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# Effects of thermally induced denaturation on technological-functional properties of whey protein isolate-based films

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## ABSTRACT

This study examined how and to what extent the degree of denaturation affected the technological-functional properties of whey protein isolate (WPI)-based coatings. It was observed that denaturation affected the material properties of WPI-coated films significantly. Surface energy decreased by approximately 20% compared with native coatings. Because the surface energy of a coating should be lower than that of the substrate, this might result in enhanced wettability characteristics between WPI-based solution and substrate surface. Water vapor barrier properties increased by about 35% and oxygen barrier properties increased by approximately 33%. However, significant differences were mainly observed between coatings made of fully native WPI and ones with a degree of denaturation of 25%. Higher degrees of denaturation did not lead to further improvement of material properties. This observation offers cost-saving potential: a major share of denatured whey proteins may be replaced by fully native ones that are not exposed to energy-intensive heat treatment. Furthermore, native WPI solutions can be produced with higher dry matter content without gelatinizing. Hence, less moisture has to be removed through drying, resulting in reduced energy consumption.
**Key words:** whey protein isolate, degree of denaturation, water vapor transmission rate, oxygen permeability, surface energy

## INTRODUCTION

Protection against negative environmental impact is the main function of food packaging (Robertson, 2013). Barrier requirements of food products depend on the composition and ingredients of the product and on their requested shelf life. Especially high amounts of unsaturated FA or oxygen-sensitive vitamins need protection against oxygen to enable a maximum shelf life of the products. For this reason, packages are often provided by petroleum-based oxygen-barrier polymers such as ethylene vinyl alcohol (Schmid et al., 2012). The facts that these plastic materials accumulate in the environment and derive from a finite resource have attracted interest in biopolymers (Ghanbarzadeh and Almasi, 2013). It is strongly desired that bio-based packaging materials used for replacement provide the same technological-functional properties. An alternative is to coat polymer films with whey protein, which is a byproduct of cheese production. Whey protein-based coatings act as excellent oxygen-barrier materials that also provide suitable mechanical and water vapor barrier properties (de Wit, 2001; Schmid et al., 2012; Bugnicourt et al., 2013).

During first investigations regarding whey protein-based films and coatings, researchers found that protein denaturation is necessary for film formation (McHugh et al., 1994). Whereas native whey proteins are globular with free thiol and hydrophobic groups buried inside the molecule, heat denaturation induces protein unfolding and the exposure of internal functional groups. Intermolecular crosslinking is promoted (de Wit, 2009). These differences in the structure of native and heat-denatured whey proteins cause different physical properties of the resulting films. Fully denatured whey protein solutions have the ability to form dense and strong films, whereas films made of native whey proteins show higher permeability for gases such as oxygen and water vapor (Schmid et al., 2013c).

Guckian et al. (2006) produced whey protein isolate (**WPI**)-based films with adjusted degrees of denaturation by mixing heated and unheated WPI solutions in different ratios. They found that decreasing the share of unheated WPI led to increasing film solubility and weakened tensile properties, with little effect on water vapor permeability. However, to our knowledge, no data have been reported so far on the influence of different shares of native and denatured WPI on oxygen permeability and surface energy. The objective of this study was to examine how and to what extent the de-

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gree of denaturation affects the technological-functional properties of WPI-based coated films, such as oxygen permeability, water vapor transmission rate, and surface energy.

Two other research papers about the relationship between the degree of whey protein denaturation and the temperature-time profile applied during convective drying exist so far. In those studies, single droplets of native WPI solution were dried in a compartment dryer at various temperatures (Haque et al., 2013a,b). Another study showed that no correlation exists between processing temperature and techno-functional properties of films made from ethylene vinyl acetate/WPI compounds and explained this phenomenon with the fact that no irreversible denaturation occurred during thermoplastic processing of the material (Schmid et al., 2013a). However, no experimental work has been reported on convective drying of WPI-based coatings. Hence, the present study also investigated the dependence of the degree of denaturation of WPI-based coatings on the applied temperature-time profiles during convective drying.

## MATERIALS AND METHODS

### Raw Materials and Chemicals

Whey protein isolate (BiPro) with a minimum protein content (on a dry basis) of 95% (total Kjeldahl nitrogen: N × 6.38) was supplied by Davisco Foods International Inc. (Le Sueur, MN) and used to produce WPI-based coatings. Glycerol was purchased from Merck Schuchardt OHG (Hohenbrunn, Germany). It was added as a plasticizer to all film-forming solutions to overcome film brittleness. Polyethylene terephthalate (**PET**) of 12-μm thickness (Hostaphan RNK 12.0; Mitsubishi Polyester Film GmbH, Wiesbaden, Germany) and polytetrafluoroethylene (**PTFE**) of 500-μm thickness (Sahlberg GmbH & Co. KG, Feldkirchen, Germany) were used as substrates for WPI coating application. Commercially purified bovine whey proteins such as α-LA, β-LG A and B, and BSA were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and applied as standards in the reversed-phase HPLC (**RP-HPLC**) tests; HPLC-grade water, acetonitrile, and trifluoroacetic acid (**TFA**) were supplied by Avantor Performance Materials B.V. (Deventer, the Netherlands), Th. Geyer GmbH & Co. KG (Renningen, Germany), and Sigma-Aldrich Chemie GmbH, respectively. Adjustment of pH was done with 0.1 *M* hydrochloric acid (Th. Geyer GmbH & Co. KG). Double-distilled water (Merck KGaA, Darmstadt, Germany), diiodomethane, and ethylene glycol (both from Sigma-Aldrich Chemie GmbH) were applied as testing liquids during determination of surface energy. All proteins and chemicals were used as received without further purification.

### Formulation Preparation

Native WPI formulations were prepared by stirring aqueous solutions of 10% (wt/wt) WPI for 30 min at 23°C and 200 rpm using a Thermomix 31-1 electric stirrer (Vorwerk Elektrowerke GmbH & Co. KG, Wuppertal, Germany). Denatured formulations were obtained by heat treatment of the WPI solutions at 90°C for 30 min using the same stirring device. After cooling down the heat-treated solutions to 23°C, glycerol was added to all formulations (66.7% on a DM basis). Formulations were stirred for another 30 min at 200 rpm with an electric overhead stirrer (WiseStir HS-100D; Witeg Labortechnik GmbH, Wertheim, Germany). In each stage, degassing was performed via ultrasonication using a Sonorex Digitec DT 514H ultrasonic bath (Bandelin electronic GmbH & Co. KG, Berlin, Germany) at a frequency of 37 kHz for 15 min. To adjust the degree of denaturation ($\mathbf{D_{Den}}$), heat-denatured (**D**) and native (**N**) WPI formulations were mixed gently for 5 min at 23°C using a magnetic stirrer (MR 3001; Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) to give the following D:N (wt/wt) ratios: 0:100, 25:75, 50:50, 75:25, and 100:0.

### Coating and Curing

Whey protein isolate formulations (pH 7.0) were applied on PET and PTFE substrates. Corona pretreatment was used to increase their surface energy and to achieve sufficient wettability and an optimum adhesion between WPI solution and substrate surface. Corona pretreatment was done with a web speed of 3 m/min and a generator power of 1,000 W using a CLNE corona treatment station (Softal Corona & Plasma GmbH, Hamburg, Germany). A surface tension of 40 mN/m was gained for PTFE and >44 mN/m for PET. In contrast to PET, the surface energy of PTFE sheets decreased more rapidly over time. Thus, it was possible to remove coated films from PTFE, whereas this was not possible with PET substrates.

Coatings were produced with the coating unit CUF 5 (Sumet Messtechnik Heinz Suttner, Denklingen, Germany), which has a built-in convection dryer. A wet film thickness of 64 μm was applied (20-N contact pressure; 20 mm/s actuation speed). Table 1 summarizes the drying conditions for WPI-based coatings. Low-temperature drying at 35°C and 1,200 s was chosen for the different D:N ratios in order not to increase the $D_{Den}$ to a greater extent (no. 1–5). These tests were