Progress in Organic Coatings 104 (2017) 161–172

Contents lists available at ScienceDirect

Progress in Organic Coatings

journal homepage: www.elsevier.com/locate/porgcoat

Effect of thermally induced denaturation on molecular interaction-response relationships of whey protein isolate based films and coatings

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ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form 9 October 2016
Accepted 26 November 2016
Available online 3 January 2017

Keywords:
Whey protein isolate
Degree of denaturation
Solubility and swelling studies
Intramolecular and intermolecular interactions
Cross-linking density
Molecular interaction-property relationships

ABSTRACT

The functional properties of whey protein isolate (WPI) based films and coatings have been the subject of several studies. However, quantitative information on the molecular interactions is rare. The solubility studies presented here allow qualitative statements to be made about the molecular interactions in WPI-based films. The other objective of this study was to determine the cross-linking density (CLD). Swelling studies were performed on WPI-based films containing different amounts of denatured WPI. The results of the swelling studies showed that the degree of denaturation has a significant influence on the CLD, which is directly proportional to the number of disulfide bonds in the WPI-based network. As a result, the structural stability of the polymer network was improved. The swelling studies showed that there was a significant linear increase in CLD of $1.17 \cdot 10^{-4}$ mol cm^{-3} on going from 50% to 100% denatured WPI-based films ($0.22 \cdot 10^{-4}$ mol cm^{-3} → $1.39 \cdot 10^{-4}$ mol cm^{-3}). The solubility study showed that WPI film solubility is mainly influence by non-covalent bonds up to a degree of denaturation of 75%. In general, there was a correlation between CLD and the mechanical properties of the films. The Youngís modulus increased by 320% on going from 25% to 100% denatured WPI-based films (26.70 MPa → 85.30 MPa). The CLD had only a minor effect on the barrier properties of the WPI-based films. Thus, this scientific paper provides new knowledge for researchers and material developers because qualitative information about the covalent intermolecular interactions in whey protein isolate based films and coatings has been obtained for the first time.

1. Introduction

Renewable raw materials and biodegradable packaging material are of increasing interest for the packaging industry due to their good film properties and growing public interest. The objective is to replace petroleum-based oxygen barrier polymers such as ethylene vinyl alcohol with biopolymers having equivalent functional properties (e.g. optical, barrier and mechanical properties). Research has been conducted on proteins such as wheat gluten, soy protein, casein, and whey protein for their use as packaging materials [1,2]. The properties of films and coatings from the before mentioned proteins and from proteins in general differ very strongly depended on their formulation, modification and processing. Therefore it is difficult to directly compare the functional properties such as barrier and mechanical properties of different protein-based films and coatings leading to a general conclusion about their performance. A comprehensive summery of the different functional properties of different proteins was recently published by Zink et al. [3]. This work focuses on whey protein isolate (WPI) as it shows good potential and has already been used for a wide range of applications due to its excellent oxygen barrier properties and suitable mechanical and water vapor barrier properties [4–6]. A further advantage is its availability in high quantities, with WP being a by-product of the cheese-making industry. Films formed from heated and native whey protein isolate (WPI) solutions have already been investigated by several authors [7,5,8]. To produce dense and strong films with low permeability for gases such as oxygen and water vapor, protein denaturation is required. Heat treatment of WPI induces unfolding of the globular protein and exposure of internal sulfhydryl and hydrophobic groups. The cohesive polymer structure is maintained by new intermolecular interactions, in particular

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http://dx.doi.org/10.1016/j.porgcoat.2016.11.032
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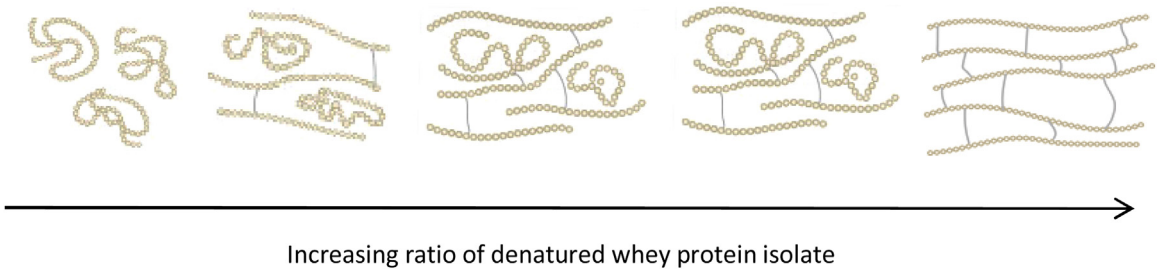

Fig. 1. Schematic illustration of protein unfolding and linearization by heat treatment and formation of disulfide bonds between the exposed cysteine units with increasing degree of denaturation.

covalent bonds between the exposed sulfhydryl groups [9,5]. Fig. 1 shows the assumed protein cross-linking process via disulfide bonds initiated by heat treatment. The mechanism was elucidated from swelling studies.

Guckian et al. [7] have already investigated the effect of thermally induced denaturation on functional properties such as the water vapor permeability and tensile properties of WPI based films. Analogous to Guckian et al. [7], Schmid et al. [8] investigated the functional properties of WPI based coatings. A significant change in material properties was observed. Thus, the results showed that both oxygen and water vapor permeability decrease with increasing degree of denaturation. In particular, significant differences were observed between coatings made of fully native WPI and ones with a degree of denaturation of 25% [8]. Thus it is already known how and to which extent thermally induced denaturation affects the functional properties (oxygen permeability, water vapor transmission rate, and surface energy), but it is still unclear what changes occur at a molecular level. Therefore, the first aim of this study is to determine the qualitative molecular interaction-response relationship due to thermally induced denaturation of WPI based coatings by means of solubility studies. Guckian et al. [7] used cast films for his solubility studies on order to qualitatively evaluate the molecular interactions in WPI-based films with different degree of denaturation and Hammann and Schmid [1] used WPI-based coatings. Thus, correlations between the tested qualitative and quantitative molecular interactions and the functional properties of WPI based films are examined and which interactions are most important for the film stability. According to Hammann and Schmid [1], a protein solubility study is a promising method for investigating molecular interactions in protein films. The use of different extracting systems allows conclusions to be drawn about the importance of covalent and non-covalent interactions for film stabilization. As part of the present study, a protein solubility study has been carried out in order to characterize the impact of the degree of denaturation on molecular interactions of WPI based coatings. The second main aim was quantitative determination of the cross-linking density (CLD) in WPI-based films with different degrees of denaturation. The quantitative identification of the CLD was determined by swelling studies in accordance with DIN EN ISO 175:2000. By means of the cross-linking density, it is possible to determine how many disulfide bonds exist in the WPI network. The CLD is calculated with the Flory-Rehner equation and is an absolute value. The CLD, however, requires further tests to determine the unknown interaction parameter χ between the swelling medium, namely distilled water, and the different WPI-based films, which is fundamental for the calculation. The parameter χ can be identified via Hansen solubility tests or from the water vapor sorption isotherm [10,11]. In addition to this two aims, the functional properties (mechanical and barrier properties) of WPI cast films made with various ratios of native and denatured WPI are determined and correlated to the SI-Unit, the CLD. In addition, a peelable monolayer WPI-based cast film was formed for the first time from 0% denatured solution (BP0:100) using PTFE evaporation dishes and a storage time of 21 days. To the authors' knowledge, no previous studies have investigated how thermally induced denaturation affects the quantitative molecular interaction-response relationship of whey protein isolate based films and coatings. The studies of Guckian et al. [7] solely concerned qualitative molecular interactions.

2. Materials and methods

2.1. Sample preparation

2.1.1. Preparation of the formulation with different degree of denaturation

The denatured formulation was prepared according to Schmid et al. [6]. 10% (w/w) WPI (BiPRO, 95% protein on a dry weight, 3% ash, 1% fat, 0.5% Lactose, Davisco Foods International Inc., Le Sureur, Minnesota, USA) were dissolved in distilled water by means of an electric stirrer (Thermomix 31-1, Vorwerk Deutschland Stiftung & Co. KG, Wuppertal, Germany) for 30 min at 23 °C and 200 rmp. For completed the denaturation formulation the aqueous solution was heated at 90 °C for 30 min under constant stirring at 200 rpm according to Schmid [12]. After cooling down to 23 °C, 6.67% (w/w protein) of glycerol (Merck Schuchardt OHG, Hohenbrunn, Germany) was added to the solution and stirred for further 30 min at 200 rpm. This recipe was previously published by Schmid et al. [6]. This formulation leads to flexible and transparent films. After filling in a laboratory bottle, the solution was degassed for 15 min in an ultrasonic bath (DT 514H, Ultrasonic peak output: 860 W, Bandelin electronic GmbH & Co. KG, Berlin, Germany) at 23 °C and 37 kHz. The native formulation was prepared in the same way, but the denaturation step (heating up to 90 °C) was skipped. Native and heat-denatured WPI formulations were mixed gently for 5 min at 23 °C using a magnetic stirrer (IKA, RH basic, Staufen, Germany), to give following denatured: native ratios of 0:100, 25:75, 50:50, 75:25, and 100:0. (later referred to as BP0, BP25, BP50, BP75, and BP100). In all cased the solutions had a slightly yellowish appearance.

2.1.2. Preparation of the cast films for the solubility studies

For the solubility test the following sample preparations were performed: To enable peeling-off of the WPI layers, polytetrafluoroethylene (clean, white PTFE 500 ± 20 μm, Sahlberg GmbH &Co. KG, Feldkirchen, Germany) was used as coating substrate and using a CLNE corona treatment station (Softal Corona & Plasma GmbH, Hamburg, Germany) to increase surface energy. To achieve a surface tension of >40 mN/m, corona treatment was performed with a web speed of 3 $m \cdot min^{-1}$ and a generator power of 1000 W. The solutions were coated on PTFE via CUF 5 coating unit (Sumet Messtechnik Heinz Suttner, Denklingen, Germany), which has a built in convection dryer. A wired rod applying a wet film thickness of 64 μm was used (20 N contact pressure; 20 $mm \cdot s^{-1}$ actua-