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Whey protein film properties as affected by ultraviolet treatment under alkaline conditions

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ABSTRACT

The effects of UV radiation treatment on film-forming solutions adjusted to two alkaline pH values (9 or 11) on the properties (water vapour permeability, solubility, mechanical properties, colour and microstructure) of whey protein concentrate films, and on some structural characteristics of proteins (free sulphydryl groups, degree of aggregation, denaturation and secondary structure) were evaluated. UV radiation increased protein aggregation at both pH values. Ultraviolet treatment of pH 9 solutions increased free sulphydryl groups and produced films with higher solubility, tensile strength, elastic modulus, and puncture properties and lower elongation at break; UV radiation at pH 11 decreased free sulphydryl groups and films showed higher solubility and elastic modulus and lower puncture deformation and elongation at break than untreated films. The treatment also affected film colour differently depending on the pH. In conclusion, ultraviolet treatment modifies the properties of the films in different ways depending on the pH of the solutions.

1. Introduction

Ultraviolet radiation has been used as a method for protein modification to obtain protein films with adequate permeability and mechanical properties for use as food packaging material. Its effects have been studied in films from several types of protein, usually combined with heat treatment; the results have been variable, depending on the protein and the film preparation conditions (Gennadios, Rhim, Handa, Weller, & Hanna, 1998; Liu, Tellez-Garay, & Castell-Perez, 2004; Micard, Belamri, Morel, & Guilbert, 2000; Otoni et al., 2012; Rhim, Gennadios, Fu, Weller, & Hanna, 1999; Rhim, Gennadios, Handa, Weller, & Hanna, 2000). Ultraviolet radiation is absorbed by double bonds and aromatic rings of some amino acids, producing free radicals and causes intermolecular covalent bonding (Rhim et al., 1999). In a previous manuscript (Díaz, Candia, & Cobos, 2016), the effects of ultraviolet radiation on the properties of whey protein films obtained from treated solutions or treated after film formation were evaluated. Ultraviolet treatment significantly affected most mechanical properties, colour and solubility when applied to the film-forming solution. Ultraviolet radiation modified the properties of the films, the concentration of free sulphydryl groups and aggregates formation in different ways that heat treatment.

That investigation was carried out with film-forming solutions adjusted at pH 7. The modification of pH could influence the characteristics of the whey proteins and films. The pH plays an important role in protein films; alkaline pH far from the pI of proteins promotes their denaturation, unfolding, and solubilisation, causing disulphide interchange reactions, noncovalent interactions and polymerisation. The negatively charged groups repel each other and produce modifications of protein chain structure and in the intermolecular bonds among molecules in films (Bourtoom, Chinnan, Jantawat, & Sanguandeekul, 2006), and gelation (Monahan, German, & Kinsella, 1995).

In protein films, the effects of both pH and heat treatment on their properties have been studied (Anker, Stading, & Hermansson, 2000; Bourtoom et al., 2006; Gennadios, Brandenburg, Weller, & Testin, 1993; Jiang, Xiong, Newman, & Rentfrow, 2012; de la Caba et al., 2012). Studies reported production of protein films from unheated solutions at alkaline pH values with variable results; soy protein isolate solutions did not produce films (Jiang et al., 2012), while faba bean protein isolate films were viable and pH modification improved mechanical properties and solubility of the films (Saremnezhad, Azizi, Barzegar, Abbasi, & Ahmadi, 2011). Quinn,

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Monahan, O'Riordan, O'Sullivan, and Longares (2003) investigated the importance of covalent and noncovalent interactions in films from unheated whey solutions at alkaline pH and found that hydrophobic interactions and hydrogen bonding were important in these films, while disulphide bonding was less important in their formation. They observed differences in mechanical properties and solubility between protein films from unheated alkaline solutions and films from heated, neutral pH solutions.

However, the effects of the increasing the pH of the film-forming solution above neutrality together with UV radiation treatment as denaturation methods on protein film properties have not been investigated. The combination of these treatments could produce films with modified properties of interest as food packaging materials.

The objective of this work was to evaluate the effect of UV radiation treatment on film-forming solutions adjusted to different alkaline pH values on the properties (water vapour permeability, solubility, colour, mechanical properties and microstructure) of whey protein films. Some structural changes induced in the proteins by UV radiation of solutions with different pH values (free sulphydryl groups, degree of aggregation and denaturation) and in the corresponding films (Fourier transform infrared spectra) were also investigated.

2. Material and methods

2.1. Film preparation

Whey protein concentrate (Protarmor 800; Armor Proteines, Saint-Brice en Coglès, France) was used for film preparation; its composition, provided by the manufacturer, was 80% protein, 4% moisture, 3.5% ash, 3.5% fat and 9% lactose. WPC film-forming solutions (8% protein, w/w) were prepared in de-ionised water by slow stirring for 30 min at 20 °C using a magnetic stirrer, then glycerol (Panreac, Barcelona, Spain), in a proportion of 2:1 protein:plasticiser, was added. The pH was adjusted to 7.0, 9.0 or 11.0 with 2 M NaOH. The solutions were stirred for additional 30 min and then poured into Plexiglas Petri dishes with an internal bottom diameter of 8.5 cm, 1.2 g total solids per dish.

Two types of films were prepared from solutions at each pH value: untreated films (from untreated solutions) and ultraviolet (UV) treated films (from solutions submitted to 12.0 J cm^{-2}). UV treatments were applied to dispersions in Petri dishes in a stainless steel exposure chamber with a microprocessor controlled UV radiation system equipped with 6 × 8-watt tubes operating at an ultraviolet wavelength of 254 nm (Bio-Link crosslinker BLX-E, Vilber Lourmat, Marne-la-Vallee, France). Film-forming solutions [8.57 g (8.4 mL) of protein solution per dish, sample thickness of 1.48 mm] were exposed to a programmed 12.0 J cm^{-2} total dose.

Both UV treated and untreated solutions were dried at 50 °C in an air forced cabinet and stored at 20 °C and 50% relative humidity for 72 h. All experiments were performed in triplicate.

2.2. Determination of free sulphydryl groups in WPC solutions

Ellman's 5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB method was carried out according to Pereira, Souza, Cerqueira, Teixeira, and Vicente (2010) with some modifications (Díaz et al., 2016), to determine the free sulphydryl groups in WPC solutions.

2.3. Size-exclusion high performance liquid chromatography analysis and residual native protein determination

The aggregation, denaturation degree and the formation of soluble oligomers and polymers of whey proteins of the various solutions were determined by size-exclusion high-performance liquid chromatography (SE-HPLC) as described by Díaz et al. (2016). Residual soluble β-lactoglobulin and α-lactalbumin values were calculated in relation to the residual soluble protein in the untreated sample at pH 7; this solution contained these proteins with the initial degree of denaturation only due to the manufacturing process of the commercial WPC. Residual soluble proteins and the ratio of residual β-lactoglobulin/α-lactalbumin were determined to evaluate the degree of denaturation and participation of WPC proteins in the treated samples.

2.4. Water vapour barrier properties, thickness, dry matter content, solubility and colour of films

Water vapour barrier properties of films were determined according to the method described by Díaz et al. (2016) based on the ASTM E96-93 method (ASTM, 1993). Film thickness, dry matter content, solubility, colour parameters (CIE L*a*b* colour space) and opacity of films were measured according to Díaz et al. (2016).

2.5. Mechanical properties

Mechanical properties (puncture and tensile tests) were measured according to the method described by Muñoz, Aguilera, Rodriguez-Turienzo, Cobos, and Diaz (2012), based on the ASTM D882 method (ASTM, 2000).

2.6. Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra of films were recorded with a ABB Bomen spectrometer (mod. MB102, Québec, Canada) using attenuated total reflectance (ATR) mode between 400 and 4000 cm^{-1}. The analyses were performed as described by Díaz et al. (2016).

2.7. Scanning electron microscopy

The microstructure of films was observed by scanning electron microscope according to the method described by Díaz et al. (2016).

2.8. Statistical analysis

Data were evaluated statistically using the SPSS version 19.0.0 for Windows (2010; SPSS Inc., Chicago, IL, USA) program. Prior to analysis, data were checked for outliers and normal distribution was tested using the Kolmogorov–Smirnov test. A two-way analysis of variance (ANOVA) was used to analyse the effects of the pH and treatment, and their interaction on the parameters determined. These analyses were carried out using the GLM procedure. The means of each pH within each group (untreated or UV treated) were compared using the least significant difference test with significance at $P < 0.05$. Comparison of means of each treatment (untreated or UV treated) within each pH was done using a *t*-test for independent samples. A significance level of $P < 0.05$ was used for all mean evaluations.

3. Results and discussion

3.1. Free sulphydryl groups and protein aggregation

Free sulphydryl groups content, protein aggregation results and evaluation of residual soluble proteins are shown in Table 1. SE-HPLC chromatographic profiles of solutions are displayed in Fig. 1. The results were significantly affected by both UV treatment and pH and interactions between these denaturing agents were detected.