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Effect of ultraviolet processing on selected properties of egg white

Lara Manzocco, Agnese Panozzo *, Maria Cristina Nicoli

Dipartimento di Scienze degli Alimenti, Università di Udine, via Sondrio 2/A, 33100 Udine, Italy

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ABSTRACT

The effect of ultraviolet processing (10.6 and 63.7 kJ m^{-2}) on selected properties of egg white (absorbance, particle size, protein fractions, free sulfhydryl content, immunoreactivity, viscosity, gelling and foaming properties) was investigated. Ultraviolet exposure induced the development of browning, the formation of large protein aggregates by disulfide exchange, and protein backbone cleavage. However, egg white proteins were differently sensitive to UV radiation. No changes in immunoreactivity, gelling temperature and gel firmness were observed. Independently on the UV dose, light treated egg white produced foams with higher stability. This effect was attributed to protein aggregates jamming in the fluid interstices between bubbles and/or to the higher viscosity of the aqueous phase. The latter was also associated to higher foam volume.

1. Introduction

Ultraviolet (UV-C) radiation is nowadays recognised as an efficient technology to non-thermally inactivate a wide range of microorganisms (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000; Falguera, Pagán, Garza, Garvin, & Ibarz, 2011; Guerrero-Beltràn & Barbosa-Cànovas, 2004). In addition to microbial inactivation, exposure of food to ultraviolet radiation may provide further advantages. The latter are associated to the development of photoreactions involving biopolymers. Polymer photoreaction is far from being a novel technology: ancient Egyptians and Babylonians exploited photoreactions to mummify corpses and waterproof papyrus boats (Decker & Bendaikha, 1998; Wondraczek, Kotiaho, Fardim, & Heinze, 2011).

The common feature of photoresponsive polymers is the presence of a chromophore inside the macromolecular matrix (Wondraczek et al., 2011). At first, the electromagnetic energy is captured by a chromophore, and converted into chemical energy allowing photoisomerisation. The chemical energy is thus transferred to the functional part of the polymer that controls its overall properties. As a consequence, conformation, size and architecture of the polymer can deeply change, leading to modifications in its properties. Proteins are major targets for photoreactions due to the abundance of endogenous chromophores within their structure. Both amino acid side-chains (e.g., thriptophan, tyrosine, phenylalanine, and cysteine) and bound prosthetic groups (e.g., flavins and heme) may act as efficient chromophores. Proteins have the additional ability to bind exogenous chromophores, and rapidly react with other excited state species. The result is the development of side-chain oxidation, backbone fragmentation, and/or formation of cross-links and aggregates (Davies, 2003).

Based on these considerations, photoinduced modification of protein structure could be exploited to produce systems with well-defined functional properties and biologic activity. Unfortunately, the potentials of ultraviolet processing to steer food protein properties have been scarcely explored. Ultraviolet treatments were reported to increase the tensile strength of gluten, zein, and albumin films (Rhim, Gennadios, Fu, Weller, & Hanna, 1999). Irradiated fish gelatin exhibited higher gel strength, marked reduction in viscosity, and significant changes in melting temperatures (Bhat & Karim, 2009). Photocrosslinking of egg white protein and sodium caseinate was associated to improved emulsifying and foaming properties (Kuan, Bhat, & Karim, 2011). As regards protein biologic activity, ultraviolet exposure was demonstrated to reduce the catalytic activity of different enzymes and the immunoreactivity of allergenic proteins (Guerrero-Beltràn & Barbosa-Cànovas, 2006; Manzocco, Dri, & Quarta, 2009; Manzocco, Quarta, & Dri, 2009; Manzocco & Nicoli, 2012).

The aim of the present paper was to study the effects of ultraviolet processing on some structure-related properties of proteins. To this end, egg white was taken as a representative example of a protein rich ingredient that is commonly exploited in the food industry for its multifunctional properties. In particular, photoinduced structural changes in egg white proteins were studied by dynamic light scattering analysis, SE-HPLC and determination of free sulfhydryl content. The effect of protein structural changes on immunoreactivity as well as on rheological, foaming and gelling properties of ultraviolet treated egg white were then evaluated.

* Corresponding author.
E-mail address: agnese.panozzo@uniud.it (A. Panozzo).

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2. Materials and methods

2.1. Materials

Eggs were obtained from a local supermarket. The egg white was manually separated from the egg yolk and the chalazae were removed. The albumen was manually gently mixed. The egg white pH was 8.2.

2.2. UV processing

Aliquots of 1.5 g of egg white were introduced into 3.5 × 6.0 cm plastic pouches (polycoupled Combiflex PA/PE 090, 20/70, Savonitti, Codroipo, Italy) and hermetically sealed (Orved, VM-16, Musile di Piave, Italy). Samples were immediately exposed for increasing time (5, 30 min) to UV-C light (15 W, OF, OSRAM, GmbH HNS, Munich, Germany, maximum emission 253.7 nm) in a thermostated cell (Climacell 222, MMM Medcenter, Einrichtungen GmbH, Graefelfing, Germany) at 8 °C. The distance between the samples and the lamp was 2.5 cm and the irradiance incident on the samples was 35.4 W m^{-2}. The dose received by the samples exposed to the light for 5 and 30 min was 10.6 and 63.7 kJ m^{-2} respectively. The lamp was allowed to stabilise by turning it on at least 15 min before use. The same samples kept under dark were the controls. No temperature changes were observed as a consequence of lighting in all samples.

2.3. Irradiance

Irradiance was measured using a portable luminometer (HD-2102.2 Delta Ohm, Padova, Italy) equipped with a UV-C light probe (LP471 UVC, Padova, Italy). The luminometer sensor was placed in the thermostated cell at 2.5 cm distance from the UV-C lamp and irradiance value recorded.

2.4. Immunoreactive egg white

The quantitative determination of immunoreactive egg white was performed at 25 °C by the enzyme immunoassay RIDASCREEN® Fast Ei/Egg Protein (R-Biopharm AG, Darmstadt, Germany) according to the manufacturer's recommendations. Analyses were performed on light treated egg white diluted to 0.1 g l^{-1} with the extraction buffer provided with the immunoassay kit. Absorbance at 450 nm was measured by using a microplate reader (Sunrise, Tecan Group, Männedorf, Switzerland). The concentration of immunoreactive egg white was determined according to the calibration curve obtained using the standard solutions provided with the immunoassay kit.

2.5. HPLC gel-permeation analysis

Egg white was diluted 1:50 (w/v) with 0.05 M tris–HCl buffer pH 9.0 containing 0.4 M NaCl (Sigma–Aldrich, Milan, Italy). The solution was filtered on 0.20 μm porosity filters (Econofilters, Agilent Technologies, Cernusco sul Naviglio, Italy). Samples were analysed using a HPLC system Jasco (model 880–02, Japan Spectroscopic Co., Tokyo, Japan) equipped with a UV/VIS detector. Two columns were used: BioSep-SEC-S 3000, 30 cm length, 7.80 mm internal diameter and BioSep-SEC-S 2000 30 cm length, 7.80 mm internal diameter, 5 μm granulometry, 125 Å porosity, with separation range among 5 and 150 kDa. Injection volume was 20 μl and the mobile phase, delivered at a flow rate of 0.6 ml min^{-1}, was 0.05 M tris–HCl buffer pH 9.0 containing 0.4 M NaCl in isocratic conditions. The detection wavelength was 220 nm. Bovine serum albumin (67 kDa), α-lactalbumin (14 kDa), β-lactoglobulin (18 kDa), albumin (44.29 kDa) (Sigma, St. Louis, MO, USA), and insulin (5.7 kDa) (Roche Diagnostic GmbH, Mannheim, Germany) were used as calibration standards. A linear relation (R^2 = 0.92) was found between retention time and molecular weight of standard proteins, expressed in logarithmic values. Peaks integration was performed by CHROM-CARD for Windows software (1.19 version).

2.6. Dynamic light scattering

Light scattering measures were made using a Particle Sizer NICOMP™ 380 ZLS (PSS NICOMP Particle Sizing System, Santa Barbara, California, USA). Samples were diluted 1:1000 (v/v) with 0.05 M tris–HCl buffer pH 9.0 containing 0.4 M NaCl. The angle of observation was 90°. The refractive index of the solution was set at 1.333 and the viscosity was approximated to that of pure water at 25 °C. The hydrodynamic radius refers to the corresponding volume distribution calculated by NICOMP Distribution Analysis.

2.7. Absorbance

The absorption spectroscopy measurements at 280, 380 and 680 nm were performed by a UV–VIS spectrophotometer (UV-2501 PC, Shimadzu Kyoto, Japan) at 25 °C with a 1 cm path-length cuvette. The egg white was diluted 1:100 (v/v) with 0.05 M tris–HCl buffer pH 9.0 containing 0.4 M NaCl.

2.8. Determination of free sulfhydryl content

The concentration of free sulfhydryl groups (SH) of the egg white samples was determined using Ellman's reagent (5′,5-dithiobis (2-nitrobenzoic acid), DTNB) (Sigma–Aldrich. Milan, Italy). Changes in free sulfhydryl groups were measured in duplicate as reported by Beveridge, Toma, and Nakai (1974). Briefly, egg white (1.5 g) was diluted to 10 ml with 1% (p/v) NaCl in tris–glycine buffer (10.4 g tris, 6.9 g glycine, 1.2 g EDTA per liter, pH 8.0) (Sigma–Aldrich, Milan, Italy). A volume of 2.9 ml of 0.5% SDS in tris–glycine buffer was added to 0.1 ml of diluted egg white and 0.02 ml of Ellman's reagent (4 mg ml^{-1} DTNB in tris–glycine buffer) to develop color. After 15 min, absorbance was measured at 412 nm by a UV–VIS spectrophotometer (UV-2501 PC, Shimadzu Kyoto, Japan). Concentration of free sulfhydryl groups (μM g^{-1}) was calculated from the following equation:

$$SH = \frac{73.53 \cdot A_{412} \cdot D}{C} \quad (1)$$

where A_{412} is the absorbance at 412 nm; C is egg white concentration (mg ml^{-1}); D is the dilution factor; and 73.53 is derived from $\frac{10^6}{1.36 \cdot 10^{-4}}$; 1.36×10^4 is the molar absorptivity (Ellman, 1959).

2.9. Foam volume and stability

Foams were processed by whipping 5 ml of egg white for 3 min at 20 °C in a 50 ml cylinder by a homogeniser (Polytron, PT 3000, Cinematica, Littau, Swiss) operating at 9500 rpm. The volumes of the foam and of the drained liquid were assessed just after whipping and after holding for 30 min at 20 °C. Foam stability was calculated as the percentage ratio between the foam volume at observation time and that detected just after whipping.

2.10. Microscopy

Just whipped foams and foams held for 30 min at 20 °C were placed onto a microscope slide. Pictures of foam bubbles were acquired as quickly as possible using a digital camera (Leica EC3, Solms, Germany) mounted on a Leica™ microscope (Leica DM 2000, Solms, Germany), magnification 100x. Images were saved