Food Hydrocolloids 55 (2016) 189–199

Contents lists available at ScienceDirect

Food Hydrocolloids

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

# Effects of ultraviolet radiation on properties of films from whey protein concentrate treated before or after film formation

Olga Díaz*, Davinia Candia, Ángel Cobos

*Área de Tecnología de Alimentos, Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias, Universidad de Santiago de Compostela, 27002 Lugo, Spain*

ARTICLE INFO

*Article history:*
Received 21 April 2015
Received in revised form
5 November 2015
Accepted 14 November 2015
Available online 18 November 2015

*Keywords:*
Ultraviolet radiation
Edible films
Whey protein concentrate
Mechanical properties
Microstructure

ABSTRACT

Properties (water vapour permeability, solubility in water, colour, mechanical properties and microstructure) of whey protein concentrate films treated with ultraviolet radiation were evaluated.

Ultraviolet treatments at different doses (0.12, 4.0 and 12.0 J $cm^{-2}$) were applied to film-forming solution or to preformed films. These films were compared to untreated films and heat treated films (from solutions subjected to heat treatment). Besides, some structural changes induced to proteins (free sulfhydryl groups, degree of aggregation and denaturation and secondary structure) by radiation have also been investigated.

Ultraviolet treatment only affected significantly most mechanical properties and solubility when was applied to film-forming solution and at the highest dose. These films showed significantly higher tensile strength, puncture strength and puncture deformation and lower solubility than untreated films. Films treated with the highest dose of ultraviolet radiation in solution showed tensile strength similar to heat-treated films. Ultraviolet radiation caused that the films were more yellow, greener and darker than untreated films, although the effect of the treatment on colour was higher when was applied to the film-forming solution. Ultraviolet radiation of solutions at high doses increased the concentration of free sulfhydryl groups and induced aggregates formation comparing to untreated films but the changes were lower than those observed in heat-treated films; the denaturation was higher in α-lactalbumin than in β-lactoglobulin. No changes in the secondary structure of proteins were detected. Differences in microstructure were found among films. According to the results, ultraviolet radiation modifies the properties of the films and in different way that heat treatment.



## 1. Introduction

Whey protein films are an interesting environmental-friendly packaging material. A thermal treatment is usually included in the solution preparation in order to denature proteins and produce films of adequate strength and permeability (Perez-Gago & Krochta, 2001; Pérez-Gago, Nadaud, & Krochta, 1999; Schmid, Krimmel, Grupa, & Noller, 2014). Heat-denatured whey proteins are also able to produce edible films with low oxygen permeability, although they show higher water vapour permeability and lower tensile strength and elongation at break than plastic films (LDPE and HDPE) (Debeaufort, 2014). Instead of heat treatment, other methods that change whey protein structure have been proposed for edible packaging preparation: chemical treatments (glutaraldehyde, formaldehyde, dialdehyde starch, carbonyldiimidazole; Ustunol & Mert, 2004), biochemical treatments such as transglutaminase addition (Schmid, Sängerlaub, Wege, & Stäbler, 2014; Yildirim & Hettiarachchy, 1998) and physical treatments such as γ-irradiation (Ciesla, Salmieri, & Lacroix, 2006), ultrasounds (Rodriguez-Turienzo, Cobos, & Díaz, 2012) and ultraviolet radiation (Schmid, Held, Hammann, Schlemmer, & Noller, 2015; Ustunol & Mert. 2004). Among physical treatments, ultraviolet (UV) radiation has been studied due to this electromagnetic radiation is absorbed by double bonds and aromatic rings, causing free radical formation in amino acids and can lead to the formation of intermolecular covalent bonds (Gennadios, Rhim, Handa, Weller, & Hanna, 1998; Rhim, Gennadios, Fu, Weller, & Hanna, 1999).

The effects of UV radiation have been studied in soy protein, corn zein, wheat gluten, peanut protein, fish gelatin, egg albumin and sodium caseinate films (Gennadios et al., 1998; Liu, Tellez-

* Corresponding author.
*E-mail address:* olga.diaz.rubio@usc.es (O. Díaz).

http://dx.doi.org/10.1016/j.foodhyd.2015.11.019
0268-005X/

Garay, & Castell-Perez, 2004; Micard, Belamri, Morel, & Guilbert, 2000; Otoni et al., 2012; Rhim et al., 1999; Rhim, Gennadios, Handa, Weller, & Hanna, 2000). In most cases, they found that UV radiation modifies the mechanical properties and colour of films, but the results were variable, probably due to the differences in the nature of the protein used and in the treatment application conditions. In these works, UV radiation was applied to pre-formed films prepared from heat-treated protein solutions, except Otoni et al. (2012) that submitted gelatin directly to radiation. The information about the use of UV radiation in whey protein films is very scarce; Ustunol and Mert (2004) applied this physical treatment to whey proteins in solution, but they were previously heat-treated and the pH was adjusted at 10, and both factors produce protein denaturation. Recently, Schmid et al. (2015) treated whey protein films, obtained from heat-treated solutions, with UV radiation and studied the influence of this treatment on film properties. However, the influence of UV radiation in film properties from whey protein solutions without heat treatment has not been previously reported in the literature.

The absorbed energy and transmittance of UV light are strongly influenced by the nature of the material and its turbidity (Koutchma, 2009; Koutchma, Forney, & Moraru, 2009), so the physical state and surface, thickness and composition of the material could affect the action of UV radiation on proteins; i.e. the changes could be different if the UV treatment is applied in the forming solution or in the pre-formed film. Besides, there is very little information (Kristo, Hazizaj, & Corredig, 2012) about the nature of the structural changes induced by UV radiation in mixed whey proteins.

The objective of this work was to evaluate the effects of the UV radiation at different doses applied to film-forming solutions or to preformed films on the properties (water vapour permeability, solubility, colour, mechanical properties and microstructure) of whey protein concentrate films. Some structural changes (free sulfhydryl groups, degree of aggregation and denaturation and secondary structure) induced to proteins by radiation of solutions and films have also been investigated.

## 2. Material and methods

### 2.1. Film preparation

Whey protein concentrate, containing 80% protein (Protarmor 800), was purchased from Armor Proteines (Saint-Brice en Coglès, France). According to the manufacturer, the composition of the product was 80% protein, 4% moisture, 3.5% ash, 3.5% fat and 9% lactose. WPC film forming solutions (8% protein, w/w) were prepared by slow stirring in de-ionized water for 30 min at 20 °C using a magnetic stirrer (VELP Scientifica, mod. ARE, Usmate, Italy). Glycerol (Panreac, Barcelona, Spain), in proportion of 2:1 protein:plasticizer, was added. The pH was adjusted to 7.0 with 2 M NaOH. The solutions were stirred for additional 30 min.

Two types of control films were prepared: untreated films (from untreated solutions) and heat treated films (from solutions subjected to heat treatment at 80 °C for 20 min in a circulating water bath). Untreated and heat treated solutions were poured into Plexiglas Petri dishes of 9 cm diameter (1.2 g total solids per dish) and were dried at 50 °C in an air forced cabinet for 6 h; after, the films were kept at 20 °C and 50% relative humidity for 24 h.

Two types of UV treated films were prepared: UV treatment in solution and UV treatment in film. In both types, the solutions were poured into Plexiglas Petri dishes. For UV treatment in films, the untreated protein solutions were dried in the same conditions that were described above. UV treatments were applied by placing the dried films (treatment in film; FUV samples) or the untreated dispersions into Petri dishes (treatment in film-forming solution; SUV samples) in the stainless steel exposure chamber of a microprocessor controlled UV radiation system (Bio-Link crosslinker BLX-E, Vilber Lourmat, Marne-la-Vallee, France), equipped with 6 x 8-W tubes operating at an ultraviolet wavelength of 254 nm. Both film-forming solutions and dried films were exposed to 0.12 J $cm^{-2}$ (SUV 0.12 and FUV 0.12, respectively), 4.0 J $cm^{-2}$ (SUV 4.0 and FUV 4.0, respectively), or 12.0 J $cm^{-2}$ (SUV 12.0 and FUV 12.0, respectively) total doses. The microprocessor constantly monitored the UV light radiation and stopped it automatically when the energy received by the samples (contained in Petri dishes) matches the programmed energy. The samples treated with UV in solution were dried in the same conditions that were described above. All films were stored and at 20 °C and 50% relative humidity for 48 h until tested. All experiments were performed in triplicate.

### 2.2. Determination of free sulfhydryl groups

Ellman's 5,5′-dithiobis-(2-nitrobenzoic acid) or DTNB method (Pereira, Souza, Cerqueira, Teixeira, & Vicente, 2010) with some modifications was used to determine the free sulfhydryl groups in WPC film-forming solutions. Samples of 250 μl of WPC untreated and UV treated solutions or of 50 μL of heat treated solution were diluted with 2.5 mL of phosphate buffer (5 mM, pH 8.0). Then, 50 μL of Ellman's reagent solution [4 mg DTNB (Sigma Aldrich, St. Louis, USA) in 1 mL of phosphate buffer] were added, mixed and incubated at room temperature for 15 min. After filtration through 0.45 μm pore size filter, absorbance was measured at 412 nm by a UV–VIS spectrophotometer (mod. PG70+, LanOptics, Labolan, Esparza de Galar, Spain). Concentration of free sulfhydryl groups was calculated using a DTNB molar extinction coefficient of 14,150 $M^{-1}$ $cm^{-1}$ (Riddles, Blakeley, & Zerner, 1983).

### 2.3. Size-exclusion HPLC analysis and residual soluble main protein determination

The degree of aggregation and denaturation of whey proteins in film forming solutions was observed by size-exclusion high-performance liquid chromatography (SE-HPLC) as described by Kazmierski and Corredig (2003). The chromatographic separations of proteins and aggregates were performed by a BioSep-SEC S 3000 HPLC column (30 cm length × 7.8 mm internal diameter; Phenomenex, Aschaffenburg, Germany) at room temperature using a Shimadzu liquid chromatograph (Shimadzu Corp., Kyoto, Japan), composed by pump model LC-10AT, a low pressure gradient flow control valve model FCV-10ALVP and a spectrophotometric detector model SPD-10AV. The mobile phase, delivered at a flow-rate of 0.5 mL/min and eluted in isocratic conditions, consisted of 50 mM $NaPO_4$ buffer at pH 7.0, containing 0.15 M NaCl. WPC film forming solution samples were diluted in elution buffer at a concentration of 8 mg/mL and were filtered through filters of 0.45 μm pore diameter before loading into the column. Injection volume was 20 μL. The detection wavelength was 280 nm. Solutions of tyroglobulin (669 kDa), bovine serum albumin (67 kDa), ovalbumin (44 kDa), β-lactoglobulin (18 kDa) at a concentration of 4 mg/mL, and α-lactalbumin (14 kDa) at a concentration of 2 mg/mL (Sigma Aldrich, St. Louis, USA) were used as external calibration standards. A linear relation ($R^2 = 0.99$) was found between retention time and molecular weight of standard proteins, expressed in logarithmic values. Peak integration was carried out using LC Solution software (Shimadzu Corp., Kyoto, Japan) and PeakFit software version 4.12 (SYSTAT Software, Richmond, CA, USA). Determination of residual soluble main proteins (β-lactoglobulin and α-lactalbumin) was calculated as % area relative to the area each protein in the untreated sample using the equation: