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Hydrophobicity, thermal and micro-structural properties of whey protein concentrate–pullulan–beeswax films

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ABSTRACT

In this research, effects of beeswax (BW) on functional properties of whey protein concentrates (WPC):pullulan (PUL) films were investigated. For this purpose, 0, 10, 20 and 30 $w/w_{glycerol}$% BW rates and 30:70, 50:50 and 70:30 w/w% WPC:PUL ratios were applied. Films containing 70% WPC:30% PUL (WPC70) and 30% BW (BW30) justified the highest contact angle (92.4°) among all films; SEM micrographs indicated that BW could come toward the surface of films during drying stage and resulted in a higher hydrophobic behavior of bilayer films compared with blend films. WPC70 supplied the lowest T_g values (36–48 °C) among different proportions of WPC–PUL; the highest melting points were just assured in the absence of BW regardless of combination ratio for WPI:PUL. BW30 films deserved lower roughness rates than BW20 (and even BW10) films, indicating more advantageous microstructure and higher hydrogen connections in BW30 films and justifying similar melting points attained for BW30 films to BW20 or 10 ones. Overall, application of WPC70 and BW30 was recommended to obtain optimum combination of final properties for WPC–PUL–BW bilayer films as SEM exhibited flexible and elastic structures of such films.

1. Introduction

Edible films are mainly prepared from polysaccharides, proteins and lipids, either individually or in combination with each other. As an example, milk protein based edible films have good mechanical strength and are excellent oxygen, lipid, and aroma barriers [1]. Whey protein derivatives have been studied frequently in the last decade for their film forming potentials due to their favorable functional properties and industrial surplus [2]. They are recognized to produce transparent, flexible, colorless, and odorless films. Edible films from whey proteins have shown moderate potential as moisture barriers and are excellent oxygen barriers [3].

However, limits of protein films in general and whey protein films in particular are highly recognized, especially their barrier properties [4]. Several approaches have shown the use of proteins with other film-forming polysaccharide materials to strengthen barrier properties of protein-based films [5]. Polysaccharides are well known for having good film-forming properties; besides, coatings made with these biopolymers or their blends are generally considered as effective gas barriers. In particular, the combination of protein with polysaccharides is an effective method in improving the performance of the films owing to their variability in physical properties and/or their interactions [2]. Pullulan (PUL) is a water-soluble microbial polysaccharide with excellent film-forming properties [6]. Unique linkage pattern of PUL provides distinctive physical properties, including excellent water solubility, adhesive properties and capacity for final PUL films [7,8].

Gounga et al. [9] prepared edible films from whey protein isolate (WPI), and characterized in order to select a best combination of protein concentration, glycerol and PUL ratio. Although addition of PUL at low concentrations was effective enough to modify physical and mechanical properties of WPI significantly, they reported that WPI–PUL combinations resulted in films with great values

Abbreviations: AFM, Atomic Force Microscopy; BW, beeswax; DSC, Differential Scanning Calorimetry; FESEM, Field Emission Scanning Electron Microscopy; LDPE, low density poly ethylene; PP, poly propylene; PUL, pullulan; RH, relative humidity; SC, sodium caseinate; SEM, Scanning Electron Microscopy; WPC, whey protein concentrate; WPI, whey protein isolate; M_p, melting point; R_q, root mean square average; T_g, glass transition temperature.

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of water vapor permeability, moisture content and film solubility. Likewise, usually polysaccharide or protein films show good mechanical properties, but are rather sensitive to moisture, due to the hydrophilic nature of these components [10]. On the other hand, films made from lipids show good water vapor barrier properties, but are opaque, only slightly flexible and brittle [11]. Thus, composite films that combine proteins or polysaccharides with fatty emulsions or fatty layers could be of particular interest, since the lipids help to lessen water vapor transmission and the proteins or polysaccharides give the necessary film strength and structural integrity [12]. Among different lipids, applicable for improving water resistance of those natural films, waxes enjoy the highest hydrophobicity [13] because of their high content in long chain fatty alcohols and alkanes [14].

Kristo et al. [15] investigated water sorption, water barrier properties and mechanical behavior of PUL and sodium caseinate (SC), as well as their blend and bilayer films plasticized with sorbitol (25% dry basis). Beeswax (BW) lamination of plain and blend films resulted in a drastic decrease in water vapor penetration whereas its effects on mechanical properties were ignorable. However, a water vapor permeability of 11.8×10^{-8} ($g\,m^{-1}\,s^{-1}\,Pa^{-1}$), obtained for SC–PUL–BW, was not preferable to that of common synthetic films such as LDPE or PP [16]. In parallel, a study on SC films had displayed them to have tensile and water barrier properties comparable to whey protein films but higher water solubility rates [17], convinced us to replace SC–PUL–BW with WPC–PUL–BW in order to improve hydrophobicity of final bilayer films.

In our previous study [8], we found that a combination of 70% WPC and 30% PUL containing 30% BW resulted in the best performance of physical and mechanical aspects as an optimum biodegradable film in terms of water solubility, water vapor permeability, color, and tensile strength. Therefore, our aim for the current study was to improve hydrophobicity of WPC–PUL blend films by application of BW. Also our objective was to investigate microstructure and thermal properties of WPC–PUL–BW since for biodegradable polymers, which are entitled to substitute synthetic ones, thermal properties should be in the range of applicable industrial films.

2. Materials and methods

2.1. Materials

WPC (70% protein) and PUL (97%) were purchased from AryaRama Co., Iran and Hayashibara Co., Japan, respectively. BW, acetic acid, glycerol, sodium borate, sodium chloride, calcium chloride, magnesium nitrate, Tween 80, and other chemicals were purchased 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 w/w% 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; just 2%) were selected to be used as emulsifiers for BW dissolution. Addition of glycerol at 20% (w/w on dry basis) led to forming a flexible and integrated film; lower and higher concentrations culminated into 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 1400 rpm and 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 ratios of 70:30, 50:50, and 30:70 (%w/w); consecutively, glycerol was added as a plasticizer in 20% ($w/w_{dry\ matter}$) and the solution was left over on the hitter-stirrer to be homogenized thoroughly for 60 min. BW was added in 0%, 10%, 20% and 30% ($w/w_{glycerol}$) rates into the previous solution 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, Memmert, Germany) for 1 h. Films were dried slowly in an oven for 48 h at 25 °C (Table 1). Consequently, it was stored in LDPE bags at refrigerator (8 °C). All films were conditioned in a desiccator at 50% RH and 25 °C for 48 h prior to tests [18].

2.4. Contact angle

Contact angles with water were measured using a goniometer (DSA 100, Kruss, Germany) equipped with an image processing software. For this purpose, a small drop of distilled water was deposited on the film surface. The contact angle was defined as the angle between the film surface and a tangent line at the point of contact between water droplet and film surface. For each film type, at least three measurements were performed and average rates were reported [19].

2.5. Differential Scanning Calorimetry (DSC)

Thermal properties of the films were studied by Differential Scanning Calorimetry (DSC 200 F3, Netzsch, Germany). 0.03 g of each sample was placed in aluminum DSC pans; the device was calibrated with Indium and Silver as standard. Each sample heated by the instrument at a heating rate of 10 °C/min to 150 °C. Characteristics of melting peaks and glass transition temperatures (T_g) were determined. The refrigerated cooling system worked with nitrogen. Glass transition temperatures were defined as the midpoint between onset and endpoint temperatures of heating curves and identified as second-order transitions [20].

2.6. Atomic Force Microscopy (AFM)

The main merit of this equipment is to measure surface properties of prepared films with high resolution rates in diverse