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Effect of pulsed light on structure and immunoreactivity of gluten

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ABSTRACT

The effect of pulsed light (from 1.75 to 26.25 J cm^{-2}) on selected properties of wheat gluten powder and aqueous suspension (absorbance, particle size and microstructure, free sulfhydryl content, protein fractions, protein electrophoretic mobility and immunoreactivity) was investigated. Gluten photoreactivity was strongly affected by hydration. While minor photo-induced structure modifications were observed in gluten powder, pulsed light induced the development of browning and promoted partial depolymerisation of hydrated gluten proteins by disulphide exchange. These changes were associated with a significant decrease in immunoreactivity, suggesting that pulsed light could be exploited to efficiently modify structure and thus functionality of gluten.

1. Introduction

Pulsed light is a non-thermal technology based on the application of a series of very short, high-power pulses of light with a broad spectrum, ranging from the UV to the near infrared electromagnetic radiation (Moraru & Uesugi, 2009). The use of pulsed light as an alternative to thermal processing for inactivating pathogenic and spoilage microorganisms in the production, processing and handling of food has been approved by the U.S. FDA (2000), provided that the treatment fluence does not exceed 1.2 kJ m^{-2}. The efficient microbial inactivation, the very short treatment times, the limited energy cost, the lack of residual compounds, and its great flexibility are some of the major benefits of pulsed light processing, giving reason for the growing interest and application of this technology by the food industry (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010).

Nowadays, pulsed light has been proposed to decontaminate the surface of fresh egg pasta, fresh-cut fruits, vegetables and ready-to-eat meat products, as well as liquid products, such as milk, fruit juices and infant food (Choi, Cheigh, Jeong, Shin, & Chung, 2010; Hierro et al., 2011; Ignat, Manzocco, Maifreni, Bartolomeoli, & Nicoli, 2014; Manzocco et al., 2013; Oms-Oliu et al., 2010).

In addition to microbial inactivation, pulsed light has been shown to affect the structure and consequently the functionalities of food biomolecules (Davies & Truscott, 2001; Wondraczek, Kotiaho, Fardim, & Heinze, 2011). The common feature of photoreactive food biomolecules is the presence of chromophores within the molecule. In this regard, proteins are major targets for photoreactions. In fact, both amino acid side-chains (e.g., tryptophan, tyrosine, phenylalanine, cysteine) and bound prosthetic groups (e.g., flavins, haem) may act as efficient chromophores. Proteins have the additional ability to bind exogenous chromophores, and rapidly react with other excited state species. The absorption of light by the protein chromophores triggers the development of side-chain oxidation, backbone fragmentation, and/or formation of cross-links and aggregates (Davies & Truscott, 2001; Pattison, Rahmanto, & Davies, 2012). In the specific case of food proteins, pulsed light was demonstrated to cause aggregation of milk and egg white proteins by disulphide exchange (Elmnasser et al., 2008; Manzocco, Panozzo, & Nicoli, 2013a) as well as to induce conformational changes in β-lactoglobulin (Fernández et al., 2012). Exposure to pulsed light was also shown to modify the structure of polyphenol oxidase and allergenic proteins (Anugu, Yang, Shriver, Chung, & Percival, 2010; Chung, Yang, & Krishnamurthy, 2008; Manzocco, Panozzo, & Nicoli, 2013b; Yang et al., 2010). Based on this literature evidence, pulsed light could be efficiently exploited to modify food protein structure, in order to obtain products/ingredients with specific functionalities.

Gluten is a viscoelastic complex mixture of proteins, containing many, probably several hundred, polypeptides, about half of the proteins being monomeric (gliadins) and the remaining being disulphide cross-linked polypeptides that form the polymeric glutenin fraction. The molecular weights (MWs) of native proteins range from around 30,000 to more than 10 million (Gianibelli,

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Larroque, MacRitchie, & Wrigley, 2001; Wieser, 2007). Glutenin forms a network of fibres in which globular gliadins are entrapped. Due to their unique technological properties and advantageous cost as compared to other proteins, gluten proteins are widely used not only in baked products, but also as an ingredient in a variety of food products (Day, Augustin, Batey, & Wrigley, 2006). However, gluten proteins are well known to cause allergenic response in people with coeliac disease, which is one of the most frequent food intolerances worldwide and affected people can only avoid symptoms by maintaining a strict gluten-free diet for their entire life (Van Eckert et al., 2010; Wieser & Koehler, 2008).

In the present study the feasibility of using pulsed light to modify the structure of gluten proteins and the influence of such structural modifications on the immunoreactivity of gluten proteins were investigated. In addition the effect of protein environmental conditions (i.e., hydration level) on gluten protein photoreactivity was also evaluated. To this purpose, gluten samples, consisting of gluten powder or aqueous suspension, were subjected to pulsed light treatments with increasing fluence. Structural changes of gluten proteins were studied by spectrophotometric analysis, dynamic light scattering analysis, determination of free sulfhydryl content, size-exclusion HPLC and SDS–PAGE. The effect of photo-induced modification of gluten protein structure on its immunoreactivity was then evaluated.

2. Materials and methods

2.1. Sample preparation

Wheat gluten (80% purity grade, Sigma–Aldrich, Milan, Italy) was suspended in 0.05 M sodium phosphate buffer containing 0.5% (w/v) sodium dodecyl sulfate (SDS) (pH 6.9) in concentration equal to 1% (w/v). Two millilitres of gluten suspension were introduced into 2 × 3 cm plastic pouches (Polycoupled Combiflex PA/PE 090, 20/70; Savonitti, Codroipo, Italy) allowing 80% of the UV radiation to reach the sample. Additional samples were prepared introducing 0.5 g wheat gluten into the pouches. In both cases, a very thin layer of sample was obtained, the maximum thickness of the filled pouches being lower than 2 mm in the case of gluten suspension and lower than 1 mm in the case of gluten powder. The pouches were hermetically sealed (VM-16; Orved, Musile di Piave, Italy) and subjected to pulsed light treatment. After pulsed light treatments, gluten suspension and gluten powder were analysed. Gluten powder was suspended in 0.5% (w/v) SDS/0.05 M sodium phosphate buffer (pH 6.9) solution in concentration equal to 1% (w/v) and analysed.

2.2. Pulsed light treatments

Pulsed light treatments were carried out at room temperature by using a pulsed light mobile decontamination unit (Claranor, Rouaine, France) equipped with 4 xenon lamps with maximum emission in the range 200–1000 nm (200–400 nm: 41%; 400–700 nm: 51%; 700–1000 nm: 8%). Lamps were positioned at each side of a quartz plaque held in the centre of the cube-shaped chamber. Two lamps were symmetrically positioned above and below the sample at a distance of 1 cm. Two lamps were symmetrically positioned at the lateral side of the sample at 1 cm distance from the quartz plaque, which corresponded to 3 cm from the sample. Samples were placed on the quartz plaque and exposed to increasing light fluence up to 26.25 J cm^{-2}, by means of an increasing number of light pulses. According to the manufacturer's instructions, each pulse delivered a light fluence of 1.75 J cm^{-2} to the surface of the pouch containing the sample. Pulse duration was 0.50 μs and repetition rate was 0.50 Hz.

2.3. Temperature

Sample temperature was measured by a copper-constantan thermocouple probe (Ellab, Denmark) connected to a portable data logger (model 502A1; Tersid, Milano, Italy). Six pouches filled with gluten suspension or gluten powder were individually subjected to PL. Immediately after the treatment, each pouch was opened and sample temperature was measured by placing the probe into the sample. Interval time between the end of the pulsed light treatment and sample temperature measurement was less than 10 s.

2.4. Absorbance

The absorption spectroscopy measurements at 280, 320, and 680 nm were performed using a UV–Vis spectrophotometer (UV-2501 PC; Shimadzu, Kyoto, Japan) at 25 °C with a 1-cm path-length cuvette. Samples were diluted with 0.5% (w/v) SDS/0.05 M sodium phosphate buffer solution (pH 6.9) to obtain absorbance signals on scale.

2.5. Dynamic light scattering

Light scattering measurements were made using a Particle Sizer NICOMP™ 380 ZLS (PSS NICOMP Particle Sizing System, Santa Barbara, CA). 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. Hydrodynamic radius refers to the corresponding volume distribution calculated by NICOMP Distribution Analysis.

2.6. Determination of free sulfhydryl content

The concentration of free sulfhydryl groups (SH) of the gluten samples was determined using Ellman's reagent (5,5′-dithiobis(2-nitrobenzoic acid), DTNB) (Sigma–Aldrich, Milan, Italy). The procedure described by Stathopoulos, Tsiami, David Schofield, and Dobraszczyk (2008) was followed. Briefly, a Tris–glycine–EDTA (TGE) buffer was prepared by dissolving Tris (80 mM), glycine (90 mM) and EDTA sodium salt (4 mM) in 800 mL distilled water. The pH was adjusted to 8.0 using concentrated hydrochloric acid and the volume was then made up to 1 L. Ellman's reagent was prepared by dissolving 20 mg of DTNB in 5 mL of dimethylformamide (4 mg/mL solution) (Sigma–Aldrich) and stored in the dark at room temperature. A working SDS–TGE solution was freshly prepared each time by mixing 45 mL of TGE stock solution with 5 mL SDS stock solution (25% w/v). The working solution was degassed in an ultrasonic bath for 30 min, and flushed with nitrogen during stirring for 15 min. Gluten (10 mg) was suspended in 3 mL TGE–SDS buffer at 20 °C and vortexed every 10 min for 30 min. Ellman's reagent (0.06 mL) was added to the suspension and the mix was held at 20 °C for 15 min. This was followed by centrifugation at 3000*g* for 15 min at 4 °C. The supernatant was then centrifuged at 20,000 *g* for 15 min at 4 °C. The absorbance of the supernatant was measured at 412 nm by a UV–Vis spectrophotometer (UV-2501 PC; Shimadzu). Both reagent and sample blanks were used.

Concentration of free sulfhydryl groups (μmol 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 gluten concentration (mg mL^{-1}); D = 5.02 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).