

Photo-Induced Modification of Food Protein Structure and Functionality

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Abstract Light processing is nowadays recognised as an efficient technology to non-thermally inactivate a wide range of microorganisms to increase food safety. However, exposure of foods to light can provide further advantages associated with the development of photoreactions involving polymers. Proteins are major target polymers for photoreactions due to abundance of endogenous chromophores, ability to bind exogenous ones and rapid reactivity with other excited state species. As food structure and functions are the result of the status of food constituents, photo-induced structural modification of food proteins can lead to new product characteristics or improved functionalities. This review paper summarises recent developments of the application of light radiation to modify protein structure, inducing changes in their technological and biological functions.

Keywords Ultraviolet · Pulsed light · Photoreaction · Proteins · Structure · Functionality

Light Processing

Light processing indicates the use of the electromagnetic radiation in the non-ionising region of the spectrum between 170 and 1,100 nm. The latter includes ultraviolet (UV, 50–400 nm), visible (VIS, 400–700 nm) and infrared (IR, 700–1,100 nm) light. Independently on the nature of the light processing, the intensity of the radiation is expressed as irradiance or intensity flux (W m^{-2}). By

contrast, the dose, which is a product of irradiance and time of exposure, is expressed as radiant exposure or fluence (kJ m^{-2}) [6].

Classification

Light treatments applied in the food industry can be classified according to radiation wavelength and operation mode (Table 1). In addition, they are carried out by applying light doses that are generally within a specific range, typical of each light technology. UV-C processing mainly exploits low- or medium-pressure mercury lamps as the source of continuous non-thermal germicidal radiation. Mercury-free lamps based on the Excimer technology are also increasingly available. However, long-life low-pressure mercury lamps are most frequently used. They are considered to provide almost monochromatic radiation at 254 nm. The latter is absorbed by highly conjugated double-bond systems in biomolecules [44, 67, 87]. It is thus able to damage microbial DNA, causing cross-linking between neighbouring thymine and cytosine in the same DNA strand [78]. DNA transcription and replication are blocked, compromising cellular functions and eventually leading to cell death [8, 46, 84]. This is generally referred to as the photochemical effect of UV-C light. Mercury lamps also emit radiation at 185 nm. The latter is responsible for the formation of ozone which oxidises organic compounds, thus exerting antimicrobial effects. UV-C-light processing is easy to use and characterised by favourable costs of equipment, energy and maintenance [3, 6, 23]. It has the undoubted advantage of not requiring heat or chemicals [20]. It is safe to apply, but some simple precautions are necessary to avoid worker exposure to light and ozone. Moreover, the technology has not been reported to form known toxic or significant non-toxic by-products.

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Table 1 Light source, wavelength range, peak wavelength and operation mode of the main light treatments applied in the food sector

	UV-C light	Pulsed light	LED
Light source	Mercury lamps	Xenon flash lamps	Light-emitting diodes
Wavelength range (nm)	200–280	100–1,100	400–700
Peak wavelength (nm)	254, 185	Polychromatic	460 (Blue LED) 520 (Green LED) 640 (Red LED)
Typical light dose (kJ m^{-2})	0.1–5	0.1–20	30–100
Operation mode	Continuous	Pulsed	Continuous

Ultraviolet processing is US-approved (US FDA [98]) and does not require authorisation according to EC novel food regulation.

Pulsed light (PL) treatment is based on the repetition of consecutive high-power light flashes emitted by xenon lamps. The radiation has a broad spectrum similar to that of the sun (170–2,600 nm) [69]. The germicidal effect of PL is primarily mediated by the photochemical effect promoted by its UV-C component [79, 101]. However, intense PL treatments can also induce localised heating leading to photothermal phenomena [24, 102]. In addition, a photo-physical effect can also result from the disturbance caused by the high-energy pulses, potentially leading to structural damages [48, 94]. Due to its high peak power, PL may provide some practical advantages over continuous UV-C when rapid disinfection is required. The intense pulses of light, which typically last a few 100 μs , can result in the application of lethal UV-C dose levels that would require mercury lamps to operate over a much longer time. The US FDA [98] has approved the use of PL provided that the treatment dose does not exceed 1.2 kJ m^{-2} .

Light-emitting diodes (LED) technology is based on the continuous exposure to the monochromatic radiation provided by LED. The radiation wavelength is in the visible range, and the peak wavelength depends on the LED colour. The technology is known to promote photodynamic phenomena. Molecules acting as photosensitiser can absorb light, producing reactive oxygen species (ROS). The latter may react with biomolecules, leading to cytotoxic effects [53]. LEDs have the advantages of low energy consumption and high durability. Their small size allows fitting most designs.

Applications

LED-induced photodynamic inactivation of microorganisms is receiving increasing attention [28, 34, 35, 54], but most information about light processing is relevant to application of UV-C and PL. The latter are successfully used for sterilisation of packaging materials, working surfaces and utensils [6, 47, 103]. Due to poor penetration depth, light radiation is traditionally applied to disinfect

transparent fluids, such as air and drinkable water [6]. UV-C and PL processing has also been proposed to extend the shelf life of different foods, such as sugar syrups, apple cider and fruit juices [11, 71, 74, 91, 96]. It is noteworthy that the presence in the fluid of soluble solids, absorbing and scattering light, may impair the process efficiency by reducing light penetration [32, 109]. For instance, the penetration depth of opaque food materials is often lower than 0.2 mm [38, 55]. For this reason, homogeneous light treatment of liquid foods requires proper technological solutions to ensure high turbulence of the fluid. By contrast, in the case of solid food, light exposure only affects a thin surface layer of the product, while a negligible light dose is able to reach its internal part. High penetration intensity is required to inactivate microorganisms in a fluid mass, but it becomes unnecessary when treating foods that exert surface contamination issues, especially if their original fresh-like appearance should be maintained. Based on these considerations, UV-C and PL has been proposed to decontaminate fresh-cut vegetables, ready-to-eat meat products and the surface of shell eggs [12, 36, 37, 65, 80].

Structural Effects of Protein Photoreaction

Proteins are well known to undergo chemical modification when they are exposed to light. Proteins photoreactivity is due to abundance of efficient chromophores (e.g. tryptophan, tyrosine, phenylalanine, cysteine, flavins, heme), ability to bind exogenous ones and rapid reactivity with other excited state species [15]. Based on these considerations, protein photoreaction is generally reported to occur via two major routes: (1) direct photo-oxidation arising from the absorption of radiation by the protein structure or bound chromophore; (2) indirect protein oxidation mediated by singlet oxygen generated by energy transfer by either protein bound, or other chromophores. The energy of the radiation absorbed by the chromophores is transferred between the initial sites of oxidation and the part of the protein that controls its structure and function. This transfer primarily involves aromatic and sulphur-containing amino acids. Tyrosine and cysteine residues

have been demonstrated to be the ultimate receptors of oxidising potential [77]. The exact transfer pathway to tyrosine is often unclear due to the presence of multiple alternative pathways [75]. By contrast, transfer to disulfide bonds can occur via both hydrogen-bonding networks and via the backbone [30]. The mechanisms of a multitude of photo-induced protein reactions have been extensively reviewed [16, 75], and only those begetting modifications in protein conformation, size and architecture will be briefly treated below.

Although light absorption can generate a number of different protein photoreactions, their structural consequences actually fall into a few classes, namely protein unfolding, aggregation and fragmentation (Table 2). Light treatment of proteins often results in their unfolding. Conformational changes are the result of light-induced modification of protein binding forces, including hydrogen bonds, hydrophobic bonds, ionic bonds or bonds that hold subunits. These modifications affect the secondary and tertiary structure of proteins that expose hydrophobic residues and free thiols. Protein exposure to light is associated with easily formation of high molecular weight aggregates (dimers and higher species) [15, 16, 75]. Aggregates may arise from radical–radical termination reactions of photo-oxidised proteins, but other reactions, mainly involving histidine residues, are also known to promote the formation of cross-links [15, 75]. In fact, the carbonyl functions of histidine oxidation products can induce cross-linking by addition with other nucleophiles, often leading to the decrease in lysine and arginine residues [1]. Aggregation by intermolecular interactions is also very common. Partially unfolded molecules can self-assemble by intermolecular hydrophobic interactions and the formation of disulfide-bonded proteins [22, 92, 105].

Although light treatments applied in the food sector (Table 1) are non-ionising radiations, high-frequency light, such as the ultraviolet one, coincides with the bond energies of protein moieties [7]. Initial oxidation would beget radicals at the α -carbon site of a peptide backbone [15]. As a result, the radiation can cleave protein chain,

potentially leading to changes not only in their conformation but also in configuration [49]. Protein photo-induced fragmentation seems to be less important than unfolding and aggregation phenomena [75]. However, there are evidences of the simultaneous occurrence of fragmentation and cross-linking of collagen upon UV irradiation [99]. Degradation and fragmentation of collagen were presumably attributed to proline oxidation. Protein cleavage has also been observed upon light treatment of egg white proteins and enzymes such as polyphenoloxidase, pectic lyase and lipoxigenase (Manzocco et al. [4, 56, 60, 62].

Main Factors Affecting Protein Photoreactivity

Protein photoreactivity and relevant structural changes are the result of the contribution of multiple concomitant and/or consequential phenomena. The role of each process during light exposure and its eventual prevalence over the others depend on several intrinsic and environmental factors (Table 3).

Intrinsic Photosensitivity

Each protein is characterised by an intrinsic photosensitivity, which depends on the presence of photoreactive sites and the overall structural arrangement of the protein. The common feature of photoreactive proteins is the presence of light-absorbing chemical sites (e.g. tryptophan, tyrosine, phenylalanine or cysteine). In addition, cross-linking by nucleophilic addition requires the presence of histidine [2].

Photoreactive sites should not only be present in the protein but also be able to initiate photoreactions leading to structural modification of the polymer. Literature has demonstrated that in random coil proteins, such as casein, the most susceptible amino acids are the exposed ones [14]. However, it is noteworthy that the oxidation of exposed individual amino acids has minor effects on the overall protein structure because they difficulty react with the other sites of the protein. By contrast, densely packed globular proteins show higher modification than random coil proteins. Globular proteins, such as lactoglobulin, polyphenoloxidase, lysozyme and egg white proteins, typically undergo unfolding [19, 56, 60, 63, 104]. The higher photosensitivity of globular proteins can be attributed to the facile transfer of oxidising equivalents among vicinal reactive sites within the molecule. In addition, globular proteins are also particularly prone to aggregation via intermolecular bond formation [19, 22]. In other words, the higher the possibility of the protein to interact at both intra- and intermolecular level, the more intense the photo-induced structural changes.

When the structural complexity of the protein increases, photo-induced changes may be sterically hindered. For

Table 2 Structural effects of the main protein photoreactions

Main protein photoreactions	Structural effects
Modification of protein binding forces (e.g. formation and cleavage of hydrogen and hydrophobic bonds)	Unfolding
Oxidative reactions (e.g. development of radical termination reactions, SH-SS exchange)	Aggregation
Hydrophobic interaction	
Oxidative reactions (e.g. radical termination reactions)	Fragmentation

Table 3 Main factors affecting protein photoreactivity

Factors	
Intrinsic photosensitivity	Protein photoreactive sites Protein structural arrangement
Environmental conditions	Ionic strength pH Protein concentration Crowding Presence of other molecules (e.g. sugars, starch)
Radiation properties	Wavelength Fluence

instance, the presence of a tertiary structure, able to oppose conformational changes deriving from photoreactions of susceptible residues, is generally associated with higher photoresistance, as observed for horseradish peroxidase [86]. Similarly, proteins organised in a fibrous structure mainly undergo degradation with minor unfolding [90]. To this regard, gluten proteins, which are embedded in a network of fibrous and globular proteins, only underwent degradation because they were spatially hampered to further photoreactions [72].

Environmental Conditions

Since photosensitivity of proteins strictly depends on their overall architecture, any structural changes induced by an alteration in environmental conditions are potentially associated with a modification in their ability to photoreact. Environmental factors (e.g. pH, ionic strength, presence/absence of other food components) influence the protein structure and hence photoreactivity.

The modulation of the ionic strength of the environment is known to allow expanded or compact protein to be achieved. It has been suggested that compact protein structures obtained at high ionic strength are particularly prone to photo-induced intramolecular rearrangement [58].

The concentration of proteins in the system also exerts a crucial role. In particular, in highly diluted protein systems, their possibility to interact with each other to form photo-induced aggregates would decrease, as observed in the case of polyphenoloxidase [62]. In this condition, photoreactions would mainly occur via intramolecular rearrangement and only to a minor extent via intermolecular cross-linking. The latter would become significant only at higher concentrations allowing photo-induced cross-linking among vicinal proteins. However, the photosensitivity of proteins would dramatically change in systems approaching the crowding concentration. Proteins are considered crowded when their concentration is higher than that associated with their accommodation in a given volume of solution without mutual structure perturbation. In this condition, proteins

are crowded into a smaller space, with less freedom of movement due to purely nonspecific steric repulsions. Protein crowding has several thermodynamic and kinetic consequences, including the inhibition of unfolding phenomena [68, 110]. For this reason, crowded proteins may result fully resistant to light. Evidence of complete photostability under crowded conditions was observed in egg white proteins exposed to UV-C light [58]. This effect was attributed to the fact that crowding opposes photo-induced unfolding, which is associated with an increase in the hydrodynamic volume of the proteins.

The presence of other molecules in the environment surrounding proteins during light exposure critically affects photoreactivity. Light treatment of protein-rich systems containing sugars has been shown to promote browning reactions [27, 57, 60, 81]. By contrast, the presence of large polysaccharides may reduce protein photoreactivity. To this regard, information has been provided by comparing the effect of PL on wheat gluten and flour [72]. The photosensitivity of gluten proteins in flour was found to be lower than that observed in the sole gluten. This was attributed to the close interaction between gluten proteins and starch granules in a complex network, which hinders photo-induced structure modifications of the protein fraction.

Radiation Properties

Protein photoreactivity strictly depends on the prevalent wavelength of the radiation applied as well as on its intensity. While the monochromatic radiation of UV-C light promotes photochemical effects, the visible light of LED technology is mainly associated with photodynamic phenomena. PL, which included not only UV-C and visible radiation but also the infrared one, is known to induce additional photothermal and photophysical effects. Based on these considerations, the nature of the structural modifications of proteins is expected to be considerably different depending on the light radiation. However, to the best of our knowledge, there is no literature information

about the wavelength dependency of structural changes induced by light in a given protein. The comparison of the effects of treatments that exploit radiation with different wavelength would be possible only keeping constant light intensity. This can be a difficult experimental condition due to the not overlapping light dose scales that are typical of the different light technologies applied in continuous or pulsed mode (Table 1).

However, it is clear that for a given light radiation, the higher the intensity of the light, the higher the extent of protein structure modifications. For instance, when the effects of both UV-C and PL were investigated on polyphenoloxidase and pectin lyase in a wide range of light doses, the same mechanism of structural modification was detected but with different extents depending on light dose [56, 63]. Similar results were also observed upon PL treatment of gluten, egg white and polyphenoloxidase [61, 62, 72]. In the case of polyphenoloxidase, a linear relation was observed between light dose and enzyme inactivation. A light dose parameter quantifying the photostability of the enzyme was defined as kJ m^{-2} required for 50 % enzyme inactivation. This result suggests that the processing variable that can be used to predict the extent of protein structural modifications is the light dose. To this regard, it is noteworthy that several research groups have provided evidence about the effects of light on proteins, but difficulties arise when comparing data. This is often due to an incomplete communication of processing parameters, which do not allow the light dose to be calculated.

Photo-Induced Modification of Protein Functionality

Protein functionality covers a range of different aspects. According to Kinsella [43], functional properties are those physical and chemical properties that affect the behaviour of proteins in food systems during processing, storage, preparation and consumption. However, as more recently noted by Foegeding and Davis [25], the most common function of food proteins is to provide and/or stabilise the characteristic structure of individual foods. Proteins are not only primary building blocks of foods but are also used to stabilise food structures, such as emulsions, foams, gels and films. In addition, they are of the great biological relevance due to physiological significance beyond the pure nutritional requirements for normal growth and maintenance. Proteins may actually exert enzymatic activity, flavours and mineral binding capacity as well as different bioactivities (e.g. antihypertensive, immunomodulatory or antioxidant). However, they may also represent a critical issue for their allergenic potential. Based on these considerations, proteins serve several technological and biological functions. The latter are known to start at the level of protein structure [25].

For this reason, any photoreaction which alters protein structure may lead to a potential modification in functionality. For instance, photo-induced unfolding or aggregation of proteins could beget systems with well-defined water adsorption capacity, optical properties, interface behaviour, rheological and mechanical properties as well as with steered enzymatic activity, digestibility or allergen reactivity. In addition, considering the close interaction between proteins and other food constituents, structural change of proteins could induce modifications in the architectural organisation of the material they are embedded in, with relevant variations in the properties of food. Protein cross-linking could also allow formation of tridimensional networks, possibly able to stabilise supramolecular structures and immobilise biomolecules with the desired nutritional or health protecting role. Similarly, also the mechanisms and the extent of protein photoreactivity could be altered, leading to unexpected changes in their functional properties.

Despite the great potential of light processing, applications to steer protein functionality are limited. In the following, literature reports on the effects of light on proteins are reviewed with reference to their main functional properties. Attention was paid to the light doses applied in the experiments or suggested for industrial applications. However, it is noteworthy that light doses were frequently missing or not computable from available processing parameters.

Rheological Properties

Light has been shown to significantly affect the rheological properties of protein-rich matrices. To this regard, the elasticity and viscosity of UV-irradiated thermal gels from sardine and pork meat were always higher than those without irradiation [39]. Scanning electron micrographs of the gels indicated that there was a large portion of densely dispersed matter in UV treated gels. In other words, gels resulted denser probably due to partial unfolding and aggregation of actomyosin. This hypothesis was confirmed by the decrease in total sulfhydryl groups of actomyosin and the increased surface hydrophobicity as well as by SDS-PAGE patterns, indicating polymerisation and aggregation of myosin by hydrophobic interactions [40, 41].

Fish gelatin powder exposed to UV-C light did not show modification of melting and gelling point as compared to the untreated sample but presented significantly higher melting enthalpy [5]. The latter was attributed to the reinforcement by cross-linking of the gel network, which exhibited improved strength. However, when UV-C-treated gelatin powder was dispersed in water, a significant decrease in viscosity was observed [5]. This effect was attributed to possible photo-induced degradation and fragmentation of proteins. In the flow measurement, the

polymer chain align in the direction of shear flow. It is thus reasonable that shorter polymer chains would easier align, accounting for lower viscosity. These apparently contradictory evidences suggest that both unfolding/aggregation and degradation/fragmentation concomitantly occur upon protein photoreaction, leading to counterbalancing effects on rheological properties. This hypothesis was confirmed by the complex dependence of viscosity from light dose observed in the case of egg white. In this protein matrix, an initial increase in viscosity was observed upon exposure to 0.1 kJ m^{-2} UV-C light, but the increase in light dose above 0.4 kJ m^{-2} was associated with rheological properties analogous to that of the untreated egg white [17, 60]. It is likely that slightly modified egg white proteins could better interact, resulting in a weak protein network with higher viscosity. The structural continuity of this weak network would be probably interrupted by the presence of large protein clusters and small fragments formed upon more intense lightning. The counterbalancing effect of networking and backbone cleavage leading to negligible changes in viscosity was also confirmed when egg white was exposed to increasing doses of PL up to 3.1 kJ m^{-2} [61]. Figure 1 schematically summarises the possible effects of light on protein viscosity as a function of light dose. The overall evolution of the rheological properties of the system is the result of the prevailing or concomitant occurrence of different photoreactions.

Film Properties

Early studies on light exposure demonstrated the possibility to modify collagen by inducing fibril cross-linking [26]. Different photo-cross-linked films are nowadays available to form surgery implants with modified biodegradation rate and tensile properties [18, 95]. Application of UV-C-irradiated collagen is also possible in the food sector to produce edible films. Miller and Marder [66] patented an edible tubular casing with improved

mechanical properties by exposure to ultraviolet light. More recently, the structural changes in collagen caused by UV-C light exposure were demonstrated to depend on the degree of hydration [106]. In particular, soluble collagen exposed to UV-C light for 40 min completely lose fibrillogenic ability. This result confirms the critical role of environmental conditions in determining protein photoreactivity (Table 3).

Tensile and puncture strength of cast soy protein films increased linearly with UV-C radiation dose from 0.1 to 1 kJ m^{-2} [27, 73]. SDS-PAGE patterns of UV-C-treated soy films revealed bands of aggregates, confirming the occurrence of cross-linking in films. The latter was also accounted for the higher tensile strength observed in gluten, zein and albumin films exposed to 0.5 kJ m^{-2} UV-C light [81].

Emulsifying and Foaming Properties

Sodium caseinate and egg white protein powders exposed to UV-C light showed improved emulsifying ability as compared to untreated samples [50]. In particular, they allowed the formation of oil-in-water emulsions with lower droplet size. Emulsions also resulted less prone to flocculation and coalescence after standing at room temperature. The improved emulsifying properties were attributed to changes in conformation and surface properties of proteins, which better form adsorption films around the fat globules and lower the interfacial tension at the oil–water interface. Kuan et al. [50] also demonstrated that UV-C irradiated sodium caseinate and egg white powders presented better foam ability and foam stability. Similar results were also reported by Fernández et al. (2012) with reference to a 0.5 mg mL^{-1} aqueous solutions of β -lactoglobulin exposed to PL up to 0.4 kJ m^{-2} . At this light dose, the foam capacity of the protein increased by 34 %. The interfacial films of treated proteins presented higher viscoelastic properties allowing to resist the shear stress resulting from liquid drainage. In addition, foaming properties resulted well correlated to the rate of protein adsorption to the air–water interface and thereby to the decrease in surface tension. These better performances were attributed to the increase in surface hydrophobicity and to the higher flexibility of partially unfolded proteins. It was also hypothesised that photo-induced protein aggregates could adsorb at the interface to form an ordered network that cross-links the two thin films of adjacent bubbles, thus stabilising the films. This hypothesis was confirmed studying the effect of ultraviolet (0.1 – 0.6 kJ m^{-2}) and PL (0.2 – 3.1 kJ m^{-2}) on egg white [60–62]. The higher foam ability and lower tendency to bubble dismutation/coalescence of light-treated egg white were tentatively attributed to the presence of high-size protein aggregates that could behave as surface-active solid

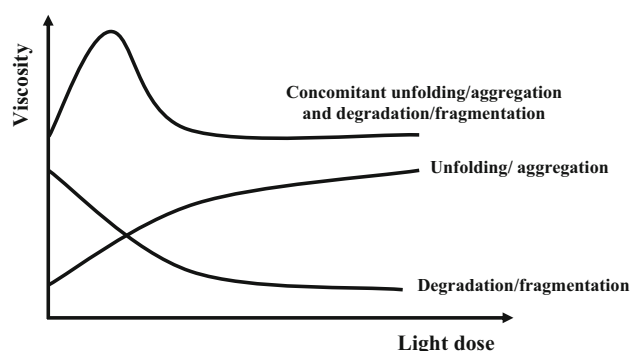


Fig. 1 Schematic representation of the effect of light dose on protein viscosity

particles and beget a Pickering foam [76]. The latter would be stabilised not only by the surfactant properties of native egg white proteins but also by the photo-induced solid protein aggregates. As suggested by Murray et al. [70], native proteins and solid particles could attract each others, increasing the particle jamming in the fluid interstice between bubbles. This would cause the formation of a firmer shell, positively affecting foaming properties.

The number of different mechanisms possibly explaining the improved foaming properties of light-treated proteins are summarised in Table 4.

Optical Properties

Protein exposed to light possibly undergo changes in their optical properties. The photo-induced formation of high-size particles was reported to cause an increase in collagen and egg white turbidity [60, 106]. However, in other cases, colour changes were also observed. Soy protein films exposed to UV-C light up to 1 kJ m^{-2} developed a yellowish colouration, which linearly increased with the light dose [27]. A similar effect was also observed in gluten, albumin and caseinate films [81]. Pigment formation upon protein photoreaction is supported by literature data indicating that UV-C irradiation of model systems containing amino acids and reducing sugars is associated with the formation of a number of different Maillard reaction products [85]. Manzocco et al. [60] reported the formation of brown pigments in egg white exposed for increasing time to 0.1 or 0.6 kJ m^{-2} UV-C light. However, when egg white was exposed to increasing doses of PL up to 3.1 kJ m^{-2} , not only brown melanoidins were formed, but they also underwent progressive degradation so that a maximum browning was detected in the sample exposed to 2.6 kJ m^{-2} . A possible schematisation of the effect of light dose on product browning is suggested in Fig. 2. A similar evolution of brown melanoidins was also detected in protein-rich foods such as fresh egg pasta [57]. It is noteworthy that in this case, the presence of photo-induced melanoidins was associated with a higher oxidative

stability of the product. It was thus postulated that protein melanoidins formed by photoreaction could also exert interesting antioxidant activity.

Enzymatic Activity

The activity of enzymatic proteins is known to depend on their structure which can be modified following photo-oxidation [21, 81, 100]. However, literature data about the effects of light on enzymes often provide controversial information. This is probably related to the different nature of the systems under study. When the effect of light on enzymatic activity is studied in plant tissues, controversial data are generally obtained. For instance, low levels of light emitted by mercury lamps ($<0.007 \text{ kJ m}^{-2}$) or LED have been reported to reduce postharvest decay in a number of fresh fruit and vegetables [45, 89, 93, 97]. These positive effects were attributed to the photo-induced modulation of enzymatic activity in the cell [29, 31]. For instance, light exposure often increases the activity of phenylalanine ammonia-lyase, which enhances the biosynthesis of phenols toxic to pathogens [64, 88]. However, Charles et al. [9] reported that PL treatment at higher fluence (80 kJ m^{-2}) did not affect the activity of this enzyme in fresh-cut mangoes and activated polyphenoloxidase. Lamikanra et al. [51] observed an increase in ascorbate peroxidase but a decrease in esterase and lipase activity in UV-C-treated fresh-cut melon. This product was also reported to show inactivation of polyphenoloxidase, peroxidase, pectin methyl esterase and polygalacturonase by exposure to UV-C light below 4.8 kJ m^{-2} [10]. It is noteworthy that in the previously described papers, authors' interest was focussed on the systemic response of plant tissue to light exposure. Enzymatic activity was thus analysed on the extract of the entire plant tissue and is likely to be the result of activation/inactivation of genes encoding enzymes rather than to a specific photoreaction involving enzymes. However, when pectolytic enzymes and polyphenoloxidase were extracted from a thin surface layer of UV-C-treated apple slices (4 kJ m^{-2}), their

Table 4 Possible mechanisms at the basis of the improved foaming properties of proteins upon photo-induced structural changes

Photo-induced structural change	Mechanism of action	Overall effect in the foam
Unfolding	Increase in protein hydrophobicity	Better protein adsorption at the interface
	Higher flexibility of partially unfolded proteins	
Aggregation	Increase in viscoelasticity of interfacial films	Firmer films
	Formation of ordered interfacial films	
	Cross-linking of interfacial films	
	Protein aggregates jamming in the interfacial films	

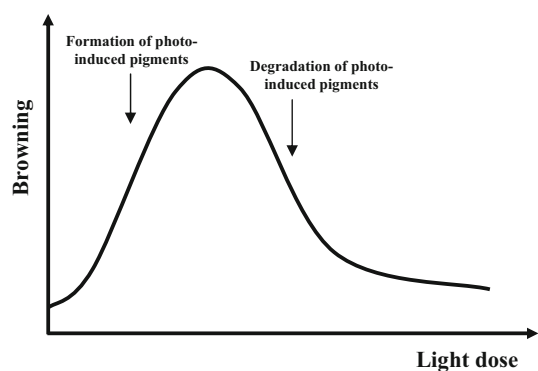


Fig. 2 Schematic representation of the effect of light dose on browning of protein-rich foods

activity was reduced by more than 60 % [56, 59, 63]. Similarly, polyphenoloxidase in mango nectar and apple juice was reported to be inactivated by UV-C treatments [33, 42, 63]. These results suggest that there is not only a metabolic response of the wound tissue to irradiation but also a direct photo-induced modification of enzyme activity. This was further confirmed by studies performed on enzymes in model systems [83]. The latter indicate that low-intensity treatments can modify the conformation of the active site of the enzyme, leading to an overall activity increase [82]. By contrast, higher intensity treatments would be associated with non-reversible structural changes, including protein aggregation and fragmentation, resulting in enzyme inactivation [56, 63]. In the case of soy lipoxygenase, the dose of PL resulted to be the main factor governing the extent of protein fragmentation [4]. However, the sensitivity of enzymes to light radiation is also strongly affected by their nature. At the same UV-C dose, pectin lyase inactivation occurred quicker than that of polyphenoloxidase, but the latter was more sensitive to the increase in light dose [56]. In addition, isoenzymes may show different susceptibility to light, as observed for UV-C-resistant forms of pectolytic enzymes identified in apple.

Allergenicity

Thanks to its potential ability to alter the structure-related biological properties, light radiation can modify the antigenic response of protein allergens. To this regard, little information is available in literature. Liquid peanut butter, soybean, almond and shrimp extracts were shown to decrease their IgE binding capacity by exposure to PL [13, 52, 107, 108]. In the case of shrimp extracts, the authors also performed *in vitro* simulated gastric and intestinal digestion and demonstrated that the lower allergen reactivity of PL-treated shrimp extract was maintained under digestive conditions [108]. A decrease in immunoreactivity was also observed in the case of gluten exposed to PL. This

was attributed to structural modifications that probably lead to a lower exposure of protein epitopes [72]. Similar results were reported for egg white proteins upon ultraviolet exposure [58]. However, when egg white was considered, light treatments were associated with no changes or even to an increase in immunoreactivity [60, 61]. These contradictory information could be due to the different environmental conditions experienced by proteins in diluted solutions or in crowded food systems. The latter could significantly affect the mechanisms of photo-induced structural modification, leading to opposite effects in terms of exposure of reactive epitopes. Further research is certainly needed to explore the possibility to use light radiation to steer allergenicity of ingredients and foods.

Final Remarks

Advances in light processing have made these technologies available, sustainable and of easy integration into food industries. Light processing is generally exploited as a non-thermal alternative to conventional disinfection methods but offers advantages well beyond it. The intense photoreactivity of proteins as well as the large variability of the deriving structures suggests a wide potential of light radiation in modifying functionality of food proteins. The modulation of rheological, film, emulsifying and foaming properties of proteins are examples of the possibilities provided by light processing to improve the technological performance of protein-rich ingredients. Similarly, the significant effects of light on activity of enzymes and reactivity of allergens reveal unexplored possibilities to use this physical process to steer protein biological properties. The key point for turning circumstantial evidence of the improvement of protein properties into a rational exploitation of light to get tailored proteins lies in the knowledge of the relation between the photo-induced structure of proteins and their functions. To reach this goal, the following research needs are still required:

- better understanding of photo-induced chemical and structural changes in food proteins;
- identifying the relation between light properties (i.e. dose and wavelength) and protein structure modification;
- studying the effects of environmental conditions experienced by the protein during irradiation;
- studying the technological, sensory, nutritional and physiological properties of photomodified food proteins.

Finally, literature data suggest that the photoresponse of proteins dramatically changes when they are embedded in crowded and complex matrices such as foods. Under these

conditions, there is plenty of possibility to photo-induce protein reactions with other food components. However, the mechanisms and effects of the photoreactivity of proteins in the presence of other compounds, such as sugars, polysaccharides or lipids, are still obscure and are expected to be further complicated by the occurrence of a food supra-molecular structure that could selectively favour or hinder specific photoreactions. These aspects certainly represent a challenging research area that would require exploration.

References

- Agon VV, Bubb WA, Wright A, Hawkins CL, Davies MJ (2006) Sensitizer-mediated photooxidation of histidine residues: evidence for the formation of reactive side-chain peroxides. *Free Radic Biol Med* 40:698–710
- Balasubramanian D, Kanwar R (2002) Molecular pathology of dityrosine cross-links in proteins: structural and functional analysis of four proteins. *Mol Cell Biochem* 234(235):27–38
- Barbosa-Cánovas GV, Pothakamury UR, Palou E, Swanson BG (1998) Nonthermal preservation of foods. Marcel Dekker Inc., New York
- Bhaskar AJ, Yang W, Marshall MR, Reyes-De-Corcuera JJ, Rababah TM (2014) Nonthermal inactivation of soy (*Glycine max* Sp.) lipoxygenase by pulsed ultraviolet light. *J Food Sci* 79:C8–C18
- Bhat R, Karim AA (2009) Ultraviolet irradiation improves gel strength of fish gelatin. *Food Chem* 113:1160–1164
- Bintsis T, Litopoulou-Tzanetaki E, Robinson RK (2000) Existing and potential applications of ultraviolet light in food industry: a critical review. *J Sci Food Agric* 80:637–645
- Blatchley ER, Peel M (2001) Disinfection by ultraviolet irradiation. In: Disinfection, sterilisation, and preservation. Lipincott Williams & Wilkins, New York, pp 823–851
- Bolton JR, Linden KG (2003) Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *J Environ Eng* 129:209–215
- Charles F, Vidal V, Olive F, Filgueiras H, Sallanon H (2013) Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. *Innov Food Sci Emerg* 18:190–195
- Chisari M, Barbagallo RN, Spagna G, Artes F (2011) Improving the quality of fresh-cut melon through inactivation of degradative oxidase and pectinase enzymatic activities by UV-C treatment. *Int J Food Sci Technol* 46:463–468
- Choi LH, Nielsen SS (2005) The effects of thermal and non-thermal processing methods on apple cider quality and consumer acceptability. *J Food Qual* 28:13–29
- Choi MS, Cheigh CI, Jeong EA, Shin JK, Chung MS (2010) Nonthermal sterilisation of *Listeria monocytogenes* in infant foods by intense pulsed-light treatment. *J Food Eng* 97:504–509
- Chung S-Y, Yang W, Krishnamurthy K (2008) Effects of pulsed UV-light on peanut allergens in extracts and liquid peanut butter. *J Food Sci* 73:C400–C404
- Dalsgaard TK, Otzen D, Nielsen JH, Larsen LB (2007) Changes in structures of milk proteins upon photo-oxidation. *J Agric Food Chem* 55:10968–10976
- Davies MJ (2003) Singlet oxygen-mediated damage to proteins and its consequences. *Biochem Biophys Res Commun* 305:761–770
- Davies MJ, Truscott RJW (2001) Photo-oxidation of proteins and its role in cataractogenesis. *J Photochem Photobiol B Biol* 63:114–125
- de Souza PM, Fernandez A (2011) Effects of UV-C on physicochemical quality attributes and *Salmonella enteritidis* inactivation in liquid egg products. *Food Control* 22:1385–1392
- De Vore DP, Kelman CD (1990) Human collagen processing and autoimplant use. Patent EP 0330389 A3
- Delincée HR (1983) Recent advances in radiation chemistry of proteins. In: Elias PS, Cohen AJ (eds) Recent advances in food irradiation. Elsevier Biomedical, Amsterdam, pp 129–147
- Devine DA, Keech AP, Wood DJ, Killington RA, Boyes H, Dumbleday B, Marsh PD (2001) Ultraviolet disinfection with a novel microwave-powered device. *J Appl Microbiol* 91:786–794
- Edwards AM, Silva E (2001) Effect of visible light on selected enzymes, vitamins and amino acids. *J Photochem Photobiol B Biol* 63:126–131
- Elmnesser N, Dalgalarondo M, Orange N, Bakhrouf A, Haertlé T, Federighi M, Chobert JM (2008) Effect of pulsed-light treatment on milk proteins and lipids. *J Agric Food Chem* 56:1984–1991
- Falguera V, Pagán J, Garza S, Garvin A, Ibarz A (2011) Ultraviolet processing of liquid food: a review. Part 2: effects on microorganisms and on food components and properties. *Food Res Int* 44:1580–1588
- Fine F, Gervais P (2004) Efficiency of pulsed UV light for microbial decontamination of food powders. *J Food Prot* 67:787–792
- Foegeding EA, Davis JP (2011) Food protein functionality: a comprehensive approach. *Food Hydrocoll* 25:1853–1864
- Fujimori E (1965) Ultraviolet light-induced change in collagen macromolecules. *Biopolymers* 3:115–119
- Gennadios A, Rhim JW, Weller CL, Hanna MA (1998) Ultraviolet radiation affects physical and molecular properties of soy protein films. *J Food Sci* 63:1–4
- Ghate VS, Ng KS, Zhou W, Yang H, Khoo GH, Yoon W-B, Yuk H-G (2013) Antibacterial effect of light emitting diodes of visible wavelength on selected foodborne pathogens at different illumination temperatures. *Int J Food Microbiol* 166:399–406
- González-Aguilar GA, Zavaleta-Gatica R, Tiznado-Hernández ME (2007) Improving postharvest quality of mango “Haden” by UV-C treatment. *Postharvest Biol Technol* 45:108–116
- Gray HB, Winkler JR (1996) Electron transfer in proteins. *Annu Rev Biochem* 65:537–561
- Green R, Fluhr R (1995) UV-B-Induced PR-1 accumulation is mediated by active oxygen species. *Plant Cell* 7:203–212
- Guerrero-Beltrán JA, Barbosa-Cánovas GV (2004) Advantages and limitations on processing foods by UV light. *Food Sci Technol Int* 28:137–148
- Guerrero-Beltrán JA, Barbosa-Cánovas GV (2006) Inactivation of *Saccharomyces cerevisiae* and polyphenoloxidase in mango nectar treated with UV light. *J Food Prot* 69:362–368
- Guffey JS, Wilborn J (2006) Effects of combined 405-nm and 880-nm light on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in vitro. *Photomed Laser Surg* 24:680–683
- Guffey JS, Wilborn J (2006) In vitro bactericidal effects of 405-nm and 470-nm blue light. *Photomed Laser Surg* 24:684–688
- Hierro E, Barroso E, de la Hoz L, Ordóñez JA, Manzano S, Fernández M (2011) Efficacy of pulsed light for shelf life extension and inactivation of *Listeria monocytogenes* on ready-to-eat cooked meat products. *Innov Food Sci Emerg Technol* 12:275–281
- Hierro E, Manzano S, Ordóñez JA, de la Hoz L, Fernández M (2009) Inactivation of *Salmonella enterica* serovar Enteritidis on

- shell eggs by pulsed light technology. *Int J Food Microbiol* 135:125–130
38. Innocente N, Segat A, Manzocco L, Marino M, Maifreni M, Bartolomeoli I, Ignat A, Nicoli MC (2014) Effect of pulsed light on total microbial count and alkaline phosphatase activity of raw milk. *Int Dairy J* 39:108–112
 39. Ishizaki S, Hamada M, Iso N, Taguchi T (1993) Effect of ultraviolet-irradiation on rheological properties of thermal gels from sardine and pork meat pastes. *Nippon Suisan Gakk* 59:1219–1224
 40. Ishizaki S, Hamada M, Tanaka M, Taguchi T (1993) Conformational-changes in actomyosins from fish and pork muscles by ultraviolet-irradiation. *Nippon Suisan Gakk* 59:2071–2077
 41. Ishizaki S, Ogasawara M, Tanaka M, Taguchi T (1994) Ultraviolet denaturation of flying fish myosin and its fragments. *Fish Sci* 60:603–606
 42. Keyser M, Müller IA, Cilliers FP, Nel W, Gouws PA (2008) Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Inn Food Sci Emerg Technol* 9:348–354
 43. Kinsella JE (1976) Functional properties of proteins in foods: a survey. *Crit Rev Food Sci Nutr* 7:219–280
 44. Koller D (1965) Ultraviolet radiation. Wiley, London
 45. Kook HS, Park SH, Jang YJ, Lee GW, Kim JS, Kim HM, Oh BT, Chae JC, Lee KJ (2013) Blue LED (light emitting diodes)-mediated growth promotion and control of Botrytis disease in lettuce. *Acta Agric Scand B SP* 63:271–277
 46. Koutchma T (2009) Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food Bioprocess Technol* 2:138–155
 47. Koutchma T, Keller S, Chirtel S, Parisi B (2004) Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. *Innov Food Scid Emerg Technol* 5:179–189
 48. Krishnamurthy K, Tewari JC, Irudayaraj J, Demirci A (2008) Microscopic and spectroscopic evaluation of inactivation of *Staphylococcus aureus* by pulsed UV light and infrared heating. *Food Bioprocess Technol* 3:93–104
 49. Kuan Y-H, Bhat R, Patras A, Karim AA (2013) Radiation processing of food proteins: a review on the recent developments. *Trends Food Sci Technol* 30:105–120
 50. Kuan Y-H, Bhat T, Karim AA (2011) Emulsifying and foaming properties of ultraviolet-irradiated egg white protein and sodium caseinate. *J Agric Food Chem* 59:4111–4118
 51. Lamikanra O, Kueneman D, Ukuku D, Bett-Garber KL (2005) Effect of processing under ultraviolet light on the shelf life of fresh-cut cantaloupe melon. *J Food Sci* 70:C534–C539
 52. Li Y, Yang W, Chung S-Y, Chen H, Ye M, Teixeira AA, Gregory JF, Welt BA, Shiver S (2013) Effect of pulsed ultraviolet light and high hydrostatic pressure on the antigenicity of almond protein extracts. *Food Bioprocess Technol* 6:431–440
 53. Luksiene Z (2009) Photosensitization for food safety. *Chem Technol* 4:62–65
 54. Maclean M, MacGregor SJ, Anderson JG, Woolsey G, Coia JE, Hamilton K, Taggart I, Watson SB, Thakker B, Gettinby G (2010) Environmental decontamination of a hospital isolation room using high-intensity narrow-spectrum light. *J Hosp Infect* 76:247–251
 55. Manzocco L, Da Pieve S, Bertolini A, Bartolomeoli I, Maifreni M, Vianello A, Nicoli MC (2011) Surface decontamination of fresh-cut apple by UV-C light exposure: effects on structure, colour and sensory properties. *Postharvest Biol Technol* 61:165–171
 56. Manzocco L, Dri A, Quarta B (2009) Inactivation of pectin lyase by light exposure in model systems and fresh-cut apple. *Innov Food Sci Emerg Technol* 10:500–505
 57. Manzocco L, Maifreni M, Anese M, Munari M, Bartolomeoli I, Zanardi S, Suman M, Nicoli MC (2014) Effect of pulsed light on safety and quality of fresh egg pasta. *Food Bioprocess Technol* 7:1973–1980
 58. Manzocco L, Nicoli MC (2012) Macromolecular crowding affects protein photosensitivity: the case of egg white immunoreactivity. *Food Chem* 132:982–988
 59. Manzocco L, Nicoli MC (2014) Surface processing: existing and potential applications of ultraviolet light. *Crit Rev Food Sci Nutr* (in press)
 60. Manzocco L, Panozzo A, Nicoli MC (2012) Effect of ultraviolet processing on selected properties of egg white. *Food Chem* 135:522–527
 61. Manzocco L, Panozzo A, Nicoli MC (2013) Effect of pulsed light on selected properties of egg white. *Innov Food Sci Emerg Technol* 18:183–189
 62. Manzocco L, Panozzo A, Nicoli MC (2013) Inactivation of polyphenoloxidase by pulsed light. *J Food Sci* 78:E1183–E1187
 63. Manzocco L, Quarta B, Dri A (2009) Polyphenoloxidase inactivation by light exposure in model systems and apple derivatives. *Innov Food Sci Emerg Technol* 10:506–511
 64. Marahaj R, Arul J, Nadeau P (1999) Effect of photochemical treatment in the preservation of fresh tomato (*Lycopersicon esculentum* Mill cv. Capello) by delaying senescence. *Postharvest Biol Technol* 15:13–23
 65. Marquenie D, Michiels CW, Van Impe JF, Schreven E, Nicolai BN (2003) Pulsed white light in combination with UV-C and heat to reduce storage rot of strawberry. *Postharvest Biol Technol* 28:455–461
 66. Miller AT, Marder RB (1998) Process for strengthening collagen casings using ultraviolet irradiation. Patent 5820812
 67. Miller R, Jeffrey W, Mitchell D, Elasm M (1999) Bacteria responses to ultraviolet light. *Am Soc Microbiol* 65:535–541
 68. Minton KW, Karmin P, Hahn GM, Minton AP (1982) Non-specific stabilisation of stress-susceptible proteins by stress-resistant proteins: a model for the biological role of heat shock proteins. *Proc Nat Acad Sci USA* 79:7107–7111
 69. Moraru CI, Uesugi AR (2009) Pulsed-light treatment. Principles and applications. In: Koutchma TN, Forney LJ, Moraru CI (eds) *Ultraviolet light in food technology. Principles and applications*. Taylor & Francis Group, Boca Raton, pp 235–261
 70. Murray BS, Durga K, Yusoff A, Stoyanov SD (2011) Stabilization of foams and emulsions by mixtures of surface active food-grade particles and proteins. *Food Hydrocoll* 25:627–638
 71. Nakayama A, Shinya R (1981) Ultraviolet sterilization of sugar solution containing spores of obligate anaerobes causing flat sour spoilage. *J Food Hyg Soc Jpn* 22:421–424
 72. Panozzo A (2014). Food biomolecule engineering by unconventional strategies. Ph.D thesis, University of Udine
 73. Park SK, Cho JM, Rhee CO (2003) Effects of ultraviolet radiation on the red colored film of soy protein isolate. *Food Sci Biotechnol* 12:385–389
 74. Pataro G, Muñoz A, Palgan I, Noci F, Ferrari G, Lyng JG (2011) Bacterial inactivation in fruit juices using a continuous flow pulsed light (PL) system. *Food Res Int* 44:1642–1648
 75. Pattison DI, Rahmanto AS, Davies MJ (2012) Photo-oxidation of proteins. *Photochem Photobiol Sci* 11:38–53
 76. Pickering SU (1907) Emulsions. *J Chem Soc* 91:2001–2021
 77. Prutz WA (1990) Free radical transfer involving sulphur peptide functions. In: Chatgililoglu C, Asmus K-D (eds) *Sulfur-centered reactive intermediates in chemistry and biology*. Plenum Press, New York, pp 389–399
 78. Rames J, Chaloupecký V, Sojková N, Bencko V (1997) An attempt to demonstrate the increased resistance of selected bacterial strains during repeated exposure to UV radiation at 254 nm. *Cent Eur J Public Health* 5:30–31
 79. Ramos-Villarreal A, Aron-Maftei N, Martin-Belloso O, Soliva-Fortuny R (2014) Bacterial inactivation and quality changes of

- fresh-cut avocados as affected by intense pulses of specific spectra. *Int J Food Sci Technol* 49:128–136
80. Ramos-Villarroel AY, Martín-Belloso O, Soliva-Fortuny R (2011) Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. *Eur Food Res Technol* 233:395–402
 81. Rhim JW, Gennadios A, Fu D, Weller CL, Hanna MA (1999) Properties of ultraviolet irradiated protein films. *LWT Food Sci Technol* 32:129–133
 82. Sampedro F, Fan X (2014) Inactivation kinetics and photoreactivation of vegetable oxidative enzymes after combined UV-C and thermal processing. *Innov Food Sci Emerg Technol* 23:107–113
 83. Sampedro F, Phillips J, Fan X (2014) Use of response surface methodology to study the combined effects of UV-C and thermal processing on vegetable oxidative enzymes. *LWT Food Sci Technol* 55:189–196
 84. Sastry SK, Datta AK, Worobo RW (2000) Ultraviolet light. *J Food Sci* 65(Supplement):90–92
 85. Sheldon SA, Jones AD, Shibamoto T (1988) Photochemical products of the cysteine/D-glucose browning model system. *J Agric Food Chem* 36:604–606
 86. Silva E, Barrera M (1985) The riboflavin-sensitized photooxidation of horseradish apoperoxidase. *Radiat Environ Biophys* 24:57–61
 87. Smith KC, Hanawalt PC (1969) Molecular photobiology, inactivation and recovery. Academic Press, London
 88. Stevens C, Liu J, Khan VA, Lu JY, Wilson CL, Igwegbe ECK, Kabwe MK, Chalutz E, Droby S (1998) Application of hormetic UV-C for delaying ripening and reduction of *Rhizopus* soft rot in tomatoes: the effect of tomatine on storage rot development. *J Phytopathol* 146:211–221
 89. Stevens C, Wilson CL, Lu JY, Khan VA, Chalutz E, Droby S, Kabwe MK, Haung Z, Adeyeye O, Pusey LP, Wisniewski ME, West M (1996) Plant hormesis induced by ultraviolet light-C for controlling postharvest diseases of tree fruits. *Crop Protect* 15:129–134
 90. Stewart EM (2001) Food irradiation chemistry. In: Molins RA (ed) Food irradiation: principles and applications. Wiley, New York, pp 37–76
 91. Stotter B (1999) UV disinfection in liquid sugar. *Int Sugar J* 101:361–363
 92. Straight RC, Spikes JD (1985) Photosensitized oxidation of biomolecules. In: Frimer AA (ed) Singlet O₂. CRC Press, Boca Raton, pp 91–143
 93. Stutte GW, Edney S, Skerritt T (2009) Photoregulation. *Hortscience* 44:79–82
 94. Takeshita K, Shibamoto J, Sameshima T, Fukunaga S, Isobe S, Arihara K, Itoh M (2003) Damage of yeast cells induced by pulsed light irradiation. *Int J Food Microbiol* 85:151–158
 95. Tomihata K, Burczak K, Shiraki K, Ikada Y (1994) Cross-linking and biodegradation of native and denatured collagen. *Polym Biol Biomed Signif* 540:275–286
 96. Tran MTT, Farid M (2004) Ultraviolet treatment of orange juice. *Innov Food Sci Emerg Technol* 5:495–502
 97. Tuan PA, Thwe AA, Kim YB, Kim JK, Lim S-J, Lee S, Chung S-O, Park SU (2013) Effects of white, blue, and red light-emitting diodes on carotenoid biosynthetic gene expression levels and carotenoid accumulation in sprouts of tartary buckwheat (*Fagopyrum tataricum* Gaertn.). *J Agric Food Chem* 61:12356–12361
 98. U.S. Food and Drug Administration (2000) 21 CFR Part 179. Irradiation in the production, processing and handling of food. *Fed Regist* 65:71056–71058
 99. Uchida K, Kato Y, Kawakishi S (1990) A novel mechanism for oxidative cleavage of prolyl peptides induced by the hydroxyl radical. *Biochem Biophys Res Commun* 169:265–271
 100. von Tappeiner H (1903) Über die wirkung fluoreszierender substanzen auf fermente und toxine. *Ber Dtsch Chem Ges* 36:3035–3038
 101. Wang T, MacGregor SJ, Anderson JG, Woolsey GA (2005) Pulsed ultra-violet inactivation spectrum of *Escherichia coli*. *Water Res* 39:2921–2925
 102. Wekhof A (2000) Disinfection with flash lamps. *PDA J Pharm Sci Technol* 54:264–276
 103. Wrigth JR, Summer SS, Hackney CR, Pierson MD, Zoecklein BW (2000) Efficacy of ultraviolet light for reducing *Escherichia coli* O157:H7 in unpasteurized apple cider. *J Food Prot* 63:563–567
 104. Wu L-Z, Sheng Y-B, Xie J-B, Wang W (2008) Photoexcitation of tryptophan groups induced reduction of disulfide bonds in hen egg white lysozyme. *J Mol Struct* 882:101–106
 105. Xie J, Qin M, Cao Y, Wang W (2011) Mechanistic insight of photo-induced aggregation of chicken egg white lysozyme: the interplay between hydrophobic interactions and formation of intermolecular disulfide bonds. *Proteins* 79:2505–2516
 106. Xing JY, Bai B, Xue WJ, Yang MY (2013) Effect of UV on stability of collagen with consideration of hydration and fibrillogenesis. *Food Sci Biotechnol* 22:1–5
 107. Yang W, Chung S-Y, Ayayi O, Krishnamurthy K, Konan K, Goodrich-Schneider R (2010) Use of pulsed ultraviolet light to reduce the allergenic potency of soybean extracts. *Int J Food Eng* 6:1–12
 108. Yang WW, Shriver SK, Chung SY, Percival S, Correll M-J, Rababah TM (2012) In vitro gastric and intestinal digestions of pulsed light-treated shrimp extracts. *Appl Biochem Biotechnol* 6:1409–1422
 109. Ye Z, Koutchma T, Parisi B, Larkin J, Forney LJ (2007) Ultraviolet inactivation kinetics of *Escherichia coli* and *Yersinia pseudotuberculosis* in annular reactors. *J Food Sci* 72:E271–E278
 110. Zimmerman SB, Trach SO (1991) Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli*. *J Mol Biol* 222:599–620