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Development and structural assessment of whey protein isolate/sunflower seed oil biocomposite film

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ABSTRACT

The overall aim of this research was to investigate effect of sunflower oil addition on formation of whey protein based biocomposite edible films. The biocomposite films were prepared through an emulsion technique by dispersing three different ratios of sunflower oil (0.05%, 0.10% and 0.15%) in whey protein isolate medium. Moisture content, solubility in water, water sorption kinetic, swelling index, contact angle, color, whiteness, and opacity index of the films were examined. Thermal, barrier, and mechanical properties were tested. Particle size distribution and surface microstructure of the films were also analyzed. The moisture content of the biocomposite films was not affected with sunflower oil addition but the solubility showed reducing tendency. Increasing oil content increased contact angle. Although color parameters, whiteness index, and total color were not affected significantly by oil addition whereas the opacity index of the biocomposite films was determined to be higher than the control film (no sunflower oil present). Glass transition value of the control film was significantly lower than those of emulsified films ($p < 0.05$). All of the film forming emulsions showed bimodal particle size distribution with two major peaks. The results showed that despite the low oil amount in biocomposite film composition, oil addition had a positive effect on the water vapor and oxygen barrier properties of the films. It was understood that low amount of sunflower oil increased the hydrophobic property of the films without any adverse change in the barrier and optical characters of the films.

1. Introduction

Edible films and coatings based on natural components ensure one of the most encouraging and recently researched approaches for quality and shelf-life enrichment of the food products (Valencia-Chamorro et al., 2011). Edible films can be used as separation layers like covers, wraps or packaging material for food products. Edible films can be used for decreasing senescence of foods and protect them from physical and microbiological injuries. Also, they can minimize some undesired physical modifications of food products because of environmental effects. The polymers used in the food industry are generally unbiodegradable and synthetic origin, and their biocompatibilities are relatively limited compared to the biopolymers. Edible films and coatings are a promising food packaging trend to protect the environment from the pollution caused by petroleum-based plastic films/packaging materials. Edible films are also non-toxic and inexpensive, they have good sensorial qualities, high barrier and mechanical properties (Debeaufort et al., 1998; Galus and Kadzińska,

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2015; Kokoszka et al., 2010). They also decrease the possibility of food quality spoilage due to prolonged shelf life of foods (Lee et al., 2008). Hydrocolloids (proteins and polysaccharides), lipids, or their combinations are the main components of the biopolymer films. Protein-based films seem to be the most fashionable and preferred among them, mainly because of their high and desired nutritional value (Cao et al., 2007). The mechanical properties of protein-based films are high typically and they are proper barriers to gases (such as oxygen) and aromas (Deeth and Bansal, 2018; Oliveira et al., 2017). Whey protein isolate (WPI) is a known material having a good film forming ability, as a good emulsifying agent and to produce transparent, stretchy and odor-free films (Gounga et al., 2007). However, because of the high hydrophilic nature of protein molecules, these films show high moisture absorption (Oliveira et al., 2017; Prodpran et al., 2007). Hence, one of the methods developed to arrange hydrophilic-hydrocolloid film properties, is forming a composite edible system that has hydrocolloids forming a continuous structure, and lipids providing high barrier property, thus giving a chance to enhance physicochemical characteristics of the films.

Except for sunflower oil-WPI combination, there are some information available in the literature about other types of protein-lipid originated films (Kokoszka et al., 2010; Ma et al., 2012; Pereda et al., 2014) which mainly focuses on developing emulsified films and improving their barrier, mechanical and physical properties. Cerqueira et al. (2012) prepared WPI based emulsion films by blending protein with different concentrations (0.25%, 0.50% and 0.75%) of corn oil (Cerqueira et al., 2012). They had reported that water vapor permeability and tensile strength were decreased significantly due to the oil addition however, it caused an increase in elongation at break. Pereda et al. (2014) had designed a composite film containing polysaccharide (chitosan), lipid (olive oil), and additionally the nanocrystals of cellulose (Pereda et al., 2012). It was stated that the cellulose nanoparticles and olive oil existence might be helpful to reduce the water vapor permeability and to improve the tensile behavior of the films. Kokoszka et al. (2010) had studied with rapeseed oil in 0, 1, 2, 3 and 4% w/w of whey protein isolate, to produce combined emulsion films (Kokoszka et al., 2010). They had found that incorporation of lipid moderately affected film thickness, and it was also reported that only 3 and 4% oil contents had influenced the maximum denaturation temperature, while there were no differences in initial temperature of denaturation and enthalpy of the films. Many lipids such as neutral lipids, fatty acids, waxes, and resins can be added into biopolymers in order to enhance their barrier properties (Galus and Kadzińska, 2016). Vegetable oils are typically liquid at room conditions, and they are readily miscible with the other polymers such as protein sources (Ghanbarzadeh and Almasi, 2011). Sunflower oil is a non-volatile, non-toxic, and non-depletable and very common oil type obtained from sunflower (Helianthus annuus) seeds. Also one of the other reason why sunflower oil was preferred in the study is that it is one of the largest source of vegetable oil in the world and ease-to-supply. It is very rich in linoleic acid (polyunsaturated fatty acids) and oleic acid as well (monounsaturated fatty acids) however, palmitic acid and stearic acid (saturated fatty acids) levels are low (O'brien, 2008). Additionally, it has high Vitamin E content (alpha-tocopherol), which is an oil-soluble antioxidant and appreciable quantities of vitamins A and D. Also sunflower seed oil contains lecithin, carotenoids, and tocopherols; it can be stated that all these are a good effect on consumers' preferences for sunflower oil.

The objectives of this study were to produce whey protein isolate-sunflower oil biocomposite films and to evaluate efficiency of emulsion film formation with the addition of such a small amount of sunflower oil. The results of low addition of sunflower oil on opacity, color, and particle size distribution were analyzed. Moreover, surface, mechanical, thermal and barrier properties; moisture content, swelling index, solubility in water, water sorption kinetic, and film microstructure were also determined to estimate hydrophilic-hydrophobic characterization.

2. Materials and methods

2.1. Chemicals

The film forming material WPI (protein content >90%) was kindly obtained by Davisco Foods International Inc. (BiPRo, Le Sueur, MN., USA). Sunflower oil was purchased from a local store in Gaziantep in Turkey. Anhydrous glycerol (84–88%) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of film forming emulsions from whey protein isolate incorporated with sunflower oil at different levels

Control and biocomposite solutions were prepared according to a method reported by Kaya and Kaya (2000). An aqueous solution of 8% (w/w) WPI (control) was prepared by blending whey protein isolate powder in distilled water, and 8% glycerol (WPI-Glycerol 1:1) was added to the solution as a plasticizer. The obtained solutions were stirred magnetically using a magnetic stirrer for 5 min. After complete dissolution of the film forming materials, the solution was heated to a final temperature of 70 °C for 25 min under continuous agitation over a hot plate. Film forming emulsions were homogenized at 20,000 rpm for 2 min using a homogenizer (IKA T18 Ultra Turrax, Staufen, Germany). Subsequently; the film forming solution was kept in an ultrasonic bath to remove dissolved air for about 20 min. Afterward, the solution casts onto glass plate at a ratio of 0.09 mL/1 cm^2 to reduce thickness alterations between treatments and spread evenly with a glass rod; they were left to dry for 24 h at room conditions (24 ± 2 °C and 50 ± 2% RH). Afterward dried films were peeled and left for conditioning in the same conditions for 48 h. Related films were used as control films. Biocomposite films were prepared as follows; 8% (w/w) WPI, 8% (w/w) glycerol and different sunflower oil concentrations (0.05, 0.10 and 0.15%) were dispersed in distilled water by constant stirring. The other steps were the same as control film productions as described above. The experimental measurements were performed in triplicate.

2.3. Thickness

Film samples were tested with a digital micrometer (Mahr GmbH., Göttingen, Poland) having a sensitivity of 1 μm. Three to five random measurements were applied for each film; the mean values were reported for each group.

2.4. Moisture content

At least three random locations of each test samples were chosen, cut uniformly in the dimensions of 2 cm × 2 cm and put

on glass petri dishes for testing. A laboratory oven at 103 °C was used to obtain a constant dry weight for film samples.

2.5. Film solubility in water

Water solubility was determined according to the procedure of Liu et al. (2004). At least three random locations of each test samples were chosen, cut uniformly as the dimensions of 2 cm × 2 cm and placed on glass petri dishes. A laboratory oven at 103 °C was used to obtain a constant dry weight for film samples (Binder, FD 115, Tuttlingen, Germany) and measured to the sensitivity of 0.0001 g. Afterward, the dried film samples were put into centrifuge tubes containing 30 mL of distilled water. These tubes were put in a water bath at 25 °C and mixed for 24 h. Then, undissolved film particles were filtered using qualitative filter paper and after that the film samples were dried at 103 °C for 24 h. Water solubility was obtained according to the equation given below (Eq. (1)):

$$WS\,(\%) = [(W_0 - W_f)/W_0] \times 100 \quad (1)$$

where WS is water solubility, W_0 is the initial dry weight of the film and W_f is the final weight of the dried undissolved film.

2.6. Water sorption kinetics

The water sorption kinetics of the film samples were evaluated by the method described by Lavorgna et al. (2010). Dried film samples were cut uniformly in a shape of square (2 cm × 2 cm) and were placed into a beaker. 30 mL distilled water was added to each one of them, and they were placed into a water bath kept at 25 °C. The three film samples of each type were periodically taken for every 30 min within 2 h. The samples were weighted to detect the water gain after the excess water over the film surfaces gently taken with a filter paper.

Water gain was calculated by the following equation (Eq. (2)):

$$WG\,(\%) = [(W_s - W_d)/W_d] \times 100 \quad (2)$$

where WG is water gain, W_s is the weight of swollen film, and W_d is the weight of dried film.

2.7. Swelling index

The swelling index of film samples were measured over three trials with a method described by Cao et al. (2007). Film samples were cut into 2 cm × 2 cm squares and weighted. Then the samples were immersed into deionized water at 25 °C for two minutes. Filter paper was used for removing excessive amount of water from wet films and they were weighed. The absorbed water amount was calculated by taking the difference of the initial and wet weight of film samples, and it was given as the percentage swelling index.

2.8. Contact angle

Contact angle values of the film samples (θ) were determined with an Easy Drop goniometer (Krüss GmbH, Hamburg, Germany). In this method (Galus and Kadzińska, 2016), using a syringe, a droplet of deionized water was put on the air and the support sides of the horizontally positioned films. At least three trials were performed for each sample. Changes of angles from both sides between the tangent at drop boundary and baseline of the drop were determined to ground on the measurement of changes in the droplet shape via image analysis software.

2.9. Color, whiteness index and opacity

A colorimeter was used to determine the color of film samples (Hunter Lab ColorFlex, Hunter Associates Laboratory Inc., Reston, VA, USA) using the CIELAB color parameters (L^*, a^*, and b^*). A white standard tile (L*:93.41; a*: −1.12; b*: 1.07) was used as a reference. For each sample, three measurements were performed and mean values were reported. The total color difference (ΔE) was calculated using the following equation (Eq. (3)):

$$\Delta E = \sqrt{\left(L_{Ref} - L^*_{film}\right)^2 + \left(a_{Ref} - a^*_{film}\right)^2 + \left(b_{Ref} - b^*_{film}\right)^2} \quad (3)$$

The whiteness index (WI) values were determined using Eq. (4) (Vargas et al., 2008):

$$WI = 100 - \sqrt{\left(100 - L^*_{film}\right)^2 + \left(a^*_{film}\right)^2 + \left(b^*_{film}\right)^2} \quad (4)$$

The opacity of the samples was evaluated according to the method described by Farhan and Hani (2017) and tested with a UV–vis spectrophotometer (UV, Shimadzu, Japan). The rectangular cut film samples were put in a spectrophotometer cell. The reference was an empty test cell. The following equation (Eq. (5)) was used for opacity calculation.

$$\text{Opacity} = Abs_{550}/X \quad (5)$$

where Abs_{550} is the absorbance value at 550 nm, X is the film thickness (mm).

2.10. Thermal properties

Differential Scanning Calorimeter (DSC) (Perkin Elmer, DSC 4000) were used to determine the thermal properties of the emulsion films. Before the experiment, DSC was calibrated with indium samples. The weight of film samples was ranged within 10 to 15 mg. Samples were put inside aluminum sample pans and they were hermetically sealed. The following program was applied for film samples: Holding for 3 min at 0 °C for stabilizing, and heating from 0 °C to 120 °C at a rate of 10 °C min^{-1}, holding for 5 min at 120 °C, cooling from 120 °C to 0 °C at a rate of 10 °C min^{-1}.

2.11. Particle size distribution measurement

The distributions of sunflower oil in film emulsions were obtained by Horiba LA-950 particle size distribution analyzer (Horiba Instruments, Inc., Kyoto, Japan) at room temperature. Optical parameters applied were: refractive indexes of sunflower oil (RI: 1.47) and water (RI: 1.33). The volume-weighted mean particle diameter ($D_{4,3}$), volume-surface diameter ($D_{3,2}$) were determined and the span was calculated with the following equation (Eq. (6)).

$$Span = \frac{Dv\,0.9 - Dv\,0.1}{Dv\,0.5} \quad (6)$$

2.12. Water vapor and oxygen permeability

Water vapor permeability of the films was gravimetrically determined (Kaya and Kaya, 2000). Before the test, the samples were conditioned at room conditions (24 ± 2 °C and 50 ± 2% RH) for ten days. Circular and glass test cups were used with 4.7 cm internal diameter and 2 cm depth. External and internal %RH of the measurement system was adjusted using saturated NaCl solution (RH = 75%) and silica gel (RH = 0%), respectively, at 24 ± 2 °C.

The given water vapor permeability values were calculated using the following equation (Eq. (7)) from the average of the three replication results of the films based on weight change versus time at the steady state.

$$WVP = \frac{\Delta m}{(\Delta t)(\Delta p)(A)} \times d \quad (7)$$

where WVP is the water vapor permeability in $g\,mm\,m^{-2}\,h^{-1}\,kPa^{-1}$, $\Delta m/\Delta t$ is the weight of moisture gain per unit of time in g/d, A is the film area exposed to the moisture transfer in m^2, ΔP is the water vapor pressure difference between the two sides of the film in kPa, and d is the film thickness in mm.

The oxygen permeability of the film samples were measured using Mocon Oxygen Permeability ML-2/21 and Ox-Tran System (Mocon Inc., Minneapolis, MN, USA) according to Standard ASTM D3985-05(2010)e1. The film sample was placed on a stainless steel mask. One side of the film was exposed to nitrogen gas flow and the other side was exposed to oxygen gas flow at 24 °C and 50% RH. The oxygen permeability was reported in $cm^3\,mm\,m^{-2}\,h^{-1}\,atm^{-1}$ for all films by converting the oxygen transmission rate values (OTR) obtained from the instrument ($cm^3\,m^{-2}\,h^{-1}$) with the use of the appropriate conversion units and the following equation (Eq. (8)):

$$P = (OTR \times d)/\Delta p \quad (8)$$

where d is the thickness of the film; Δp is pressure differential acting on the film.

2.13. Atomic force microscopy (AFM)

Atomic force microscope (Park XE-100 AFM, Schaefer Technologies GmbH) was used in order to observe the surface microstructure of samples. Film samples were uniformly cut in square shapes and put on the sample chamber. Measurements ($5 \times 5\,\mu m^2$ and $20 \times 20\,\mu m^2$) were taken from at least three areas of the film surface for each side to capture acceptable images. The values of Rq and Ra gives information about roughness of the film surfaces: Rq is defined as root-mean square average of height deviations taken from the mean data plane; Ra is defined as average of the absolute value of the height deviations from a mean surface (Shojaee-Aliabadi et al., 2014).

2.14. Mechanical properties

Texture Analyzer (Stable Microsystems, Godalming, UK, Model: TA-XT2i) was utilized to analyze tensile strength and percent elongation of the samples according to ASTM Standard Method D 882 (ASTM, 1993).

Table 1 – Moisture content and water solubility values of the control and biocomposite films.

Film type	Moisture content (%)	Water solubility (%)
WPI-C	40.0 ± 1.9^a	49.2 ± 2.4^a
WPI-0.05	38.0 ± 1.9^a	44.7 ± 2.2^b
WPI-0.10	37.0 ± 1.8^a	43.1 ± 2.1^b
WPI-0.15	37.0 ± 1.8^a	45.2 ± 2.2^b

WPI-C (control film), WPI-0.05 (0.05% sunflower oil added WPI film), WPI-0.10 (0.10% sunflower oil added WPI film), WPI-0.15 (0.15% sunflower oil added WPI film). Values were given as mean ± standart deviations. Different letter in the same column indicates statistical differences (Duncan's test, $p < 0.05$).

Mechanical properties were calculated with averages of at least three measurements. Mechanical properties were calculated with the following equations (Eqs. (9) and (10)).

$$TS = N/A \quad (9)$$

where TS is the tensile strength in MPa; N is the force maximum at rupture of the film; A is the initial cross sectional area of the film (mm^2).

$$E\% = \frac{(D_f - D_i)}{D_i} \times 100 \quad (10)$$

where E% is the percent elongation at break; D_f is the distance elongation at break (mm); D_i is the initial distance between the baselines (mm).

2.15. Statistical analysis

Statistical analyses of the data were analyzed using the SPSS statistical program (SPSS Version 10.0 for Windows, SPSS Inc., Chicago, USA) using analysis of variance (ANOVA). In order to determine the significant difference between group samples, the confidence interval was chosen as 95% ($p < 0.05$). Post-hoc Tukey and Duncan tests were used to perform to determine significant differences for film properties. A Pearson correlation test was applied to evaluate the presence of correlation between mechanical, barrier, color and water absorption properties of the films using the SPSS statistical program.

3. Results and discussions

3.1. Moisture content and solubility in water

The moisture content and solubility in water of the samples are given in Table 1. The moisture content of the control film had the highest value (40%) among the other film samples and moisture content of the composite films were not statistically different from control film ($p > 0.05$). However, there was a study had been reported for olive oil incorporated gelatin based films (olive oil/protein weight ratios of 5, 10, 15 and 20%), moisture content values of films significantly decreased due to the inclusion of olive oil (Ma et al., 2012). The differences between our results and their reports might be related with the oil concentrations added, protein kinds used and processes applied during film production.

There was a significant difference between water solubility values of the biocomposite and the control films ($p < 0.05$), however oil concentration did not cause any significant difference ($p > 0.05$, Table 1). Galus and Kadzinska (2016) had found

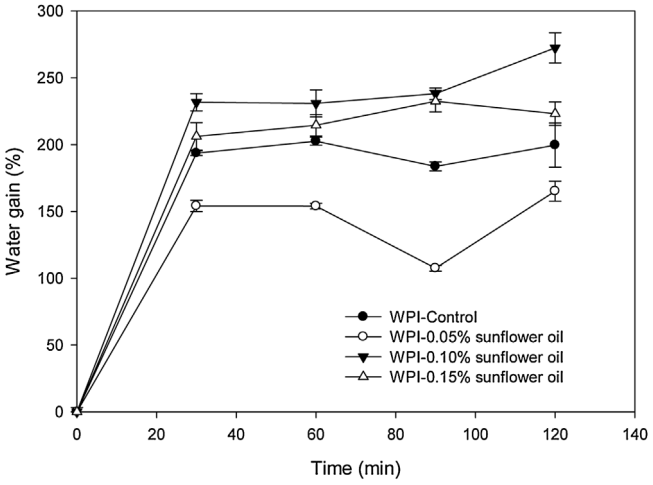

Fig. 1 – Water sorption profiles of control and biocomposite film samples. Error bar shows standart deviation. WPI: whey protein isolate.

the WPI film solubility in water to be 42.4% that was close to our result (Galus and Kadzińska, 2016). According to their study walnut oil addition (0.5 and 1.0%) slightly reduced the solubility of whey protein film but almond oil addition did not cause any decrease in the solubility. They were supposed that oil and protein chemical interactions result in a smooth structure of emulsion films and this situation possibly increased the solubility. Similar to our results, decreasing solubility had been reported in other studies for examples for pistachio globulin protein-fatty acid emulsion films (Zahedi et al., 2010) and gelatin-based edible films incorporated with olive oil (Ma et al., 2012). Actually water solubility property of films could be related to the film's hydrophilicity and/or hydrophobicity directly. If the solubility of film into water is high, it means that the hydrophilic character of the film is high. Meanwhile, when coated foods contact with water, like during osmotic dehydration, high solubility can cause the disintegration of the coating (Farhan and Hani, 2017; Gontard et al., 1994). Therefore, water insolubility is generally preferred for more resistant packaging films.

3.2. *Water sorption kinetics*

Sorption kinetics of control and biocomposite film samples were tested by determining film water uptake versus time at 25 °C and are presented in Fig.1. It can be stated that water uptake of all film samples was divided into mainly two zones as firstly water absorption zone and secondly equilibrium zone. Fig.1 shows that all films were rapidly taken water to their structure in the first 30 min and reached their highest water content after 40 min of immersion in water; after that time, no more water uptake was determined. The existence of sunflower oil in the whey protein film matrix did not drastically modify sorption behavior despite their non-polar character. It was possible to say that all film samples showed similar behavior except 0.05% sunflower oil added WPI film showed lower water absorption than control film. This situation can be explained with the hydrophobic characteristic of liquid sunflower oil. WPI-0.10 and 0.15% sunflower oil added WPI film samples showed higher results than the control. This phenomenon was possible due to the homogenization was not enough in the film production or the formation of more hydrogen bonds in the film matrix was contributed with the presence of glycerol (water-holding agent). In the current study, it was understood visually that all films protected their integrity and remained stable even after water intake for all periods of test time (120 min), indicating a functional interaction between film forming materials include sunflower oil.

Table 2 – Contact angle (θ) and swelling index of control and biocomposite films.

Film type	Air side (θ°)	Support (θ°)	Swelling index (%)
WPI-C	86.2 ± 3.1^{a}	84.6 ± 2.3^{a}	49.6 ± 1.5^{a}
WPI-0.05	81.2 ± 7.5^{a}	88.1 ± 2.1^{a}	47.0 ± 1.5^{a}
WPI-0.10	88.5 ± 8.5^{a}	84.5 ± 7.4^{a}	48.0 ± 1.5^{a}
WPI-0.15	103.3 ± 5.1^{b}	104.0 ± 5.7^{b}	48.5 ± 1.5^{a}

WPI-C (control film), WPI-0.05 (0.05% sunflower oil added WPI film), WPI-0.10 (0.10% sunflower oil added WPI film), WPI-0.15 (0.15% sunflower oil added WPI film). Values were given as mean ± standart deviations. Different letter in the same column indicates statistical differences (Duncan's test, $p < 0.05$).

3.3. *Swelling index*

The swelling indexes of whey protein films containing sunflower oil and control films were represented in Table 2, and it was found that they were not found different from each other, statistically ($p > 0.05$). Swelling index, indicating hydrophilic characteristics, of the control film were found similar to the reported value (51.60%) by Galus and Kadzińska (2016). This behavior could be explained by the water retention capacity of the hydrocolloid films due to their hydrophilic property. Mayachiew et al. (2010) had found that swelling index could change due to the differences in the amount and nature of intermolecular chain interactions (Mayachiew et al., 2010). For example, it was proposed that film drying temperature and/or addition of an extract (Indian gooseberry) had affected chain interactions, after which swelling index of edible chitosan films was also changed (Mayachiew et al., 2010).

3.4. *Contact angle*

The contact angle value of the control film was 86.23° for the air side and 84.62° for the support side, which was close to the contact angle value of 0.10% sunflower oil added WPI film. The highest contact angle values for both the air and the support sides were determined (0.15% sunflower oil added WPI film) as 103.33° and 104°, which were significantly different from other films ($p < 0.05$). It was observed that, regardless of the film side, increased oil content caused an increase in contact angle value because of higher hydrophobic character of the lipid molecules (Abdollahi et al., 2019). Galus and Kadzinska (2016) had prepared whey protein isolate films with almond and walnut oil (0.5 and 1%). The initial contact angles of whey protein isolate (control film) for the air and the support sides were found 26.2° and 29.9°, respectively. Their values were lower than our observations (Table 2); the differences might be generated from the processes applied in film production (Ramos et al., 2013). They reported that the contact angle of the film containing walnut oil had higher values on the air side in comparison with the support side. Additionally, they found that contact angle values increased with oil content for all trials and oil types. It was suggested that due to the density differences between oil and water, oil could be located on the air side of the film and thereof, air side of the film gained more hydrophobic character (Galus and Kadzińska, 2016). Karbowiak et al.

Table 3 – L*, a*, b* values, whiteness index (WI) and opacity of whey protein control and biocomposite films.

Film type	L*	a*	b*	ΔE	WI	Opacity
WPI-C	96.3 ± 3.0^{a}	-0.17 ± 0.01^{a}	2.2 ± 0.07^{ab}	3.3 ± 0.17^{a}	95.7 ± 3.07^{a}	2.62 ± 0.13^{a}
WPI-0.05	96.5 ± 3.1^{a}	-0.41 ± 0.01^{b}	2.1 ± 0.06^{a}	3.3 ± 0.17^{a}	95.8 ± 3.08^{a}	2.76 ± 0.14^{b}
WPI-0.10	96.5 ± 3.1^{a}	-0.14 ± 0.01^{a}	2.4 ± 0.08^{b}	3.5 ± 0.18^{a}	95.7 ± 3.06^{a}	2.88 ± 0.14^{b}
WPI-0.15	96.6 ± 3.1^{a}	-0.18 ± 0.01^{ac}	2.0 ± 0.06^{a}	3.5 ± 0.18^{a}	96.0 ± 3.08^{a}	3.01 ± 0.15^{b}

WPI-C (control film), WPI-0.05 (0.05% sunflower oil added WPI film), WPI-0.10 (0.10% sunflower oil added WPI film), WPI-0.15 (0.15% sunflower oil added WPI film). Values were given as mean ± standart deviations. Different letter in the same column indicates statistical differences (Duncan's test, $p < 0.05$).

(2006) had interpreted this situation such that helical-three dimensional structure of polymer molecules, in which hard internal molecular hydrogen bonded down the film surfaces, and thus, no motion of polar groups took place at the film surface (Karbowiak et al., 2006). Contradicting observations were obtained in other studies on hydrocolloid films and emulsion films, in which the support side angle values were found higher than the air side (Hambleton et al., 2009; Karbowiak et al., 2006). In the current study, the obtained values for the both sides of the films were found very close to each other. This could be because of the characteristics of oil and its molecular distribution in the whey protein matrix. Nevertheless, increasing of film hydrophobicity due to the addition of lipids was previously reported for whey protein-rapeseed oil composite film (Abdollahi et al., 2019; Kokoszka et al., 2010; Ojagh et al., 2010) as well as for other hydrocolloid films (Karbowiak et al., 2006; Pereda et al., 2012). It was interesting to note that the contact angle values determined were similar to those of nonabsorbent hydrophobic reference surfaces (93.9–100.2° for low-density polyethylene film and 91.5 for Plexiglas) (Galus and Kadzińska, 2016). It was understood that whey protein isolate films, even when modified with the addition of sunflower oil, exhibit hydrophilic character (Karbowiak et al., 2006). Kokoszka et al. (2010) had found that contact angle of whey protein films increased significantly when rapeseed oil content was increased in the film matrix. They indicated that lipid addition affected wettability properties of whey protein films (Kokoszka et al., 2010).

3.5. *Color and whiteness index*

Optical parameter and whiteness index (WI) emulsion films were tabulated in Table 3. Lightness (L* parameter) values were found quite high regardless of the sunflower oil content. All the films had negative values of a* and positive values of b* parameters. There was no significant effect of oil addition on the color of the films obtained ($p > 0.05$). All of the films displayed fairly translucent appearance and whiteness index, and there was no statistical difference between them depending on oil content; however, opacity values of the biocomposite films were statistically different from that of control films ($p < 0.05$). It was predicted that this behavior could be attributed to higher polymer chain mobility and intermolecular spacing which could facilitate the permeability of the light through films.

3.6. *Thermal properties*

The glass transition temperature (T_g) and the enthalpy (ΔH) values are summarized in Table 4.

Probably due to the presence of crosslinks between the blend components, T_g value of the biocomposite films was significantly higher than those of control films ($p < 0.05$). Emulsion films the T_g values changed between 69–93 °C and the heat of fusion were between 0.073 and 0.079 J/g. The T_g of control was found to be 50 °C and heat of fusion was 0.023 kJ/kg. Osés et al. (2009) reported that the T_g of WPI film should be within 20–50 °C. Generally, in a DSC scan a single T_g for blended polymers indicates good compatibility of the component polymers. Johnston (1999) had also reported that T_g of films based on β-lactoglobulin and WPI were 45 °C (De Mulder-Johnston, 1999). Probably due to the presence of crosslinks between the blend components, T_g value of the emulsified films were significantly higher than those of control films ($p < 0.05$). Emulsion films T_g values changed between 69–93 °C and the heat of fusion values were between 0.073 and 0.079 J/g. Water content, type and amount of plasticizers used and film forming processes applied might affect the thermal properties of the films. Incorporation of sunflower oil into WPI films caused increasing in T_g and heat of fusion. Su et al. (2010) had supported that the formation of the crosslinks inevitably would be caused of increasing T_g (Su et al., 2010). The T_g values of 0.05% and 0.10% sunflower oil added WPI film were the same but 0.15% sunflower oil added WPI film was found to be the highest among them. It should be accepted that there are some difficulties in getting homogenous film and sample amount for the DSC scan was only about 10 mg, they may have contributed to the variability in the results (De Mulder-Johnston, 1999).

Table 4 – Glass transition temperatures and heat of fusion values of control and biocomposite films.

Film type	T_g (°C)	Heat of fusion (ΔH) (J/g)
WPI-C	50 ± 2.50^{a}	0.023 ± 0.001^{a}
WPI-0.05	69 ± 3.45^{b}	0.078 ± 0.004^{b}
WPI-0.10	71 ± 3.55^{b}	0.073 ± 0.004^{b}
WPI-0.15	93 ± 4.65^{c}	0.079 ± 0.004^{b}

WPI-C (control film), WPI-0.05 (0.05% sunflower oil added WPI film), WPI-0.10 (0.10% sunflower oil added WPI film), WPI-0.15 (0.15% sunflower oil added WPI film). Values were given as mean ± standart deviations. Different letter in the same column indicates statistical differences (Duncan's test, $p < 0.05$).

3.7. *Particle size measurement*

Particle size distribution in the area, $D_{3,2}$ and $D_{4,3}$ of oil droplets in film forming solutions containing sunflower oil at different levels (0.05, 0.10 and 0.15%) are presented in Fig. 2 and Table 5. McHugh and Krochta and Debeaufort and Voilley had reported that when the diameter of the lipid globules smaller, the better the film properties are and more homogeneous distribution occur (Debeaufort and Voilley, 1995; McHugh and Krochta, 1994b). The 0.05% sunflower oil added WPI film sample represented bimodal particle size distribution with a sharp

Table 5 – Mean diameters and span values of oil particles of control and biocomposite films.

Film forming dispersion type	$D_{3,2}$ (μm)	$D_{4,3}$ (μm)	Span
WPI-C	–	–	–
WPI-0.05	1.35 ± 0.07^{a}	15.28 ± 0.76^{a}	8.94 ± 0.45^{a}
WPI-0.10	14.39 ± 0.72^{b}	41.37 ± 2.07^{b}	3.27 ± 0.16^{b}
WPI-0.15	12.08 ± 0.60^{c}	23.92 ± 1.20^{c}	6.19 ± 0.31^{a}

WPI-C (control film), WPI-0.05 (0.05% sunflower oil added WPI film), WPI-0.10 (0.10% sunflower oil added WPI film), WPI-0.15 (0.15% sunflower oil added WPI film). Values were given as mean ± standart deviations. Different letter in the same column indicates statistical differences (Duncan's test, $p < 0.05$).

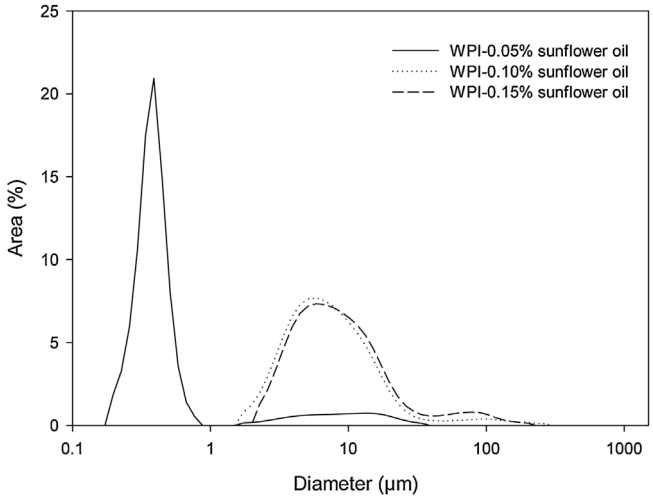

Fig. 2 – Particle size distributions of film forming dispersions as a function of sunflower oil concentration. WPI: whey protein isolate.

shoulder peak at 0.37 μm and a wider second peak from 1 to 50 μm. The most of the oil droplet population of 0.05% sunflower oil added WPI film had small particles (<1 μm). After the emulsification process, the coalescence of smaller droplets into larger ones might be the reason of the bimodal nature of the droplet size distribution. However, 0.10% and 0.15% sunflower oil added WPI films had bigger particles than others and they existed in the range of 1–400 μm. It was interesting to note that the distribution changed significantly with increasing oil concentration from 0.05 to 0.10 with the increase in size. 0.10% and 0.15% sunflower oil added WPI films showed similar distributions (Fig.2). Atarés et al. (2010) were examined particle size of a film solution containing sodium caseinate and two different essential oils (clove and ginger), they found three-modal and wider distribution (approximately from 0.01 to 800 μm) than our results (Atarés et al., 2010). They had suggested that essential oils are liquid at room temperature so they are small in size, but small particles had a tendency to get the aggregated structure after emulsification. Galus and Kadzińska (2016) had found $D_{3,2}$ between 3.11 and 3.47 μm for almond and walnut oils (0.5 and 1.0%) added whey protein films. Whereas in another study cinnamon essential oil (protein to oil mass ratios as 1:0.025, 1:0.050, 1:0.075 and 1:0.100) had added into sodium caseinate based films and $D_{3,2}$ values were between 0.63 and 19.4 μm; $D_{4,3}$ were found between 44 and 69 μm (Atarés et al., 2010). It was possible to state that some of the possible reasons for the existence of such different results in particle size and size distributions were the differences in the amount and/or type of oil used.

3.8. *Water vapor and oxygen permeability*

Water vapor permeability (WVP) and oxygen permeability (OP) values are given in Table 6 for the control and emulsion films as a function of sunflower oil concentration. Regardless of the concentration, sunflower oil addition decreased the water vapor permeability, and lower values were determined when compared to control film samples. Decreased water vapor permeability values with sunflower oil incorporation may be explained by generation of an interconnecting lipid network within the protein matrix, which caused high hydrophobicity and ultimately reduced the absorption of water through the film (Lee et al., 2019; Valenzuela et al., 2013). Previously similar trends were reported in different studies for whey protein films that were added olive oil (Javanmard and Golestan, 2008), rapeseed oil (Kokoszka et al., 2010), oregano oil (Lee et al., 2019) and waxes (Talens and Krochta, 2005). They suggested that incorporation of lipids into the whey protein film matrix significantly improved the water barrier properties. Srinivasa et al. (2007), had similar observations for the fatty acid-added chitosan films, they reported that water vapor permeability of emulsified films were found higher than that of pure chitosan film. Additionally, water vapor and/or oxygen permeation could also be influenced by film morphology and existence of pores, which could increase the water vapor migration, because gas permeation contributes to the capillary mechanism (Srinivasa et al., 2007).

The oxygen permeability values (Table 6) found as $1.17\ cm^3\ mm\ m^{-2}\ h^{-1}\ atm^{-1}$ for the control film, which is a close figure to those reported for whey protein films (Ramos et al., 2013). Whey protein based films are excellent oxygen barriers because of their highly polar nature due to the high degrees of hydrogen bonding of highly polar proteins (Chen, 1995). It was accepted that hydrogen bonding creates limited polymer chain motion, resulting in low gas permeability. It is known that oxygen permeability of protein-based films is lower than that of conventional synthetic films such as low density polyethylene (LDPE) and high density polyethylene (HDPE) (Galus and Kadzińska, 2016). The oxygen permeability of WPI based film samples (Table 2) was about seven times lower than that of LDPE ($7.77\ cm^3\ mm\ m^{-2}\ h^{-1}\ atm^{-1}$) (McHugh and Krochta, 1994a). Hernandez and Giacin (1998) had suggested that higher cohesive energy between protein molecules and lower free volume among polymer chains can be directly related with the oxygen barrier property of the films (Hernandez and Giacin, 1998). A similar trend was observed in our study since it was determined that changed concentration of the oil, increasing from 0.05 to 0.10%, caused an increase in oxygen permeability values, as well. It was predicted that oxygen solubility is higher in the lipid phase than hydrocolloids. This assumption was supported by a research study that when lipid concentration increased in the hydrocolloid film matrix,

Table 6 – Water vapor and oxygen permeability of control and biocomposite films.

Film type	Thickness (mm)	WVP (g mm m^{-2} h^{-1} kPa^{-1})	OP (cm^3 mm m^{-2} h^{-1} atm^{-1})
WPI-C	0.23 ± 0.01^a	2.57 ± 0.13^a	1.17 ± 0.06^a
WPI-0.05	0.15 ± 0.01^a	0.62 ± 0.03^b	0.41 ± 0.02^b
WPI-0.10	$0.21 \pm 0{,}01^a$	1.66 ± 0.08^c	1.15 ± 0.06^a
WPI-0.15	0.16 ± 0.01^a	1.42 ± 0.07^c	0.62 ± 0.03^c

WPI-C (control film), WPI-0.05 (0.05% sunflower oil added WPI film), WPI-0.10 (0.10% sunflower oil added WPI film, WPI-0.15 (0.15% sunflower oil added WPI film). Values were given as mean ± standart deviations. Different letter in the same column indicates statistical differences (Duncan's test, $p < 0.05$).

Table 7 – Mechanical properties of control and biocomposite films.

Film type	Percent elongation (%)	Tensile strength (MPa)
WPI-C	10.5 ± 0.53^a	4.1 ± 0.21^a
WPI-0.05	21.7 ± 1.09^b	1.7 ± 0.09^b
WPI-0.10	32.3 ± 1.62^c	1.2 ± 0.06^b
WPI-0.15	39.9 ± 1.18^c	1.1 ± 0.05^b

WPI-C (control film), WPI-0.05 (0.05% sunflower oil added WPI film), WPI-0.10 (0.10% sunflower oil added WPI film), WPI-0.15 (0.15% sunflower oil added WPI film). Values were given as mean ± standart deviations. Different letter in the same column indicates statistical differences (Duncan's test, $p < 0.05$).

higher oxygen permeability values were found (Pan et al., 2014; Valenzuela et al., 2013). According to relevant research studies, when the lipid phase incorporated in a protein matrix, more heterogeneous structures and higher gas permeation capacities were observed. It was predicted that the existence of lipid in biopolymer matrix might lead to a decrease in crystalline spacing by matrix restructuring, that resulted in channels and pores probably facilitating oxygen permeability (Bertan et al., 2005), as previously reported in the water vapor permeability results. Since many factors were affecting the permeability properties of the films, it was hard to explain further decrease in oxygen permeability with changing concentration from 0.10 to 0.15%. It was possible to state that incorporation of lipid was advisable to get lower water vapor and oxygen permeability compared to the control film.

3.9. *Mechanical properties*

Mechanical properties of the tested films are shown in Table 7. Control film had the lowest E% value with 10.54%, and E5 values of the films rose linearly with the addition of sunflower oil. It can be possible to state that percent elongation increased proportionally with increasing oil content in emulsion matrix. Since sunflower oil is liquid at room temperature and it contains a high content of mono-unsaturated fatty acids, it provides more soft and elastic film structure. All film samples containing sunflower oil had lower tensile strength values than that of the control film (Table 7). This behavior could be explained by the replacement of polymers due to lipid existence in the film matrix, since stronger interactions can take place between polar-polar (control film samples) molecules compared to interactions between non-polar (lipid) and polar (protein) molecules (Lee et al., 2019; Yang and Paulson, 2000). Furthermore, decreasing TS values might be attributed to structural heterogeneity of the film and oil incorporation, which caused negative effect on the cohesion forces of the WPI film samples (Azevedo et al., 2019; Petersson and Stading, 2005) therefore resulted in featuring discontinuities. Javanmard and Golestan (2008) had suggested that increased soya oil in whey protein films (oil to protein ratio: 0.0, 0.2, 0.3, and 0.4) had caused a decrease in TS; they also reported that decreased TS could be due to the plasticizing effect of the lipids in hydrocolloid films (Javanmard and Golestan, 2008). Galus and Kadzińska (2016) had studied the effects of 0.5 and 1.0% walnut and almond oil addition into whey protein films and they had indicated that using a low amount of oils in matrix was more effective on modifying mechanical properties of the films compared to higher oil addition (Galus and Kadzińska, 2016). In contrast to these studies, there are also studies reporting adverse effects of oil content on TS values of different types of edible films (Bonilla et al., 2012; Ghasemlou et al., 2011), such as gelatin (Bertan et al., 2005), gellan (Yang and Paulson, 2000), sodium caseinate (Chen, 1995), wheat gluten (Gontard et al., 1994) or whey protein isolate (Shellhammer and Krochta, 1997).

3.10. *Morphology of films*

Atomic force microscopy (AFM) analysis was examined in order to observe morphological structure of emulsified films. In the AFM analysis, there are two different image surfaces examined which were contacting with air called air side and contacting with glass substrate called support side. They were taken to evaluate the surface properties and understand the phases in film composition better. AFM analysis showed that biocomposite films induced slight differences in the structural arrangement of the film surface compared to the control film surface. The support side of all films generally showed more smooth and flat surfaces than the air sides, but 0.15% sunflower oil added WPI film displayed an irregular and rough surface with branched and some aggregated lines compared to the other tested films (Fig. 3). This result might be related to naturally high intermolecular chain interaction forces occurring between whey protein isolate and sunflower oil matrix that cause the formation of complex entanglements. AFM analysis results revealed that the support sides had relatively uniform and smooth surfaces compared to the air sides of the films. On the other hand, some agglomerations occurred probably during the heat denaturation of proteins, and a few cracks were seen locally on some samples, but neither the dispersion of particles nor the individual particles were clearly observed. In the results, generally Rq and Ra values showed an increasing tendency to oil content for both the air and the support sides. High Rq and Ra values indicated more rough surface whereas lower values indicated smoother surfaces, which were confirmed with the image results (Galus and Kadzińska, 2016). The principle reason of increase in the surface roughness and existence of holes & cracks might be occurrence of coalescence and creaming of oil droplets (Ma et al., 2012; Song et al., 2018) and/or phase separation between the oil and WPI

Table 8 – Pearson's correlation coefficients (p-value) between tensile strength (TS), elongation (E%), water vapor permeability (WVP), oxygen permeability (OP), whiteness index (WI), contact angle (CA) and swelling index (SI) values of the films, respectively.

	TS	E%	WVP	OP	WI	CA (air side)	SI
TS	1						
E%	−0.901**						
WVP	0.726**	−0.407					
OP	0.507	−0.284	0.870**				
WI	0.045	0.120	0.072	0.073	1		
CA (air side)	−0.222	0.595	0.307	0.239	0.646	1	
SI	−0.092	−0.246	−0.727**	−0.702	0.187	−0.510	1

** $p < 0.05$.

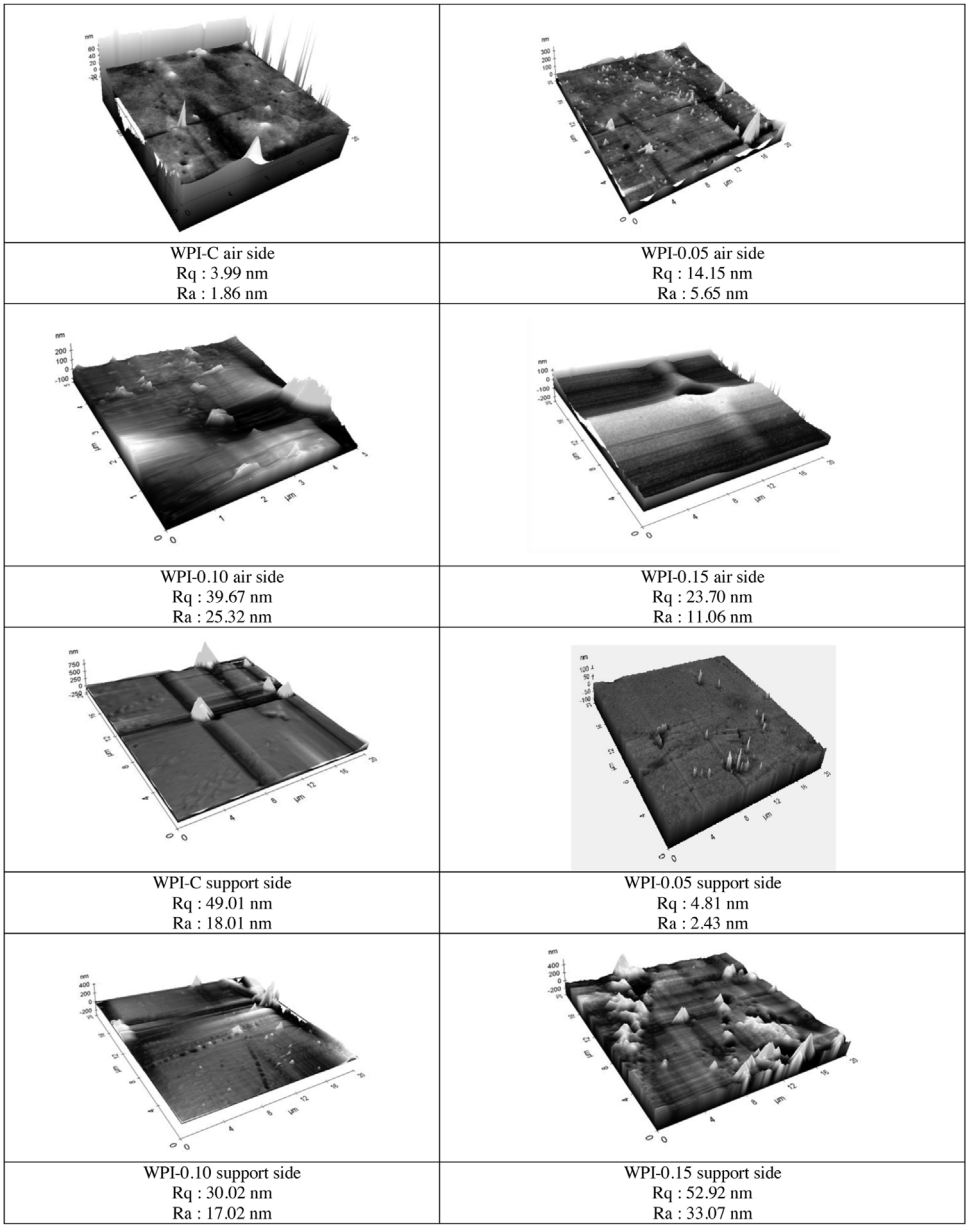

Fig. 3 – AFM topography images of the film samples (20 × 20 μm). WPI-C (control film), WPI-0.05 (0.05% sunflower oil added WPI film), WPI-0.10 (0.10% sunflower oil added WPI film), WPI-0.15 (0.15% sunflower oil added WPI film).

matrix (Azevedo et al., 2019). Atarés et al. (2010) and Lee et al. (2019) observed similar results on sodium caseinate-ginger oil emulsion films and HPMC-oregano essential oil nanoemulsions respectively, in which oil underwent aggregation during the step of drying, thus causing irregularities on the films surface (Atarés et al., 2010; Lee et al., 2019). Abdollahi et al. (2019) was found that heterogeneity of surface microstructure of the CMC-agar film was further increased by increasing summer savory essential oil content to 1.5%.

3.11. *Pearson correlation*

Correlation matrix between mechanical properties (tensile strength (TS), percent elongation (E%)), barrier properties (water vapor permeability (WVP), oxygen permeability (OP)), color (whiteness index (WI)), and water absorption properties (swelling index (SI)) of the film samples were given in Table 8. This method gives information about the correlation, as well as the direction and statistical relationship between variables. In the study, a negative and significant correlation was found between the TS and E% ($r = -0.901$; Table 8). In other words, when TS value decreased, E% value increased. On the other hand, there was a positive and strong correlation between water vapor and oxygen permeability ($r = 0.870$, Table 8). It is possible to say that heterogeneity in the structure due to an increase in the oil content increase both water vapor and oxygen permeability. Also the microstructure test of AFM supported these assumptions that 0.10% and 0.15% sunflower oil added films were showed more tangled, heterogenic and aggregated images.

4. Conclusions

Effects of addition of sunflower oil on some of the properties of whey protein isolate based films were investigated. Moisture content of the control film had the highest value among the other film samples, however there was no statistical difference between moisture content of the biocomposite and control films ($p > 0.05$). The presence of sunflower oil in the whey protein film matrix did not drastically modify sorption behaviour despite their non-polar character. Control film had the lowest opacity, thereof higher degree of transparency compared with the biocomposite films produced were determined. Biocomposite films featured a higher hydrophobic nature compared to the control films, which was confirmed by lower water vapor permeability and higher contact angle measurements. Total color and whiteness index values were not affected by lipid addition. Microstructure of films showed that there were no drastic changes due to oil existence. Incorporation of sunflower oil into WPI films caused increasing in glass transition and heat of fusion. All emulsions had a bimodal particle size distribution, there were significant differences in the mean diameters of dispersions without any direct relation with oil concentrations ($p < 0.05$). These findings demonstrated the produced films' potential usage in packaging industry whereas further studies would be required to investigate performance improvement for industrial usage in commercial food systems.

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