



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, 30:70w/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 $3 \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$), 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.

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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 & Oromieh, 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 $0.04 \times 10^{-12} \text{ mol m m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$); 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

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

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.

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 $3 \times 1 \text{ cm}^2$ 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

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