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Physical and mechanical properties in biodegradable films of whey protein concentrate–pullulan by application of beeswax

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ABSTRACT

Different ratios of whey protein concentrate (WPC):pullulan (PUL) (70:30, 50:50, 30:70%w/w) and various rates of beeswax (BW) (0, 10, 20, and 30%w/$w_{glycerol}$) were applied to prepare biodegradable WPC–PUL films containing glycerol as a plasticizer, for the first time. Thickness, moisture content, water solubility, water vapour permeability, colour, and mechanical properties of prepared films were measured. Higher ratios of WPC:PUL led to more desirable physical and mechanical properties; in other words, lower rates of thickness, moisture content, water solubility and water vapour permeability, and higher elongations were achieved. Application of BW (especially in higher contents) could successfully improve colour indices, diminish water solubility (nearly 12%) and water vapour permeability (approximately 3×10^{-11} g m^{-1} s^{-1} Pa^{-1}), and increase tensile strength (by about 7 MPa) of WPC–PUL blend films. Our edible films enjoyed great whiteness and ignorable yellowness indices, making it a suitable alternative for application in food products. Overall, WPC70-PUL30 containing 30% BW resulted in the best performance of physical and mechanical aspects as an optimum film.

1. Introduction

Recently, studies on edible films have been intensified, since the potential benefits of these films are both environmental and cost-related. Components of edible films can be divided into three categories: hydrocolloids, lipids and composites (Fabra, Talens, & Chiralt, 2008b). Hydrocolloids include proteins and polysaccharides, such as starch, alginate, cellulose derivatives, chitosan, and agar. Lipids include waxes, acyl-glycerols, and fatty acids. Composites contain both hydrocolloid components and lipids. A composite film could be in the form of dried emulsions, or bilayers. The choice of materials for a film is largely dependent on its desired functions (Kokoszka, Debeaufort, Hambleton, Lenart, & Voilley, 2010a; Kokoszka, Debeaufort, Lenart, & Voilley, 2010b). Pullulan (PUL) is produced extracellularly by the fungus-like yeast, *Aureobasidium pullulans*. PUL is a water-soluble polysaccharide with excellent film-forming properties. Its films are colourless, tasteless, odourless, flavourless, transparent, flexible, highly impermeable to oil and oxygen and heat-sealable (Kristo & Biliaderis, 2007). The $\alpha(1 \rightarrow 6)$ linkages that interconnect maltotriose units are responsible for its irregularly ordered chains and result in an amorphous structure in this polysaccharide (Kristo & Biliaderis, 2006). These properties determine the application of PUL films as food coatings, packaging materials or an encapsulating matrix. However, the wider application of PUL is still limited, due to its high price (Gniewosz et al., 2014). On the other side, application of whey protein for production of films has received a large attention in the last few years. This is quite understandable, since these films are edible, biodegradable, based on a waste stream from the cheese industry, and have interesting mechanical properties (Kokoszka et al., 2010a,b). Whey, the by-product of cheese-making, is one of the biggest reservoirs of food proteins, remained largely outside human consumption channels. Hence, great efforts are made to find new uses for whey proteins, for example, as edible and biodegradable films. The use of pure whey protein can be rather expensive for commercial purposes (Ghanbarzadeh & Oromiehi, 2008, 2009). As a result, combination of whey protein concentrate (WPC) and PUL could be effective to omit problems exist for each component, having been examined in this article.

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Films made from polysaccharides or proteins usually show good mechanical properties, but are rather sensitive to moisture, due to hydrophilic nature of these components. For example, Gounga, Xu and Wang (2007) prepared edible films from different ratios of whey protein isolate (WPI), PUL and glycerol. WPI–PUL films had a good appearance and 1:1 WPI:PUL resulted in films with greatest values of oxygen permeability, moisture content, and film solubility among different ratios applied. However, although prepared edible films enjoyed similar rates of tensile strength and elongation properties compared with common synthetic films such as LDPE or HDPE but water vapour permeability of WPI–PUL films was several times higher than that of synthetic ones (0.014 or 0.04×10^{-12} mol m m^{-2} s^{-1} Pa^{-1}); at the same time, SEM micrographs showed many pinholes and a favourable structure for their low barrier ability. On the other hand, films made from lipids showed good water vapour barrier properties, but were opaque, only slightly flexible and brittle (Fabra, Talens, & Chiralt, 2008a). There have been a couple of reports on successful applications of beeswax in preparing diverse biodegradable films and coatings where it was proved to reduce water vapour permeability of soy protein isolate based films (Monedero, Fabra, Talens, & Chiralt, 2009), increased water vapour barrier properties of chitosan/zein/sodium alginate–beeswax bilayer coatings (Zhang, Xiao, & Qian, 2014), diminished water vapour permeation and enhanced tensile modulus (in water content of less than 5%) of caseinate–pullulan films laminated by beeswax (Kristo, Biliaderis, & Zampraka, 2007) and improved mechanical resistance, oxygen and moisture barrier properties of Hydroxypropyl methylcellulose films (Navarro-Tarazaga, Massa, & Pérez-Gago, 2011). Thus, composite films which combine protein–polysaccharide films with fatty layers could be of particular interest, since lipids help to lessen water vapour transmission and proteins or polysaccharides give the necessary strength and structural integrity.

It seems that more research could be performed in the field of blend films since their different parts play diverse special roles and impart particular assignments into a composite film; thus, our first aim was to prepare a composite film incorporating all types of biological macromolecules to benefit from those parts simultaneously. To our knowledge, there isn't any research dealing with composite films based on whey protein concentrate and pullulan, blended with a type of lipid, while it seems necessary (as mentioned above) to improve those films (particularly, their water vapour permeability) by application of lipids. Accordingly, our second objective was to modify mechanical and structural properties of WPC–PUL by application of beeswax.

2. Material and methods

2.1. Materials

WPC (70% protein and 20% lactose) and PUL (99% purity) were purchased from AryaRama Co., Iran and Hayashibara Co., Japan, respectively. BW (constituted of cerotinic acid, palmitic acid, merossinic acid, and hexaeicosan acid), acetic acid, glycerol, sodium borate, sodium chloride, calcium chloride, magnesium nitrate, Tween 80, and other chemicals were prepared from Tetrachem Co., Iran.

2.2. Pre-treatments

Preparation of a suitable WPC film requires appropriate studies in order to select a desirable level of matrix context and a given temperature range for heating process applied on the protein in an aqueous context. The best concentration of WPC in distilled water was opted for 5% since a gel complex was obtained at higher levels and no film was formed at lower levels than 5%. After trial and error procedure, Tween 80 and borax (sodium borate) were selected as emulsifiers for BW dissolution. The BW emulsion was prepared by preparing a ratio of 2:2:25:50 g for borax:Tween:BW:water. Addition of glycerol at 20% (w/w on solid content) led to forming a flexible and integrated film; lower and higher concentrations culminated in brittle and sticky films, respectively.

Table 1
Different ratios of three components for preparing films and their symbols in the current research.

PUL (%w/w)	WPC (%w/w)	BW (%w/w$_{glycerol}$)	Film symbol
30	70	0	WPC70-BW0
		10	WPC70-BW10
		20	WPC70-BW20
		30	WPC70-BW30
50	50	0	WPC50-BW0
		10	WPC50-BW10
		20	WPC50-BW20
		30	WPC50-BW30
70	30	0	WPC30-BW0
		10	WPC30-BW10
		20	WPC30-BW20
		30	WPC30-BW30

2.3. Film preparation

WPC was dissolved in distilled water and heated by a hotplate-stirrer (VWR, Germany) for 30 min at 90 °C; then, the solution was cooled to room temperature. PUL was also dissolved into distilled water in another beaker; there was no need to apply heating treatment for PUL dissolving process. After cooling, WPC and PUL solutions were mixed in three different ratios of 70:30, 50:50, and 30:70 (%w/w); then, glycerol was added as a plasticizer in 20% (w/w$_{drymatter}$) and the solution was left over on the hitter-stirrer to be homogenized thoroughly. BW was added in 0, 10, 20 and 30% (w/w$_{glycerol}$) rates of glycerol content into the previous solution (Table 1) and it was mixed by a rotor stator homogenizer (IKA®T25 digital, Ultra-Turrax®, Germany) at 13,500 rpm for 1 min and then, at 20,500 rpm for 3 min; finally, this solution poured on the Teflon plates after deaeration in a thermostat vacuum oven (VO400, Memert, Germany) for 1 h. Films were dried slowly in an oven for 48 h at 25 °C. Next, it was stored in LDPE bags at low temperature of 8 °C. All films were conditioned in a desiccator at 50% RH and 25 °C for 48 h prior to tests (Kristo & Biliaderis, 2007).

2.4. Physical properties

2.4.1. Thickness

Thickness of different films was determined by a digital micrometer (IP54, QLR digit, China) to the nearest 0.0001 mm. For each sample, 5 random positions on the film were measured and mean values were used.

2.4.2. Moisture content

Empty capsules were placed in the oven at 110 °C for 1 h to reach the constant weight. Film samples were cut into 3×1 cm^2 pieces; then, they were placed into capsules and weighed by a digital balance (XS 105, Mettler Toledo, Germany) and finally, the capsules were placed in the oven at 110 °C to reach the constant weight (Dehnad, Emam-Djomeh, Mirzaei, Jafari, & Dadashi, 2014). After cooling in the desiccators, the whole films and capsules were

weighed to obtain the dry sample weights. Moisture contents of the films were calculated on the basis of wet weight as follows:

$$MC_{wb} = \left(\frac{\text{Wet sample weight} - \text{Dry sample weight}}{\text{Wet sample weight}} \times 100\right) \quad (1)$$

2.4.3. Water solubility

The dried films were immersed in 50 mL of distilled water for 12 h at 25 °C (Ghasemlou, Khodaiyan, Oromiehie, & Yarmand, 2011). Next, the films were taken out from the water by blotting with filter papers previously dried and weighed to remove the surface adsorbed water. Remaining pieces of films were dried at 110 °C to constant weight (final dry weight). Water solubility (WS) was calculated by using the following equation:

$$WS = \left(\frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}} \times 100\right) \quad (2)$$

2.4.4. Water vapour permeability

This test was conducted gravimetrically using ASTM procedure E96-95. At first, the measurement cells filled with anhydrous calcium chloride desiccant to create a 0% RH storage condition and the surfaces of cells were covered with the films and sealed with molten paraffin. To maintain a 75% RH gradient across the film at 25 °C, a sodium chloride-saturated solution was used in the desiccator. The RH difference between two sides of the films creates a vapour pressure equal to 1753.55 Pa. The cells were weighted at certain intervals by a digital balance (XS 204, Mettler Toledo, Germany) nearest to the 0.00001; the slope of weight loss versus time was obtained by linear regression. A control cell lacking hygroscopic substance but having a film on its surface was considered to calculate rate of the moisture which was absorbed by the film surface and not transmitted to internal space of the cells. Water vapour transition rate (WVTR) and water vapour permeability (WVP) were calculated by the following equations, respectively:

$$WVTR = \left(\frac{\text{Curve slope}}{\text{Film area}}\right) \quad (3)$$

$$WVP = \left(\frac{\text{Thickness} \times \text{WVTR}}{\text{Pressure difference}}\right) \quad (4)$$

2.4.5. Colour

Regarding recent promotes in consumers information and ongoing complexities created in their demands, application of new approaches for quality control seems inevitable. Colour values of packaging films were determined by a colorimeter (D25-9000, Hunterlab, U.S.). The CIELab scale was used, L^* (brightness) and chromaticity parameters of a^* (red to green) and b^* (yellow to blue) were measured. For each film, three different points were selected and following indices, indicating yellowness (Y_i), whiteness (W_i), and total colour difference (ΔE), were calculated (Bourtoom & Chinnan, 2008):

$$Y_i = \frac{142.86b}{L} \quad (5)$$

$$W_i = 100 - \sqrt{\left[(100 - L)^2 + a^2 + b^2\right]} \quad (6)$$

$$\Delta E = \sqrt{\left[(L - L^*)^2 + (a - a^*) + (b - b^*)^2\right]} \quad (7)$$

2.5. Mechanical properties

The films were cut into $1 \times 10\,cm^2$ pieces and maintained for moisture equilibrium in desiccator at 25 °C and 50% RH for 24 h. Saturated magnesium nitrate solution was used to meet required relative humidity. Mechanical properties of the films were measured using a texture analyser (SMS, Surrey, U.K.) according to the ASTM method D882 (ASTM, 1995); initial grip separation and crosshead speed were set at 50 mm and 5 mm/min, respectively. Tensile strength (TS) and elongation-at-break (E_b) were calculated from Force-Extension curves. E_b value was calculated as the ratio of final length at the point of sample rupture to the initial length of a specimen and expressed as a percentage. TS value was calculated by the following Eq. (8); three replicates of each film were tested.

$$TS = \frac{\text{Maximum force}}{(\text{Film thickness}) \times (\text{Film width})} \quad (8)$$

Table 2
Thickness and moisture contents of WPC–PUL-BW films.

Film type	Thickness (mm)	Moisture content (%)
WPC70-BW0	0.093 ± 0.0018^{bc}	7.32 ± 1.49^{cd}
WPC70-BW10	0.066 ± 0.0067^{a}	8.82 ± 1.73^{ef}
WPC70-BW20	0.076 ± 0.0067^{ab}	9.30 ± 1.34^{g}
WPC70-BW30	0.073 ± 0.0033^{ab}	10.05 ± 0.84^{h}
WPC50-BW0	0.100 ± 0.0024^{c}	7.63 ± 0.93^{d}
WPC50-BW10	0.100 ± 0.0017^{c}	8.40 ± 0.69^{f}
WPC50-BW20	0.093 ± 0.0045^{bc}	8.84 ± 1.11^{ef}
WPC50-BW30	0.093 ± 0.0029^{bc}	9.25 ± 2.11^{fg}
WPC30-BW0	0.086 ± 0.0067^{abc}	6.02 ± 0.55^{a}
WPC30-BW10	0.090 ± 0.0011^{bc}	6.63 ± 1.17^{b}
WPC30-BW20	0.090 ± 0.0033^{bc}	6.86 ± 1.24^{bc}
WPC30-BW30	0.093 ± 0.0033^{bc}	7.45 ± 1.46^{cd}

2.6. Field emission scanning electron microscopy (FESEM)

All the specimen were examined by a FESEM (SU-70, Hitachi, Japan) equipment at an accelerating voltage of 15 kV and different magnitudes. First, the samples were cryo-fractured by immersion in liquid nitrogen and mounted on aluminium stubs. Then, the portions were gold coated with a sputter coater for 5 min. The resolution was 500 to 2000 μm.

2.7. Statistical analysis

Different treatments were applied according to a completely randomized factorial design at three replications. Data analysis and mean comparison were performed with application of SPSS 9.1 (SPSS Inc., Chicago, IL) and Duncan test in the level of $P < 0.05$, respectively. EXCEL software was applied for plotting data.

3. Results and discussion

3.1. Physical properties of WPC–PUL-BW films

3.1.1. Thickness

Thickness rates of the films were variable between 0.066 and 0.1 mm (Table 2). Different ratios of components led to nearly same thickness rates of films; of course, WPC70 films justified lower thickness than their counterparts. Lower thickness of edible films improves their digestibility and solubility in mouth, arising overall desirability of final packaged products. Increasing PUL ratio enlarged thickness of the films to some extent, distinctive than BW. Our results indicated that changes in BW and glycerol levels didn't leave behind any significant changes in thickness of prepared films ($P > 0.05$); the results were in accordance with similar research carried out by Monedero et al. (2009) on plant-protein based films containing beeswax as a lipid component.

3.1.2. Moisture content

The moisture contents of the prepared films are listed in Table 2. As clear in the table, moisture contents were dependent on BW levels and variable between 6.02 and 10.05% w/w. Increasing WPC and decreasing PUL levels increased moisture content of the films

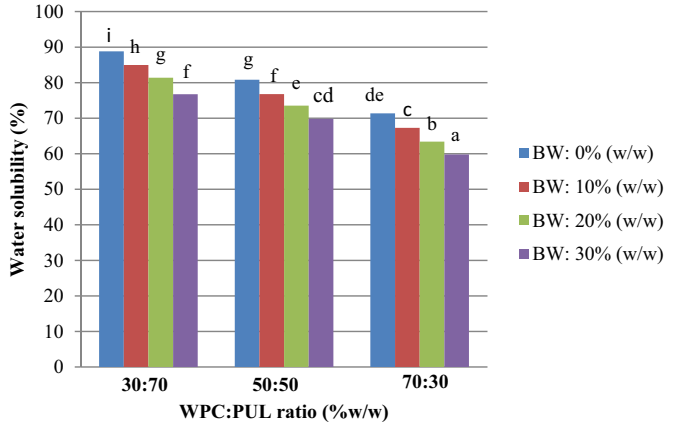

Fig. 1. Water solubility of WPC–PUL-BW films.

significantly ($P<0.05$). According to the results, higher levels of BW culminated in higher moisture contents of final films, possibly due to higher contribution of water molecules retained within BW emulsions in the films. Overall, moisture content of PUL–WPC-BW films was lower than normal edible films prepared by researches, which guarantees high stability of these films for later usage. Our results were in agreement with those obtained by Osés, Fernández-Pan, Mendoza, and Maté (2009) who studied WPC based films; minor observed differences are probably due to different glycerol levels applied in our work.

3.1.3. Water solubility

Fig. 1 compares solubility rates of different combined films in water; this index was between 59% (in the best state) and 88.83%. Increasing PUL proportions augmented water solubility rates of films, in accordance with other studies carried out by Gounga et al. (2007) and Wu, Zhong, Li, Shoemaker, and Xia (2013), due to PUL amorphous structure. Increasing BW decreased water solubility of films considerably because of its hydrophobic nature, reminding one of our initial goals of BW application. Specifically, WPC70-BW30 is offered to attain lower water solubility for most favourable films since a desirable (biodegradable) film should be resistant against unfavourable ambient conditions e.g. wet environments.

3.1.4. Water vapour permeability (WVP)

Fig. 2 stands for WVP values of the films, assorted from 6.95 to 14.53 ($\times 10^{-11}$ g m^{-1} s^{-1} pa^{-1}). Increasing PUL ratio led to higher WVP values since PUL enjoys more internal OH groups than WPC, which is in accordance with the report of Gounga et al. (2007). There were significant differences ($P<0.05$) between WVP values

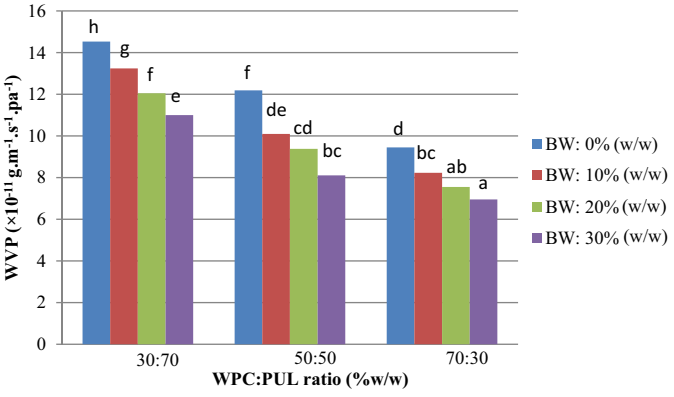

Fig. 2. Water vapour permeability values of WPI-PUL-BW films.

Table 3
Color indices of WPC–PUL-BW films.

Film type	Y_i	W_i	ΔE
WPC70-BW0	0.058 ± 0.0029^{e}	89.75 ± 0.13^{c}	6.68 ± 0.75^{a}
WPC70-BW10	0.021 ± 0.0011^{ab}	88.33 ± 0.21^{bc}	7.51 ± 0.14^{ab}
WPC70-BW20	0.078 ± 0.0015^{f}	86.88 ± 0.07^{a}	9.45 ± 0.02^{d}
WPC70-BW30	0.074 ± 0.0021^{f}	89.05 ± 0.88^{bc}	7.41 ± 0.15^{abc}
WPC50-BW0	0.018 ± 0.0000^{a}	87.88 ± 0.12^{abc}	9.29 ± 0.10^{cd}
WPC50-BW10	0.075 ± 0.0011^{f}	87.66 ± 0.09^{ab}	9.01 ± 0.13^{cd}
WPC50-BW20	0.047 ± 0.0070^{cde}	88.98 ± 0.19^{bc}	7.05 ± 0.13^{ab}
WPC50-BW30	0.055 ± 0.0000^{ed}	87.87 ± 0.16^{abc}	8.01 ± 0.04^{abcd}
WPC30-BW0	0.071 ± 0.0028^{f}	88.14 ± 0.03^{bc}	8.90 ± 0.01^{cd}
WPC30-BW10	0.033 ± 0.0030^{abc}	88.56 ± 0.02^{abc}	8.13 ± 0.05^{bcd}
WPC30-BW20	0.035 ± 0.0018^{bc}	88.41 ± 0.21^{abc}	7.54 ± 0.20^{abcd}
WPC30-BW30	0.041 ± 0.0030^{cd}	89.46 ± 0.04^{c}	6.56 ± 0.02^{a}

of the films having various components or beeswax percentages. Recent studies have suggested that hydrocolloid films are potentially efficient barriers against gas transmittance in spite of their high water vapour permeability values. The main cause of their poor water vapour barricade is hydrophilic nature of carbohydrate compounds; proteins have a low resistance against water vapour permeation, too. Escalating BW reduced WVP values of the films due to its hydrophobic nature. In particular, WPC70-BW30 deserved the minimal WVP rates of all prepared films, recommending higher WPC or BW ratios/rates to obtain an optimum blend film. These WVP rates were lower than that of similar biodegradable films e. g. caseinate–PUL laminated by beeswax (Kristo et al., 2007) or whey protein isolate-glycerol (Kokoszka et al., 2010a,b) justifying at least 9×10^{-10} or 2×10^{-10} (g m^{-1} s^{-1} Pa^{-1}) water vapour permeation, respectively.

3.1.5. Colour

Table 3 signifies total colour difference, yellowness and whiteness indices of prepared films. Generally, ΔE didn't follow a same trend for different quotients of WPC:PUL. However, at WPC30 levels, increasing BW concentration was associated with a significant decrease in ΔE values of the films but at two other WPC:PUL levels, ΔE values remained nearly constant at various BW levels. W_i values of prepared films were in the range of 86.88–89.75; hinting high brightness of WPC–PUL-BW films and their fitness for food applications. As obvious, W_i index levelled out in different ranks of BW, indicating insignificant influence of beeswax addition on brightness of films. Although Y_i values of dissimilar films covering miscellaneous ingredient ratios differed with each other, this index was too low for all cases, indicating final bright films as declared above. Overall, WPC70 films deserved better colour parameters including lower ΔE values and higher W_i indices than other types of films; although WPC30-BW30 treatment justified the best colour parameters, advocating application of higher BW rates to improve colour attraction of packaging films in food industries.

Table 4
Mechanical properties of WPC–PUL-BW films.

Film type	E_b (%)	TS (MPa)
WPC70-BW0	291.3	2.11
WPC70-BW10	198.6	4.85
WPC70-BW20	160.9	7.63
WPC70-BW30	121.2	9.28
WPC50-BW0	282.5	2.85
WPC50-BW10	193.7	5.15
WPC50-BW20	154.3	8.00
WPC50-BW30	114.9	10.10
WPC30-BW0	276.4	3.19
WPC30-BW10	189.6	5.75
WPC30-BW20	150.1	8.32
WPC30-BW30	109.4	10.73

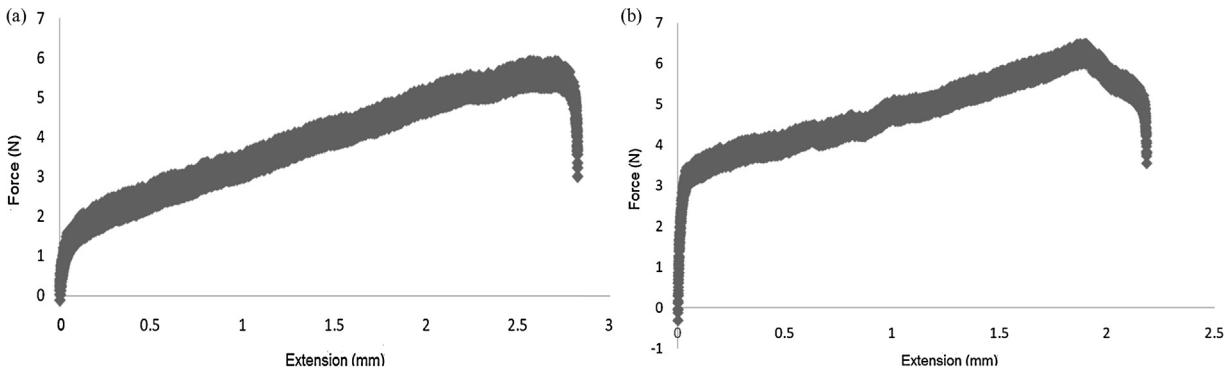

Fig. 3. Force-Extension curves of composite films containing 50%WPC, 50% PUL, and (a) 0 or (b) 30%BW.

3.2. Mechanical properties of WPC–PUL-BW films

One of the most important criteria for evaluating an industrial biodegradable film is its mechanical properties. This feature is affected by chain length and molecular weight of involved biomacromolecules and extent or position of branched chains. These properties influence biopolymer capability to form intermolecular connections. More elevated and stronger the intermolecular connections, superior the structural integrity or better mechanical resistance. It shouldn't be left unsaid that some prepared films benefited from nearly 400% elongation rates, which can be invaluable for industrial films with food applications. Increasing WPC:PUL

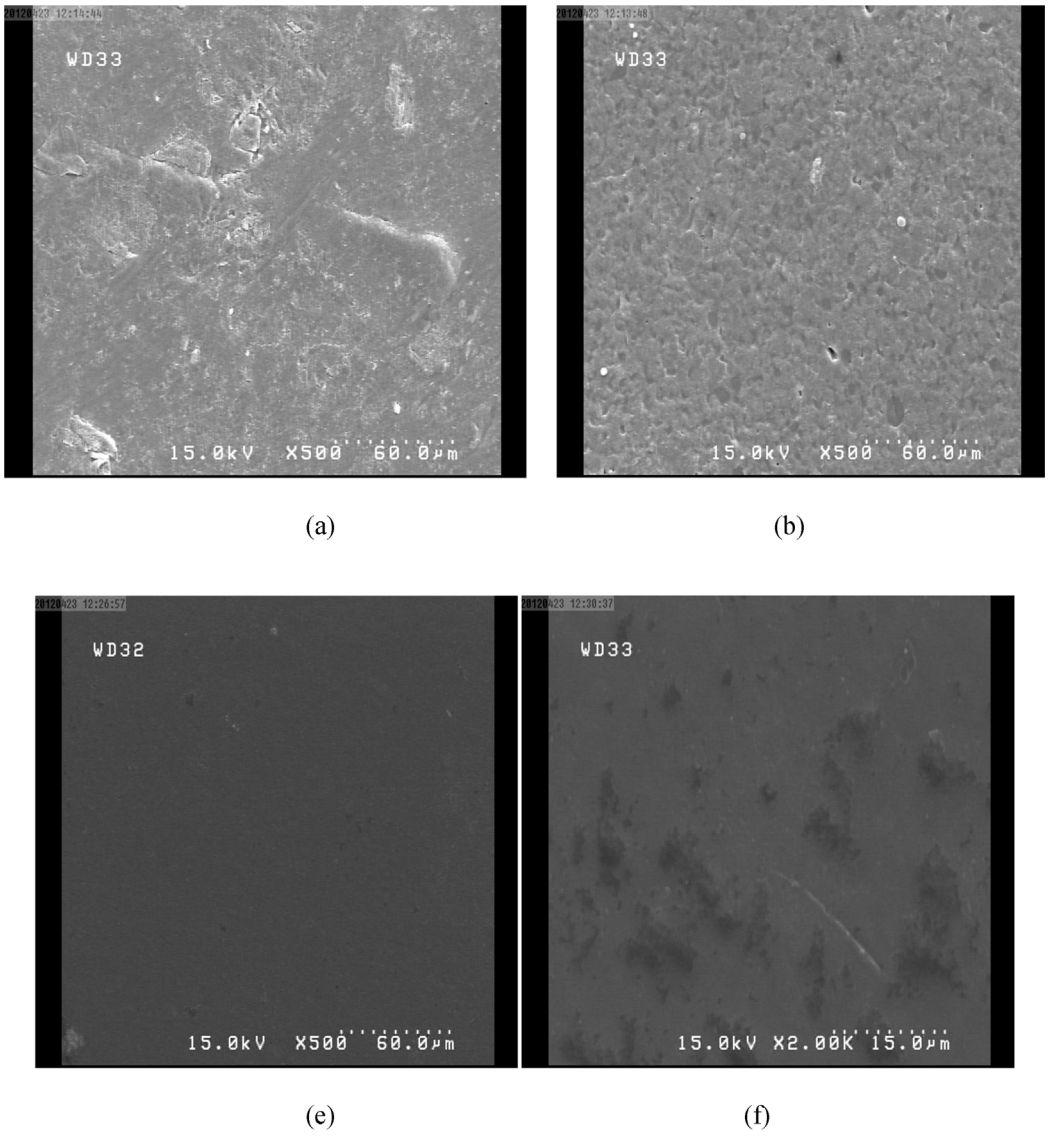

Fig. 4. Micrographs of WPC–PUL-BW blend films. (a) WPC70-BW0, (b) WPC70-BW10, (c) WPC30-BW20, and (d) WPC30-BW30.

ratio was able to increase E_b percentage (Table 4). Addition of BW diminished intermolecular spaces and increased physical interactions including hydrogen connections between molecules into the films; therefore, it can justify lower flexibility and higher tensile strength for the films containing beeswax. The results obtained in this research were in accordance with previous findings by Fabra, Talens, and Chiralt (2009), but high elongation rates achieved hasn't been reported in previous similar researches. For example, Soazo, Rubiolo, and Verdini (2011) reported just 2–4% elongation percentage for WPC–BW films dried at 25 °C whereas WPC70-BW0 or WPC70-BW10, entitled to be considered as the best blend films in terms of E_b, justified 291.3 or 198.6% elongation liability. It is to be noted that incorporating glycerol in protein–polysaccharide-based films might interfere with polymer chain-to-chain interactions and provide flexible domains within the film; hence, it could reduce strength and increase flexibility of original films (Talens & Krochta, 2005).

Although E_b percentages (109–199%) of WPC–PUL-BW films was much higher than a lot of novel edible films such as alginate (6.5–13), gelatine (10–65) or pea starch (3–45), Tensile strength (TS) of these films were between 2 and 10 MPa which were lower than that of normal polymers such as LDPE (9–17 MPa), polystyrene (35–55 MPa) or cellophane (114 MPa) (Azerado et al., 2010). The reverse trend of changing in TS compared with E_b is obvious regarding data listed in Table 4; increasing PUL ratios and BW rates could improve TS index of WPC films. Specifically, WPC30-BW30 guaranteed the highest TS rates among different ratios assumed for components. Fig. 3 illustrates two force-extension diagrams attained for prepared films by a texture analyser, exemplifying higher peak area for composite film containing 30% BW, compared with blend film lacking BW component.

3.3. FESEM

Fig. 4 represents surface microstructures of prepared films with different ingredients/ratios. It deserves to point out that while films without BW displayed smooth and regular structure justifying low hydrophobic trend of that film (Fig. 4a), films containing BW indicated different kinds of unevenness in their structures (Fig. 4b). It could be concluded that during drying stage, BW came towards the surface of film and led to less WVP values of those films. Surface microstructures of WPC30-BW20 and WPC30-BW30, enjoying high TS rates, have been represented in Fig. 4, too; desirable uniting of different layers and lacking agglomeration are evident in this figure.

4. Conclusion

Rising BW ratios could moderate water solubility and WVP values of WPC–PUL films due to its hydrophobic nature. Addition of BW was capable of lessening ΔE and raising W_i values of WPC–PUL blend films, assisting them to be comparable with an increasing volume of transparent edible films being prepared from different sustainable resources these days. Supreme elongation capacity of 291.3% was achieved by application of higher WPC:PUL ratios and lower BW rates although applying of upper BW levels guaranteed the highest TS rates among different films. To sum up, composite films of WPC–PUL (containing BW) were promising films in terms of physical, mechanical and structural properties for food applications when compared with other biodegradable and synthetic films.

References

ASTM. (1995). Standard test methods for water vapour transmission of material. In *Standard E 95-96*. Philadelphia, PA: American Society for Testing and Materials.

Azerado, H. M. C., Mattoso, L. H. C., Avena-Bustillos, R. J., Filho, G. C., Munford, M. L., Wood, D., et al. (2010). Nanocellulose reinforced chitosan composite films as affected by nanofiller loading and plasticizer content. *Journal of Food Science*, *75*, N1–N7.

Bourtoom, T., & Chinnan, M. S. (2008). Preparation and properties of rice starch–chitosan blend biodegradable film. *LWT—Food Science and Technology*, *41*(9), 1633–1641.

Dehnad, D., Emam-Djomeh, Z., Mirzaei, H., Jafari, S. M., & Dadashi, S. (2014). Optimisation of physical and mechanical properties for chitosan–nanocellulose biocomposites. *Carbohydrate Polymers*, *105*, 222–228.

Fabra, M. J., Talens, P., & Chiralt, A. (2008a). Effect of alginate and λ-carrageenan on tensile properties and water vapour permeability of sodium caseinate–lipid based films. *Carbohydrate Polymers*, *74*(3), 419–426.

Fabra, M. J., Talens, P., & Chiralt, A. (2008b). Tensile properties and water vapour permeability of sodium caseinate films containing oleic acid–beeswax mixtures. *Journal of Food Engineering*, *85*(3), 393–400.

Fabra, M. J., Talens, P., & Chiralt, A. (2009). Microstructure and optical properties of sodium caseinate films containing oleic acid–beeswax mixtures. *Food Hydrocolloids*, *23*(3), 676–683.

Ghanbarzadeh, B., & Oromiehi, A. R. (2008). Biodegradable biocomposite films based on whey protein and zein: Barrier, mechanical properties and AFM analysis. *International Journal of Biological Macromolecules*, *43*(2), 209–215.

Ghanbarzadeh, B., & Oromiehi, A. R. (2009). Thermal and mechanical behaviour of laminated protein films. *Journal of Food Engineering*, *90*(4), 517–524.

Ghasemlou, M., Khodaiyan, F., Oromiehie, A., & Yarmand, M. S. (2011). Characterization of edible emulsified films with low affinity to water based on kefiran and oleic acid. *International Journal of Biological Macromolules*, *49*, 378–384.

Gniewosz, M., Synowiec, A., Kraśniewska, K., Przybył, J. L., Bączek, K., & Węglarz, Z. (2014). The antimicrobial activity of pullulan film incorporated with meadowsweet flower extracts (*Filipendulae ulmariae flos*) on postharvest quality of apples. *Food Control*, *37*, 351–361.

Gounga, M. E., Xu, S. Y., & Wang, Z. (2007). Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation. *Journal of Food Engineering*, *83*(4), 521–530.

Kokoszka, S., Debeaufort, F., Hambleton, A., Lenart, A., & Voilley, A. (2010). Protein and glycerol contents affect physico-chemical properties of soy protein isolate-based edible films. *Innovative Food Science & Emerging Technologies*, *11*(3), 503–510.

Kokoszka, S., Debeaufort, F., Lenart, A., & Voilley, A. (2010). Water vapour permeability, thermal and wetting properties of whey protein isolate based edible films. *International Dairy Journal*, *20*(1), 53–60.

Kristo, E., & Biliaderis, C. G. (2006). Water sorption and thermo-mechanical properties of water/sorbitol-plasticized composite biopolymer films: Caseinate–pullulan bilayers and blends. *Food Hydrocolloids*, *20*(7), 1057–1071.

Kristo, E., & Biliaderis, C. G. (2007). Physical properties of starch nanocrystal-reinforced pullulan films. *Carbohydrate Polymers*, *68*(1), 146–158.

Kristo, E., Biliaderis, C. G., & Zampraka, A. (2007). Water vapour barrier and tensile properties of composite caseinate–pullulan films: Biopolymer composition effects and impact of beeswax lamination. *Food Chemistry*, *101*(2), 753–764.

Monedero, F. M., Fabra, M. J., Talens, P., & Chiralt, A. (2009). Effect of oleic acid–beeswax mixtures on mechanical, optical and water barrier properties of soy protein isolate based films. *Journal of Food Engineering*, *91*(4), 509–515.

Navarro-Tarazaga, M. L., Massa, A., & Pérez-Gago, M. B. (2011). Effect of beeswax content on hydroxypropyl methylcellulose-based edible film properties and postharvest quality of coated plums (*Cv. Angeleno*). *LWT—Food Science and Technology*, *44*(10), 2328–2334.

Osés, J., Fernández-Pan, I., Mendoza, M., & Maté, J. I. (2009). Stability of the mechanical properties of edible films based on whey protein isolate during storage at different relative humidity. *Food Hydrocolloids*, *23*(1), 125–131.

Soazo, M., Rubiolo, A. C., & Verdini, R. A. (2011). Effect of drying temperature and beeswax content on moisture isotherms of whey protein emulsion film. *Procedia Food Science*, *1*, 210–215.

Talens, P., & Krochta, J. M. (2005). Plasticizing effects of beeswax and carnauba wax on tensile and water vapour permeability properties of whey protein films. *Journal of Food Science*, *70*(3), E239–E243.

Wu, J., Zhong, F., Li, Y., Shoemaker, C. F., & Xia, W. (2013). Preparation and characterization of pullulan–chitosan and pullulan–carboxymethyl chitosan blended films. *Food Hydrocolloids*, *30*(1), 82–91.

Zhang, W., Xiao, H., & Qian, L. (2014). Enhanced water vapour barrier and grease resistance of paper bilayer-coated with chitosan and beeswax. *Carbohydrate Polymers*, *101*, 401–406.