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Influence of pulsed light treatment on the aggregation of whey protein isolate

Md Abu Bakar Siddique[a], Paola Maresca[b], Gianpiero Pataro[a,*], Giovanna Ferrari[a,b]

[a] Department of Industrial Engineering, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, SA, Italy
[b] ProdAl S.c.ar.l., Via Ponte don Melillo, 84084 Fisciano, SA, Italy

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ABSTRACT

The effect of pulsed light (PL) on the aggregation of whey protein isolate (WPI) solutions was investigated. PL fluence values from 4 to 16 J/cm^2 were used to treat WPI (1% w/v) solutions in sodium phosphate buffer (pH = 7.5). Whey protein structural modification and aggregation were assessed through the determination of free SH-groups and UV-absorption spectra. Additionally, covalent and non-covalently linked protein-protein interactions were identified through the measurement of turbidity, aggregation index, particle size distribution, and SDS-PAGE.

WPI upon PL treatment showed structural changes as demonstrated by the immediate increase of free SH-group content (unfolding) and the subsequent formation of a small fraction of aggregation of unfolded proteins, due to both hydrophobic interactions and the formation of disulphide bonds. Turbidity, mean particle size, and aggregation index increased in samples treated at PL fluence from 4 to 16 J/cm^2. Furthermore, particle size distribution analysis of samples treated at higher fluence indicated that WPI dimer dissociation and formation of larger particles were likely to occur. The association of intermediate and larger protein molecules as well as the formation of soluble aggregates between β-lactoglobulin and α-lactalbumin were also observed in gel electrophoresis analysis. In conclusion, the results of this investigation demonstrated the potential of PL treatments to induce protein denaturation, with a minimal formation of soluble protein aggregates.

1. Introduction

Whey proteins (WP) are a by-product of cheese making process, which represent a rich and cheap source of a variety of proteins with high nutritional value and unique functional properties including gelation, emulsification, foaming, and ligand binding capacity (Kinsella & Whitehead, 1989; Ni et al., 2015; Rodrigues et al., 2015). As a consequence, WP in the form of dry whey, such as whey protein concentrate (WPC) and whey protein isolate (WPI), are widely used as major ingredients in several food formulations and dietary supplement products, such as beverages, bakery products, nutritional bars, dairy foods, muscle foods, infant formula, and protein and/or energy drinks (de Wit, 1989; Luyten, Vereijken, & Buecking, 2004).

The major WP components are β-lactoglobulin and α-lactalbumin, bovine serum albumin (BSA) and immunoglobulins. In their native form, they exist as compact, globular proteins with hydrophobic groups located inside the globular structure (Fox, 2003).

It is well known that the functional properties of proteins and their behavior in food formulations are determined by their structural properties (Segat et al., 2014). These properties markedly depend on the extent of denaturation or aggregation phenomena (Ni et al., 2015).

The denaturation of globular WP comprises conformational changes, backbone fragmentation, and partially unfolds of the protein native structure resulting in the exposure of hydrophobic and thiol (SH) groups. Then unfolded WP can form aggregates with other molecules through covalent and non-covalent interactions (Fernández et al., 2012). The formation of intermolecular disulphide bonds, by sulfhydryl-disulphide (SH/S\S) interchange reactions, is considered one of the major mechanisms of protein aggregation (Havea, Singh, & Creamer, 2002). However, other mechanisms of proteins aggregation are typically involved, such as protein self-aggregation, non-covalent, electrostatic, hydrophobic interactions and hydrogen bonding (Havea et al., 2002; Zhu, Frankema, Huo, & Kok, 2005).

Depending on the specific application, the formation of aggregates and its extent may impair functionality or improve processing performance of proteins by affecting protein solubility and texture of protein-enriched products (Ni et al., 2015), as well as increase the turbidity and viscosity of beverages, or foster protein precipitation and gelation

* Corresponding author.
E-mail address: gpataro@unisa.it (G. Pataro).

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(Lefevre, Subirade, & Pezolet, 2005; Ryan et al., 2012; Schmidt, 1976).

Thus, it is important to gain insight on the aggregation process of a variety of proteins to prevent this phenomenon or exploit it for specific applications. Several studies have shown that protein unfolding and aggregation occur to different levels depending on intrinsic molecular structural factors and extrinsic factors, including protein composition/concentration, pH, ionic strength, concentration of calcium, fat content, and presence of lactose, among others (de Wit, 1989; Ni et al., 2015). Moreover, the changes observed at molecular level typically occur also during processing of WP-containing food products. To this regard, a great deal of information comes from heating studies. Heat-induced unfolding and aggregation of pure protein fraction of β-lactoglobulin or mixtures of other WP and caseins have been widely studied in the past years (de la Fuente, Singh, & Hemar, 2002; Livney, Corredig, & Dalgleish, 2003; Rodrigues et al., 2015). Protein aggregation is assumed to be mainly driven by β-lactoglobulin, the most abundant protein in whey (i.e. 50% of whey protein composition) (Verheul, Roefs, & de Kruif, 1998), even though the overall functionality depends on the combined properties of all WP components (Dalgleish, Senaratne, & Francois, 1997). β-Lactoglobulin native molecule contains two intra-molecular disulphide bonds and one thiol group that, in aqueous solutions at pH between 5.5 and 7.5, can form dimers and oligomeric structures by non-covalent reversible association (Verheul et al., 1998). Upon heating above 60 °C, the protein molecule undergoes denaturation phenomena (Iametti, Degregori, Vecchio, & Bonomi, 1996) that can be followed by irreversible aggregation reactions (Sawyer, 1968). The formation of WP or β-lactoglobulin aggregates significantly modify protein functional properties, namely gelation, foaming and emulsifying characteristics, thereby decreasing the availability of protein to form films and stabilize emulsions (Phillips, Schulman, & Kinsella, 1990). Moreover, heat-induced aggregation of WP can also result in excessive turbidity, increased viscosity, phase separation, precipitation, and gelation (Ryan et al., 2012), causing substantial and undesired changes in the nutritional, organoleptic or technological properties of food products (Fernández et al., 2012).

More recently, non-thermal technologies have gained increasing interest by food scientists and manufacturers, not only because they can prevent adverse thermal effects and produce safe food products (Barbosa-Cánovas, Góngora-Nieto, & Swanson, 1998), but also because they can be employed to induce targeted structural modifications of proteins, which could allow producing different formulated foods with appropriate functional properties (Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011; Kim, Randolp, Seefeldt, & Carpenter, 2006; Kristo, Hazizaj, & Corredig, 2012; Segat et al., 2014; Xiang, Ngadi, Ochoa-Martinez, & Simpson, 2009).

Among these technologies, pulsed light (PL) can be individuated. It consists of the exposure of a food product to successive repetition of short (100 ns–1 ms), high-intensity pulses (flashes) of polychromatic light (200 nm–1100 nm), including UV (180–400 nm), visible (400–700 nm) and near infrared region (700–1100 nm), produced by a xenon flash lamp with approximately 40% of the emitted light corresponding to the UV spectrum (Oms-Oliu, Martin-Belloso, & Soliva-Fortuny, 2010). Although the inactivation of a number of pathogenic and spoilage micro-organisms in vitro or in food products (Oms-Oliu et al., 2010), including liquid dairy foods (Artíguez & Martínez de Marañón, 2015; Krishnamurthy, Demirci, & Irudayaraj, 2007; Miller, Sauer, & Moraru, 2012), has been extensively studied, very few works have been focused on the analysis of the effects of PL treatments on the proteins structural modifications and formation of aggregates. It has been found that PL treatments cause some aggregation in milk proteins by disulphide bonds, although no changes in amino-acid composition was observed (Elmnasser et al., 2008). Fernández et al. (2012) observed conformational changes of β-lactoglobulin related with PL fluence, which led to the formation of high molecular proteins species improving surface and foaming properties of β-lactoglobulin solutions. Manzocco, Panozzo, and Nicoli (2013) demonstrated that the exposure of egg whites to PL induced the occurrence of browning, the formation of large protein aggregates by disulphide exchange, as well as protein backbone cleavage. However, no changes in viscosity and gel strength were observed. Only recently, the effects of PL on the structural changes of WPI have been described (Siddique, Maresca, Pataro, & Ferrari, 2016). However, to date, the impact of PL processing on the formation of aggregates in WPI solutions has not been investigated yet.

The aim of this work was to investigate the effects of PL treatments at different fluence on the intermolecular and intra-molecular changes of WPI, with the attempt to provide some insight into the mechanisms of protein aggregation as a result of light absorption by the food protein chromophores. Free SH-groups, UV–Vis absorption spectroscopy, turbidity, particle size distribution, and non-reducing gel electrophoresis were determined to assess the formation of WPI aggregates.

2. Materials and methods

2.1. Materials

WPI powder (UltraWhey 90 instant), derived from sweet cheese whey, was obtained from a commercial supplier (Volac International Ltd., Orwell, UK) and stored at 4 °C until use.

As per manufacturer specification, the weight composition of the powder was as follows: 90% proteins, 1.0% fat, 2.0% lactose, 2.2% ash, and 4.0% moisture. The protein fraction included β-lactoglobulin (43–48%), α-lactalbumin (14–18%), Bovine Serum Albumin (1–2%), Immunoglobulin G (1–3%), and Lactoferrin (< 1%).

All chemicals used in this study were from Sigma Aldrich (Milan, Italy) unless otherwise stated. MilliQ water was used to dilute samples and prepare all reagents and buffers.

2.2. Preparation of WPI solutions

WPI powder was dissolved in 50 mM sodium phosphate buffer (pH = 7.5) and maintained under gentle mixing in a water-ice bath to obtain a homogenous solution. The WPI solution with a final concentration of 1% (w/v) was then stored at 4 °C until use. As previously stated (Siddique et al., 2016), this value of WPI concentration was selected in order to ensure treatment uniformity, minimizing any possible light attenuation effect throughout the processed sample volume.

2.3. PL treatments

PL treatments were performed in a bench-top PL unit (RS-3000C SteriPulse-XL system, Xenon Corp., Wilmington, Mass., USA), described in detail in Pataro, Sinik, Capitoli, Donsì, and Ferrari (2015). The system included a power/control module connected to a linear 40.6 cm xenon flash lamp, which emitted high intensity pulses of polychromatic light in the wavelength range between 200 and 1100 nm at a pulse rate of 3 pulses/s and 360 μs duration. For the experiments, samples were placed on a stainless steel tray at 16.4 cm vertical distance below the lamp source. As per manufacturer specifications, operating at this distance and for an input voltage of 3800 V, the fluence delivered to the sample per each pulse was 0.43 J/cm^2.

According to the protocol described in a previous work (Siddique et al., 2016), before PL treatment, 2 mL of the refrigerated (~4 °C) WPI solution were poured in a Petri dish (3.5 cm in diameter) to cover the entire dish surface to a depth of 2 mm. In order to minimize the temperature increase of the sample during light treatments due to the absorption of light by processed sample or by lamp heating (Pataro et al., 2015), Petri dish containing the sample was placed at the center of the tray into another Petri dish (9.5 cm in diameter) containing ice flakes. Samples were then exposed to irradiation with PL for 3.1, 6.2, 9.3 and 12.4 s, corresponding to total fluence values of 4, 8, 12, and 16 J/cm^2, respectively.

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