disruption, and sedimentation during storage (McClements & Gumus 2016). Solid particles such as graphene (Wang et al. 2021), clay (Yu et al. 2021), and chitosan (Mwangi et al. 2016) have also been used as emulsion stabilizers instead of surfactants because of toxicity concerns. These emulsion systems, including solid particles, bind irreversibly at the interface producing Pickering emulsions that have been found more stable over a long period (Shah et al. 2016). Cellulose is the most abundant biopolymer in nature, and due to its unique characteristics, such as biodegradability, biocompatibility, non-toxicity, high flexibility, and mechanical strength, it has been used as drug delivery systems, biosensors, and packaging films and to obtain nanocellulose (NC) particles (Mihaly-Cozmuta et al. 2017). NC is one of the efficient stabilizers used at oil/water interfaces and has been successfully used to prepare various nanoemulsions (Asabuwa Ngwabebhoh et al. 2018, Saidane et al. 2016, Sogut 2020). It has been reported that the effectiveness of NC to stabilize the emulsions is due to its amphiphilic character, which results from hydroxyl groups on the surface and the hydrophobic face (Fujisawa et al. 2017). Thus, the stabilization of BO with NC particles to obtain a nanoemulsion can be used in aroma carrier systems for food applications.

Biodegradable polymers obtained from renewable sources such as whey protein isolate (WPI) and starch (S) have been often studied due to their biodegradability, biocompatibility, and the potential application of these materials in various industrial sectors with sustainability and environmental protection issues (Fonseca-García et al. 2021, Rodriguez Llanos et al. 2021, Seydim et al. 2020). However, the addition of essential oils into film-forming solutions has some limitations due to their low miscibility, phase separation, increasing sensitivity to environmental factors, and adverse effects on optical properties (Atarés & Chiralt 2016). Recent studies have shown that the entrapment of essential oils within a polymeric matrix via emulsions has successfully enhanced their effectiveness or controlled the release rate promoting its use during the shelf life of food products (Acevedo-Fani et al. 2015). Researchers have also reported that controlling aroma release has been possible by modifying the emulsion characteristics such as using biopolymers, changing the particle size and shape of the emulsifier (Doi et al. 2019, Ren et al. 2018). However, such systems' design is still a challenge due to their complex structures, requiring more studies. It was assumed that the amphiphilic nature of NC may help oil droplets to a position at the interface and might be a suitable candidate as BO based emulsion stabilizer for its ability to adsorb at the emulsion interface. Polysaccharides and proteins can retain aroma compounds and be structurally modified to favor the release of aroma compounds, thus being suitable aroma carriers. Therefore, one polymer was chosen from a protein source (WPI) and another from polysaccharides (starch) to evaluate their potential as carriers for an aroma compound (BO), stabilized by NC to control its release. In this way, the impact of the polymer support characteristics contributing to flavor release may be elucidated. Besides, the interaction between aroma compounds and biopolymeric matrix and both controlling release and retention of aroma compounds are challenging points. Therefore, this study aimed to prepare

nanocellulose-based bergamot essential oil nanoemulsions and then incorporate them into the polymeric matrix, whey protein isolate, and starch, potentially used as aroma transferring mediums with better miscibility. The results of this study can be used to prepare emulsion-based encapsulation/stabilization systems to control the release of volatile flavors in packaged foods during storage.

MATERIALS AND METHODS

Materials

Whey protein isolate (WPI) (90% w/w protein) was supplied by Proteinocean Gıda Inc. (Ankara, Turkey). Nanocellulose (NC) was purchased from Blue Goose Biorefineries Inc. (BGB ULTRA™, Canada) as an aqueous suspension formed a gel at 8.0 % (w/w) with a crystal length of 100 nm and crystal diameter between 9 and 14 nm.

Bergamot oil (BO) was kindly supplied from the Turkish Ministry of Food and Agriculture, Western Mediterranean Agricultural Research Institute (Antalya, Turkey). The obtained BO was analyzed and characterized by a high content of d-limonene (45.21%), α-linalool (11.51%), linalyl acetate (18.83%), c-terpinene (9.10%), and α-pinene (5.81%).

Candelilla wax was provided from Strahl and Pitsch Inc. (S&P-99, West Babylon, NY, USA). Starch (S) (from potato), glycerol, magnesium nitrate 6-hydrate, ethanol, sodium hydroxide (NaOH), and calcium chloride ($CaCl_2$) were all of the analytical grades and supplied from Sigma-Aldrich (St. Louis, Missouri, USA).

Formation of nanoemulsions

The BO nanoemulsion was prepared with NC aqueous suspension (2 mg NC/mL of distilled water) in the 3 mM $CaCl_2$ solution (continuous phase) to reduce the possible repulsive forces occurring on the surface to the charged groups. The performance of the emulsion also depends on the ratio between NC and BO. In our preliminary studies, lower NC concentrations (less than 0.5 mg/mL) caused a coalescence of oil droplets, while higher concentrations than 2 mg/mL resulted in less effective NC accumulation. Therefore, the concentration of NC in the aqueous suspension containing 3 mM $CaCl_2$ was maintained at 2 mg/ml, which is sufficient to cover the interfacial area. BO was then added to the NC suspension with a weight ratio of 2/8 (BO/NC aqueous suspension), and the pH of this mixture was adjusted to 5.0, followed by homogenization at 15000 rpm for 15 min (Kasiri & Fathi 2018). The film materials used components show over a broad pH range, the pH of the dispersion was adjusted to study at a stable pH. The weight ratio of 2/8 was selected after the preliminary studies measuring the zeta potential of prepared emulsions in different ratios (1/9, 2/8, 3/7) with a nanoparticle analyzer (Nanopartica, SZ-100V2, Horiba Scientifica, Germany). The selected ratio presented a zeta potential lower than –30 mV, which are accepted as stable systems to be sufficient for ensuring the physical stability of nanoemulsion (Gurpreet & Singh 2018). The success of the prepared nanoemulsion was also controlled by particle size distribution analysis, and the average particle size was found as 95±7 nm.

Film preparation

Whey protein isolate (WPI) films were prepared according to the method described by Seydim & Sarikus (2006). Briefly, WPI at 5% w/w was dissolved in distilled water, including glycerol at 50% w/w (based on the dry weight of WPI), and the pH of the film solution was adjusted to 8 with NaOH (2N). Then, the solutions were heated to 90 °C while adding candelilla wax at 0.8% w/w and stirring continuously for 25 min. The film-forming solution was filtered three

times and cooled before adding nanoemulsion and casting.

Starch (S) films were also prepared by the casting method. S at 5% was dissolved in distilled water, and glycerol at 30% (based on the dry weight of S) was added. The S film-forming solution was obtained by heating at 85-90 °C for 15 min and then cooled for further applications.

BO nanoemulsion was added to WPI and S film-forming solutions with a final BO concentration of 2% (based on WPI and S content) and homogenized at 10000 rpm for 5 min before removing air bubbles. In the preliminary studies, it was discovered that higher BO concentrations caused the loss of film integrity. Thus, the threshold concentration of BO inside the film-forming solutions was selected as 2%. Film-forming solutions were cast onto Teflon® coated Petri-plates (Ø=15 cm) with controlled weight to adjust the thickness and dried at 25 °C. The solid content per cm^2 of petri dishes was adjusted for film samples and the pouring amount of each film-forming solution was measured based on their solid content.

WPI and S films, including BO nanoemulsion, were coded as WPI-BO and S-BO, respectively. Final film samples were conditioned at 25 °C and 53% relative humidity (RH) for 1 week before the physical characterization analyses. At least 6 random points were selected to measure each film's thickness using a digital micrometer (Digimatic Micrometer, Mitutoyo, Japan).

Characterization of film samples

ASTM standard method (D882) (ASTM 2018) was used to determine the elastic modulus (EM), tensile strength (TS), and elongation at break (E, %) values of film samples. Film samples (2.5 cm wide and 5 cm long) were stretched at 50 mm/min until break by a universal testing machine (Lloyd LR5, AMETEK, Inc, UK), and the corresponding values were determined from strain stress curves, estimated from force-distance data. At least ten replicates were obtained from each sample.

ASTM E96/E96M-16 gravimetric method (ASTM 2016) (cup method) was used to measure water vapor permeability (WVP) values of film samples. A 5 mL of distilled water was placed in permeability cups (3.5 cm in diameter), and then the cups were transferred to a desiccator having 53% RH (including oversaturated magnesium nitrate) at 25 °C. During the drying process, the air contact side of the film sample was exposed to 53% RH, and the other side was exposed to 100% RH at 25 °C. The decrease in permeability cups' weight was recorded (every 1.5 h) to calculate the permeability rates using linear regression.

The opacity of film samples was determined by a spectrophotometer (Shimadzu, UV-1601, Japan). Films were cut into 1×4 cm size, and the opacity (absorbance units per film thickness, AU nm/mm) was calculated from the absorption spectrum of films obtained between 400-800 nm. The color values of film samples were determined with Minolta Chroma Meter (CR-400, Konica Minolta, Inc., Japan) using a white standard calibration plate (Y=92.7, x=0.3160, y=0.3321) as a background. Results were expressed as CIE L^* (lightness), a^* (red-green), and b^* (yellow-blue) coordinates in the color space.

Estimation of BO release from films

The food simulant D1 (50 %, v/v ethanol) was selected as a release medium for film samples (European Commission 2011). BO is more soluble in ethanol than water, and an increase in ethanol concentration will increase the solubility of BO. Our preliminary study revealed that an increase in ethanol concentration limited the hydration of the film samples in ethanol, and BO diffusion from the film matrix has become difficult due to the polymer network's attenuating effect.

Therefore, to determine the highest release of BO from the film samples, D1 simulant was chosen as the release medium in this study. Film samples (0.1 g) were immersed into 10 mL of simulant and stirred at 25 °C for 48 h. The absorbance of the medium was recorded after various exposure times of released BO concentration measurements by using the corresponding calibration curve. The calibration curve of d-limonene obtained by the analysis of standard solutions prepared in different concentrations was used to measure the concentration of BO at the determined time interval for release studies.

The following equation proposed by Peleg (1988) was used to model the release kinetics of BO in the D1 simulant.

$$M_t = \frac{t}{k_1 + k_2} \quad (1)$$

where M_t is the amount of BO released at each time, and the kinetic constants, k_1 and k_2, are the inverse of the initial release rate and the asymptotic release value, respectively.

Statistical analysis

Each experiment was replicated two times with at least three observations for each sample. The differences between samples were determined with an analysis of variance (ANOVA) and Tukey's multiple comparison tests at a 95 % confidence level. The statistical analysis was performed using Minitab 17 software (Minitab Inc., Brandon, UK).

RESULTS AND DISCUSSION

Physical properties of film samples

The effect of BO nanoemulsion on the thickness, WVP, and mechanical properties of film samples was evaluated, and the results are shown in Table I. BO nanoemulsion incorporated S film showed higher thickness values. In contrast, the addition of nanoemulsion caused a decrease in the thickness of WPI film compared to corresponding neat films ($p<0.05$). The thickness of films is related to the interaction between compounds and polymer chains; thus, a decrease in the thickness might be related to the better interaction and compatibility between WPI and BO nanoemulsion. On the other hand, the differences in water uptake behaviors and holding capacities of WPI and S films might have caused different thickness values, affecting the water permeability and mechanical stability. Kalateh-Seifari et al. (2021) reported that the addition of nettle essential oil nanoemulsion caused an increase in the thickness of starch/chitosan films.

The addition of BO nanoemulsion resulted in an increase in EM and TS values ($p>0.05$) with a concomitant decrease in % E ($p<0.05$), leading to more rigid and less flexible films. These results suggest an adequate adhesion

Table I. WVP, thickness, and mechanical properties of film samples.

Sample	Thickness (μm)	EM (MPa)	TS (MPa)	E (%)	WVP (g mm/kPa h m^2)
WPI	151±9^{a}	172.3±16.1^{a}	13.1±1.6^{a}	18.8±3.8^{a}	60.49±0.45^{ab}
S	140±1^{ab}	146.6±30.7^{a}	7.9±2.9^{a}	2.7±0.4^{b}	86.87±16.86^{a}
WPI-BO	129±6^{b}	190.2±10.7^{a}	14.1±1.5^{a}	16.3±2.2^{a}	21.57±2.14^{c}
S-BO	146±2^{a}	204.3±32.5^{a}	10.1±1.1^{a}	1.8±0.3^{b}	43.73±5.76^{bc}

$^{a–c}$ **Different letters in the same column indicate significant differences among the film samples ($p < 0.05$) (Mean ± SD).**

was obtained through the interaction between functional groups of polymer matrices and BO nanoemulsion at the interface (Zhu et al. 2018). Similarly, de Oliveira Filho et al. (2021) reported improved mechanical properties for starch-based films when incorporated with carnauba wax nanoemulsion, cellulose nanocrystals, and essential oils. Kong et al. (2020) have also stated an increase in EM and TS values of starch/polyvinyl alcohol films when incorporated with carvacrol nanoemulsions. On the other hand, Norcino et al. (2020) observed a decrease in EM and TS with an increase in % E values of pectin films due to the plasticizing effect of copaiba oil nanoemulsions leading to weakening the intermolecular interactions between polymer chains. The incorporation of BO nanoemulsion into S films resulted in a 27-40% increase in TS and EM values, whereas approximately 10% of the increase was observed in WPI films for EM and TS values. The lowest % E values were found in S-based films with a 32% reduction in elasticity upon the addition of BO nanoemulsion, while only a 14% decrease was observed in BO nanoemulsion added WPI films. Elongation is a capacity of film flexibility desired for easy handling of films, while the elastic modulus and tensile strength are related to the resistance required to maintain the structural integrity of films. Nanoemulsion droplets might provoke hydrogen bonding of polymer and increase the rigidity and resistance while decreasing flexibility and mobility in polymer chains (Almasi et al. 2020). A carrier film should maintain the integrity of itself and the added emulsion during the handling and processing. It can be concluded that the incorporation of BO nanoemulsion had a more pronounced effect on the mechanical properties of S based films than those obtained for WPI based films.

In contrast to the results obtained in this study, Hasheminya & Dehghannya (2021), Li et al. (2020), and Lee et al. (2019) reported a decrease in strength and an increase in elongation values for nanoemulsion included film samples. Lee et al. (2019) attributed the decrease in mechanical properties of hydroxypropyl methylcellulose films when incorporated with oregano nanoemulsion to the replacement of polymer-polymer interaction with polymer-nanoemulsion interactions causing discontinuities in the polymer. Besides, Li et al. (2020) stated that thymol nanoemulsion addition resulted in weakened hydrogen bonds and decreased internal network and cohesiveness of the gelatin film.

An improvement in WVP of film samples was observed with BO nanoemulsion. The permeability of water molecules through polymer depends on the number of polar groups present in the structure, such as hydroxyl groups, which affect intermolecular attraction (Hasheminya & Dehghannya 2021). BO nanoemulsion caused a decrease between 50-65%, according to the relative neat film. The decrease in WVP values upon the addition of BO nanoemulsion might be due to the contribution of nanocellulose based emulsion to the establishment of a tortuous pathway for water molecules. The nanoemulsion droplets could increase certain tortuosity and reduce the diffusivity of water molecules due to the amorphous regions of the polymer matrix that have hydrophilic and hydrophobic molecules. The ratio between the hydrophobic and hydrophilic parts of the film determines the diffusion of water molecules through the film matrix. The homogeneous distribution of nanoemulsions with hydrophobic nature might lower the adsorption and diffusion of water molecules within the film by increasing the hydrophobicity of films. Besides, the polymer chains could become less mobile, reducing the diffusion of water molecules at the interface (Almasi et al. 2020). The lower WVP values

observed for WPI films compared to S films can be explained by the differences in components and concentration used for plasticizing due to the different requirements of films. These results agree with the studies reported by Shen et al. (2021), who studied the properties of clove essential oil loaded nanoemulsion and Pickering emulsion activated pullulan-gelatin based edible film. Norcino et al. (2020) studied the potential use of copaiba oil nanoemulsion in pectin films and reported a decrease in WVP. The improvement in the water barrier of WPI and S-based carrier films could avoid deterioration arising from water activity for the nanoemulsion as the water-resistance increased by strengthening the interaction between polymer chains while reducing the chain mobility by filling the free spaces in the polymer matrix (Zhu et al. 2018). Similarly, Oliveira Filho et al. (2020) have found a reduction in WVP values of arrowroot starch films, including carnauba wax, due to its high-water repellent properties and high fatty alcohol contents and reported that carnauba wax nanoemulsions showed a higher reduction in WVP when compared to their macro emulsion forms. The decrease of WVP upon the addition of a nanoemulsion into WPI-based films was also confirmed by Ghadetaj et al. (2018).

The optical properties of film samples are shown in Table II. WPI based films had lower opacity values than those obtained for S based films ($p<0.05$). The addition of BO nanoemulsion significantly increased the opacity of film samples ($p<0.05$). These results might be attributed to the light absorption characteristics of phenolics found in BO, especially at lower wavelengths (Chen et al. 2016). The increase in opacity could also be related to the increase in the light scattering throughout the film with the oil droplets in the film structure. With the help of the decrease in light transmittance, the light barrier effect might show a protective impact on the oxidative reactions of BO. The addition of nanoemulsion might scatter the light and block the light passage depending on the concentration and volume fractions of the lipid phase of the emulsion (Hasheminya et al. 2019).

Besides, the addition of BO nanoemulsion into films caused a decrease in lightness ($p>0.05$) and a^* values ($p<0.05$) while increasing the b^* values ($p<0.05$). The changes observed in a^* and b^* values depend on the nature of the essential oil used to form nanoemulsions, as confirmed by Kalateh-Seifari et al. (2021). Similarly, Hasheminya & Dehghannya (2021) reported a decrease in lightness and light transmittance values of Cordia dichotoma gum-based films when incorporated with *Salvia mirzayanii* essential oil nanoemulsion. The color values of S films did not change significantly upon the addition of BO nanoemulsion. Almasi et al. (2020) also reported that the addition of nanoemulsion did not significantly change the color of pectin films due to the smaller droplet size of the emulsion.

Table II. The optical properties of film samples.

Sample	T (%)	Opacity (AU nm/mm)	*L**	*a**	*b**
WPI	74.7±0.6^{a}	198.6±22.2^{c}	95.91±0.32^{a}	-0.18±0.06^{a}	4.36±0.18^{b}
S	62.5±0.5^{b}	352.7±12.5^{b}	95.68±0.18^{a}	-0.35±0.02^{b}	3.00±0.07^{c}
WPI-BO	64.6±3.3^{b}	414.9±22.9^{b}	94.81±0.03^{a}	-0.58±0.06^{c}	7.33±0.36^{a}
S-BO	52.1±1.1^{c}	632.7±104.3^{a}	95.76±1.27^{a}	-0.45±0.02^{b}	3.22±0.12^{c}

$^{a–c}$ **Different letters in the same column indicate significant differences among the film samples ($p < 0.05$) (Mean ± SD).**

The differences observed for the color values between WPI and S films can be explained by the differences in the light absorption behavior of each film, the differences in surface roughness of film samples, or differences in compatibility and homogeneity of the constituents within different polymer matrices (Acevedo-Fani et al. 2015). The color film samples also depend on the phenolic composition, natural color, and concentration of added essential oil in the nanoemulsion formulation. Similar results have been reported by Lee et al. (2019) and Agudelo-Cuartas et al. (2020) for hydroxypropyl methylcellulose and WPI films when enhanced with oregano essential oil nanoemulsions and α-tocopherol nanoemulsions, respectively.

The estimation of BO release from film samples

A moiety is released from a polymeric matrix by a three-stage process: i) solvent penetration through the polymer, ii) swelling of the polymer, and iii) diffusion of a constituent from swollen polymer (Zhu et al. 2018). The release of BO from WPI and S films was examined, and the related kinetics with release profiles are shown in Figure 1 and Table III.

The release rate of BO from S films was higher than WPI films. The interaction between BO nanoemulsion and S film matrix might be weaker than in WPI films, leading to a more open structure and more relaxed polymer matrix related to the polymer solubility (Chen et al. 2016). Similarly, Sogut (2020) reported an initial faster release and subsequent slower release rates for nanocellulose stabilized BO from WPI based films. Chu et al. (2020) reported that the retention of cinnamon essential oil inside pullulan-based films had been increased by nanoemulsion formation. The cumulative release rates obtained after 48 h were 29.28±0.17% for WPI-BO and 44.91±2.61% for S-BO films. The presence of nanocellulose within the polymer matrix might also slow down the release of BO due to the interaction at the interface (Sogut & Seydim 2018). An initial fast release followed by a slower release rate of nanoemulsion of eugenol from chitosan was demonstrated by Das et al. (2021), and the authors stated that the hydrophilic nature of chitosan and possible weak interactions might affect the release behavior of eugenol, resulting in rapid nanoparticle hydration. Sheorain et al. (2019) also reported a fast dissolution followed by a sustained release of thymol from chitosan-tragacanth nanoparticles with biphasic release kinetics. The obtained cumulative release rates for BO from WPI and S after 48 h showed a potential opportunity in the controlled release of bioactive components such as aroma compounds, suggesting the potential of selected polymers as wall matrix for controlling aroma release.

As shown in Table III, the absorbance data and measured cumulative release rates were fitted to the selected model equation to calculate the release kinetics. The release behavior of BO from films to the selected food simulant exhibited a good fit with the model proposed by Peleg (1988) ($R^2 > 0.93$). The initial release rate ($1/k_1$) and the equilibrium/asymptotic value ($1/k_2$) of WPI-BO and S-BO films were obtained by this equation and are shown in Table III. The release rate of an active agent such as flavors from film matrix is significant for its potential use in food

Table III. Peleg's model parameters of film samples.

Sample	$1/k_1$ (wt.%/min)	$1/k_2$ (mg/g film)	R^2
WPI-BO	0.048±0.002[b]	27.38±3.63[b]	0.93
S-BO	0.267±0.024[a]	46.59±2.76[a]	0.98

[a–b] Different letters in the same column indicate significant differences among the film samples ($p < 0.05$) (Mean ± SD). k_1 is related to the release rate at the beginning of the process and k_2 is related to the asymptotic value, which is obtained from the inverse of M∞, the amount of active agents released at equilibrium.

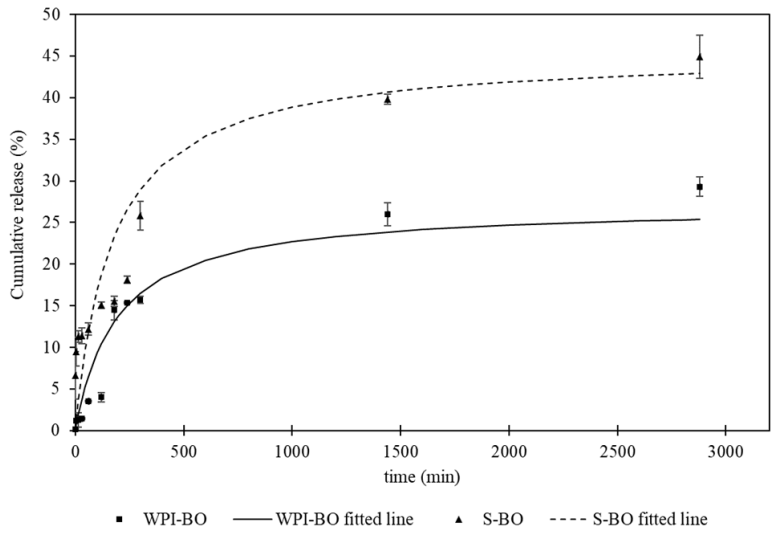

Figure 1. Cumulative release rates of film samples.

applications, active films, or coatings. The higher $1/k_1$ and $1/k_2$ (M_∞) values were observed in S-BO films ($p<0.05$). The total BO released from WPI films was lower than S-BO films, which coincided with the lower $1/k_2$ values obtained for WPI-BO films ($p<0.05$). Peleg (1988) proposed that the lower concentrations reached equilibrium were related to the higher k_2 values.

CONCLUSION

BO is one of the widely used flavors in the food industry; however, its volatile nature limits its use. The stabilization of BO using polymeric matrices and nanoparticles for packaging applications may provide a broader application of BO. Thus, in this study, BO nanoemulsion was prepared with NC before adding into WPI and S film-forming solutions and aimed to test the potential of selected film samples to be used as carriers of BO, which stabilized with NC. The film samples had improved mechanical and water vapor barrier properties when incorporated with BO nanoemulsion. The release rate of BO from S films was faster than WPI films, which might be due to the partial solubility of S films in the selected food simulant, as confirmed with WVP values. This study showed that essential oils could be added to biopolymeric film solutions via nanoemulsion formation without phase separation, miscibility problems. Besides, these results can be used to control the release of aroma compounds commonly used in food products such as BO. However, further studies are needed to understand the compatibility between components and their effectiveness in packaged foods.

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