JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Structural Changes Imposed on Whey Proteins by UV Irradiation in a Continuous UV Light Reactor

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ABSTRACT: The objective of this study was to investigate the structural changes of whey proteins during exposure in a continuous-flow UV reactor. Varying UV irradiation dosages were obtained by controlling the flow rate and the mixing speed. Whey protein isolate (WPI) solutions at concentrations of 1% and 5% (w/v) were circulated at flow rates ranging from 30 to 800 mL·min^{-1}, and changes in physicochemical properties of the proteins were investigated. Intrinsic fluorescence spectra and surface hydrophobicity measurements suggested changes in the tertiary structure of the proteins with UV exposure. The UV treatment also increased the concentration of total and accessible thiol groups in 1% WPI solutions, while no change was measured in 5% WPI solutions. Size-exclusion chromatography demonstrated the formation of UV-induced aggregates and oxidation products (*N*-formylkynurenine and dityrosine) of aromatic amino acids. Furthermore, the UV-induced changes in protein conformation increased the susceptibility of whey proteins to pepsin hydrolysis.

KEYWORDS: *UV irradiation, UV reactor, whey proteins, denaturation*

INTRODUCTION

Short-wave UV irradiation (UV-C, 200−280 nm) is known for its lethal activity against most microorganisms including bacteria and viruses.[1] UV irradiation technology has been successfully used in water disinfection[2] and pasteurization of fruit beverages like cider.[3] However, far-UV light has a limited ability to penetrate liquids, as it is affected by UV-C liquid absorptivity, soluble solutes, and suspended particles.[4] For decades, there has been an interest in UV-C irradiation as a nonthermal technology for pasteurization of milk; however, its success has been limited by the low effective penetration of UV-C light.[5]

Scarce research data are available on the effect of UV irradiation on the physicochemical properties of milk components. Early work has been mainly focused on the effect of UV irradiation on microbial load[5,6] and did not report off-flavors or alterations of the organoleptic properties of treated milk or derived products. A recent study[7] focused mainly on the effect of UV-C irradiation on the chemical and sensory properties of whole goat milk. A UV dose of ∼16 mJ·cm^{-2}, sufficient to achieve a greater than 5 log reduction of *Listeria monocytogenes*, caused off-flavors and increased the concentration of thiobarbituric acid-reactive substances, an indication of lipid oxidation and hydrolytic rancidity.

The available knowledge on the effect of UV irradiation on the structure of milk proteins comes from studies on purified components of whey proteins (*β*-lactoglobulin and human or goat *α*-lactalbumin) subjected to UV light under quiescent conditions.[8−10] Very diluted protein solutions were subjected to a wavelength between 270 and 295 nm and illumination times between 2 and 24 h. These studies reported changes to the structure of whey proteins and increased concentration of free thiol groups with UV exposure; however, the conditions used are far from realistic. In the present study, whey protein solutions were exposed to UV irradiation at 254 nm in a Taylor−Couette-type UV reactor. The Taylor−Couette reactor allows for uniform mixing throughout the fluid without causing mechanical damage to the fluid,[11] which in turn improves UV light penetration and dose distribution. Furthermore, there are no reports on the UV-induced structural changes of mixed whey proteins. A great deal of information on such mixed protein systems comes from heating studies, which indicate that whey protein components behave differently when heated alone than when heated in a mixture. Thus, heating of *α*-lactalbumin and *β*-lactoglobulin separately resulted in no aggregates and large aggregate formation, respectively, whereas heating in the presence of each other brought about formation of heat-induced soluble and insoluble complexes.[12]

The objective of this work was to investigate the effect of UV-C irradiation on the structure of whey proteins in whey protein isolate (WPI) solutions during continuous flow through a Taylor−Couette-type UV reactor designed to minimize poor UV penetration and heterogeneous UV dose distribution.

MATERIALS AND METHODS

Materials. Trizma base, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), and 1-anilinonaphthalene-8-sulfonic acid (ANS) were purchased from Sigma−Aldrich (Oakville, ON, Canada). WPI was obtained from Davisco Food International Inc. The rest of the chemicals were purchased from Fisher Scientific (Mississagua, ON, Canada).

Preparation of WPI Solutions and Treatment in the UV Reactor. WPI solutions (5 L) were prepared to a final concentration of 1% or 5% (w/v) by dissolving the protein in ultrapure water. The solutions were then filtered sequentially through 0.8 and 0.45 *μ*m filters (47 mm diameter, Millipore Corp., Fisher Scientific, Mississauga, ON, Canada). Sodium azide was added at 0.02% concentration to act as a bacteriostatic, and the solution was kept overnight in the refrigerator at 4 °C to allow complete hydration of

Received: January 30, 2012
Revised: May 7, 2012
Accepted: May 25, 2012
Published: May 25, 2012

proteins. The protein content of WPI solutions was measured using DC Bio-Rrad protein assay (Bio-Rad, Hercules, CA). The protein content after filtration was 7.6 ± 0.3 and 38.7 ± 1.5 mg·mL^{-1} for 1% and 5% WPI solutions, respectively, and did not change after UV treatment. The pH of the solutions was measured before and after UV treatment and remained unchanged at pH 7.0 ± 0.1.

WPI solutions were exposed to UV irradiation by use of a Taylor−Couette-type UV reactor from Trojan Technologies (London, ON, Canada), equipped with a FEP Teflon fluid conduit and a static mixing element. The UV lamp, operating at 254 nm, was placed on the inner core of the reactor, and the mixing element could be rotated at various mixing speeds. The UV dosage was varied by changing the flow rate of the liquid through the reactor: a slower flow rate translated into longer residence times and longer times of UV exposure. WPI solutions were pumped through the reactor at 30, 40, 70, 130, and 800 mL·min^{-1} with a variable-speed peristaltic pump Masterflex (Cole-Parmer Instrument Co., Barrington, IL). The UV treatments were named UV 30, UV 40, UV 70, UV 130, and UV 800 to indicate the various flow rates of the solutions. Furthermore, to study the effect of the mixing speed, in another set of experiments the solution of 1% WPI was stirred in the reactor at either 50 or 250 rpm while simultaneously exposed to UV irradiation by being pumped through the reactor at 30 mL·min^{-1}.

Fluorescence Spectroscopy. The fluorescence measurements were performed with a Shimadzu RF-5301PC spectrofluorometer (Shimadzu Corp., Tokyo, Japan). For intrinsic fluorescence measurements, the solutions were exposed to an excitation wavelength of 280 nm and spectra were collected between 300 and 400 nm, with emission and excitation slits of 3 and 5 nm, respectively. Surface hydrophobicity of proteins was determined by titration of WPI solutions with the fluorescent probe ANS according to Bonomi et al.[13] Protein concentration of WPI solutions was adjusted to 0.8 mg·mL^{-1} and appropriate volumes of WPI solution were mixed with ANS stock solution of 1 mM prepared in 20 mM Tris-HCl buffer (pH 7.0) containing 5 mM $CaCl_2 \cdot 2H_2O$ to obtain ANS concentrations of 0.1−20 μM. Spectrofluorometric experiments were carried out with an excitation wavelength of 390 nm and an emission spectrum ranging from 400 to 650 nm, under the same conditions as above. Overall binding capacity of proteins to the probe (the fluorescence intensity at saturating ANS concentration, F_{max}) and the apparent dissociation constant of the protein−ANS complex (K_d^{app}) were obtained from the titration curves by fitting a simple ligand binding equation using SigmaPlot 10 software (Systat Software Inc., San Jose, CA). The protein surface hydrophobicity index (PSH) was calculated as the ratio of F_{max} corrected for protein content over K_d^{app}.

Size-Exclusion Chromatography. Size-exclusion chromatography was employed to determine changes in protein aggregation. The native proteins were estimated by determining the amount of residual soluble protein after acidification. WPI solutions (1 mL) were mixed with 100 μL of acetic acid/sodium acetate buffer (0.5 M, pH 4.7) and centrifuged at 13000*g* for 30 min in an Eppendorf centrifuge (Brinkmann Instruments Inc.). Both nonacidified and acidified solutions were then diluted with phosphate buffer [50 mM NaH_2PO_4 (anhydrous) containing 0.15 mM NaCl, pH 7] to a final protein concentration of 7.1 ± 0.5 mg·mL^{-1}. All the samples were filtered through 0.22 μm membrane filter units (Millipore Corp.), and 100 μL was injected in a Superdex 75 size-exclusion column connected to an AKTApurifier 10 system (GE Healthcare, Uppsala, Sweden). The samples were eluted from the column with 50 mM NaH_2PO_4 containing 0.15 mM NaCl (pH 7) as mobile phase at flow rate of 1 mL·min^{-1}. The elution was followed at 280 nm for detection of proteins and 321 nm for detection of products of oxidation of aromatic amino acids.

Determination of Concentration of Thiol Groups. The concentration of total and DTNB-accessible thiol groups of proteins was determined according to the Ellman assay.[14] Thiol groups react with DTNB to produce 5-thio-2-nitrobenzoic acid (TNB). The concentration of TNB was measured at 412 nm using a UV/vis spectrophotometer (Ultraspec 3100 pro, Biochrom Ltd., Cambridge, U.K.) and an extinction coefficient of 13600 M^{-1}·cm^{-1}. DTNB was prepared by dissolving 10.75 mg DTNB in 10 g of 50 mM Tris-HCl buffer containing 1 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.5), and 0.25 mL of DTNB solution was added in a quartz cuvette along with 50 μL of sample and 2.70 mL of 50 mM Tris-HCl buffer containing 1 mM EDTA (pH 7.5). In the reference cuvette, the sample was replaced with buffer. For measurement of the concentration of total thiol groups, the buffer contained 8 M urea. All samples were measured after they reached a plateau in absorbance, corresponding to 4 h and 10 min for the sulfhydryl groups accessible to DTNB and total sulfhydryl groups, respectively. For the purpose of this paper, the sulfhydryl groups that were accessible to DTNB at pH 7.5 were named accessible sulfhydryl or thiol groups.

Pepsin Hydrolysis. Pepsin from porcine gastric mucosa (Sigma-Aldrich, P7000-25 g, enzyme activity 874 units·mg^{-1}) was dissolved in 0.01 M HCl. The pepsin solution (3 mL) was mixed with WPI samples (2 mL) at final concentration of 1 mg of pepsin·(mg of protein)$^{-1}$. The pH of the mixture was further adjusted to ∼2.2 and the mixture was incubated at 37 °C for 1 h in a shaking water bath. The reaction was stopped by increasing the pH to 7. The sample was filtered through a 0.45 μm Millex-GV filter (Fisher Scientific, Mississauga, ON, Canada) and analyzed by reverse-phase (RP) chromatography to obtain the profile of peptides released during treatment with pepsin. All the samples were diluted to a final protein concentration of 3.0 ± 0.1 mg·mL^{-1}. Reverse-phase HPLC was carried out with a SpectraSystem HPLC (Thermo Fisher Scientific, Mississagua, ON, Canada) by use of a 20 μL loop and a UV detector set at 214 nm. The column (Nova-Pak C18 4 μm, 3.9 × 150 mm with a C18 guard column, Waters Ltd., Mississauga, ON, Canada) was eluted with a nonlinear gradient of 0.1% trifluoroacetic acid in water (buffer A) and 0.1% trifluoroacetic acid in 90% acetonitrile and 10% water (buffer B) at a flow rate of 1 mL·min^{-1}. Gradient conditions were as follows: buffer B concentration increased from 2% to 70% between 0 and 40 min and increased further to 100% B in 1 min, where it was kept constant for 6 min. Then buffer B concentration decreased to 2% in 1 min and was kept constant for 6 min. Total time of the run was 54 min.

Statistical Analysis. All runs were performed in duplicate. The results are reported as mean ± standard deviation. Data were analyzed for significance between means at $P < 0.05$ by use of a general linear model and Tukey's pairwise comparisons. Minitab 15 software (Minitab Inc., State College, PA) was used for the statistical analysis.

RESULTS AND DISCUSSION

Control of UV dosage in the reactor was achieved by varying the flow rate. Although an accurate UV dosage was not determined in these experiments, the flow rates used were within the range that caused 2−4.5 log reduction per milliliter of *Salmonella* phages in model glycerol solutions tested under similar reactor conditions.[15] WPI at two different concentrations was used in the present experiments as model systems because of the low light scattering of its solutions and the well-documented changes in the structure of whey proteins occurring during other processing treatments (i.e., heating).

Figure 1 shows intrinsic fluorescence emission spectra of 1% and 5% WPI solutions, pumped through the UV reactor at flow rates varying from 30 to 800 mL·min^{-1}. This emission spectrum can be considered, to a first approximation, as that of tryptophan amino acid. A shift in the maximum intensity of the fluorescence toward longer wavelengths (red shift) was observed for both solutions as the flow rate decreased (i.e., the UV exposure time increased). In addition, there was a decrease in the intensity of the emission with decreasing flow rate. The red shift was larger for 1% WPI than for 5% WPI solutions. For example, the maximum fluorescence intensity of 1% WPI UV 30 shifted from 327 (in untreated WPI) to 339 nm, corresponding to a red shift of 12 nm, whereas for 5% WPI the shift was only 7 nm from the initial maximum at 327 nm.

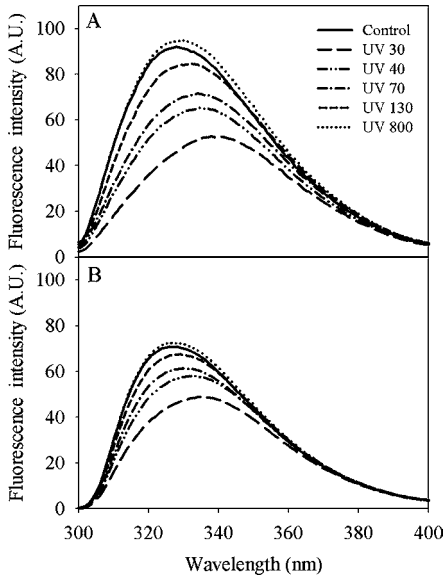

Figure 1. Effect of UV treatment on tryptophan fluorescence spectra of (A) 1% and (B) 5% whey protein isolate solutions. The UV irradiation dosage varied by changing the flow rate of solution. The legend is the same for both graphs; the numbers represent the flow rate in milliliters per minute. The spectra are representative runs.

The red shift in tryptophan fluorescence indicated the increased polarity of the immediate environment surrounding tryptophan, as a result of unfolding of protein structure and increased exposure of the fluorophore to the aqueous solvent.[8−10] Furthermore, the decrease of fluorescence intensity in the treated samples could be attributed to quenching of tryptophan fluorescence, again indicating a structural change, aggregation, or oxidation of the protein with increased UV exposure.[8,16] Tryptophan fluorescence can be quenched by water molecules[17] and peptide bonds[18] as well as side-chain amino acids, with tyrosine, cysteine, positively charged hystidine, and cystine being the best quenchers.[19] Another possible cause for the decrease in the intensity of intrinsic fluorescence may have been the oxidation of tryptophan and formation of N-formylkynurenine.[8,16] By comparison of the spectra in Figure 1, it was possible to conclude that UV irradiation had a greater effect on 1% WPI than 5% WPI solutions, possibly because of the lower penetration of UV light in the higher concentration samples.

The effect of UV irradiation on the concentration of accessible thiol groups is shown in Table 1. The UV treatment on 1% WPI solutions increased the concentration of total SH groups in the samples subjected to higher UV dosages (UV 30 and UV 40). It has been previously reported that electron transfer from the excited tryptophan amino acid to a neighboring disulfide bond could lead to cleavage of the disulfide bridge and a higher content of total thiols.[10] The increase in concentration of total thiol groups of UV 30 and UV 40 samples was accompanied by a significant increase in the concentration of accessible sulfhydryl groups compared to untreated 1% WPI control (Table 1). Previous studies[9,10,20] showed an increase in accessible SH groups in UV-treated cutinase, human α-lactalbumin, and goat α-lactalbumin. Although there was a clear increase in total and accessible SH in 1% WPI samples, there were no significant changes in the concentration of both total and accessible thiol groups in 5% WPI solutions, regardless of the flow rate used. The reduced effect in the more concentrated protein solution may be related to the lower penetration of UV irradiation in these samples compared to the diluted WPI solution.

Exposure of milk whey proteins to UV irradiation may cause structural changes that could affect the distribution of hydrophobic regions of proteins. Overall protein surface hydrophobicity (PSH) can be determined by analyzing the maximum fluorescence intensity of proteins as a function of ASN concentration, which fluoresces when in a hydrophobic environment.[13] The values of PSH are shown in Figure 2 panels A and B for 1% and 5% WPI solutions, respectively. In accordance with the increased red shift and changes in thiol group concentration, in the case of surface hydrophobicity, a significant change in 1% WPI solutions pumped through the UV reactor at 30, 40, and 70 mL·min^{-1} was also observed (Figure 2A). The decrease in surface hydrophobicity in 1% WPI was to a certain extent due to aggregation of proteins caused by longer exposure to UV irradiation of UV 30, UV 40 and UV 70 samples compared to control. In these samples, F_{max} (fluorescence intensity at the saturation concentrations of ANS) and K_d^{app} (apparent dissociation constant of protein−ANS complex) increased in comparison to control untreated samples. During UV treatment, proteins may unfold, causing the exposure of hydrophobic sites that become accessible to ANS, causing the increase in F_{max} values. However, the increase of K_d^{app} indicated a decrease in the affinity of hydrophobic sites for the probe, which may result from a different distribution of hydrophobic residues on the protein surface.[21] In contrast to what was observed in Figure 2A, in the case of 5% WPI, the values of PSH were not significantly affected by exposure to UV light in comparison to the untreated samples (Figure 2B). These results once again confirmed the reduced effect of UV treatment on the more concentrated WPI solutions.

The aggregation behavior of whey proteins induced by UV treatment was followed by analyzing the solutions via size-

Table 1. Concentration of Total and DTNB-Accessible Sulfhydryl Groups of Whey Proteins as Affected by UV Treatment[a]

	1% WPI		5% WPI	
treatment (mL·min^{-1})	total SH (μmol·g^{-1})	accessible SH (μmol·g^{-1})	total SH (μmol·g^{-1})	accessible SH (μmol·g^{-1})
control	40.9 ± 3.5 a	24.2 ± 3.5 a	38.8 ± 0.5 a	23.8 ± 3.2 a
UV 30	49.9 ± 1.9 b	33.5 ± 3.1 b	39.8 ± 0.4 a	27.5 ± 2.0 a
UV 40	50.0 ± 1.8 b	33.9 ± 1.4 b	37.8 ± 0.5 a	25.7 ± 3.4 a
UV 70	44.1 ± 2.6 a	28.3 ± 1.3 a	39.8 ± 1.0 a	26.7 ± 2.1 a
UV 130	43.7 ± 1.7 a	27.7 ± 1.6 a	37.9 ± 0.9 a	24.6 ± 2.5 a
UV 800	42.9 ± 0.7 a	26.4 ± 1.2 a	37.8 ± 2.4 a	24.7 ± 3.0 a

[a]UV dosage was varied by changing the flow rate of the solution through the reactor. DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); WPI, whey protein isolate. Different letters within a column indicate statistically significant differences at $P \leq 0.05$.

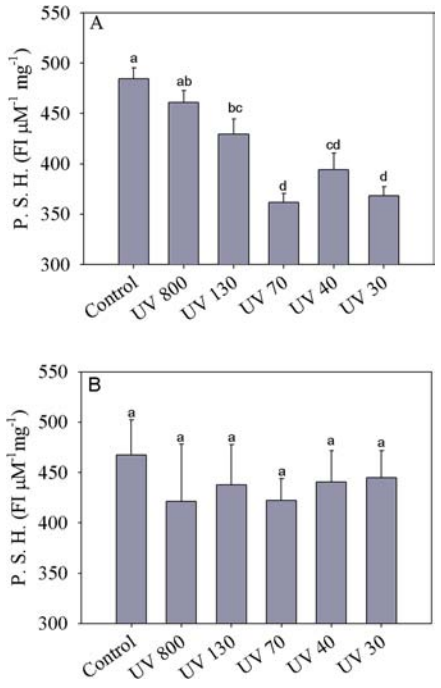

Figure 2. Protein surface hydrophobicity index (PSH) of (A) 1% and (B) 5% whey protein isolate solutions as a function of UV irradiation treatment. Different letters indicate statistically significant differences ($P < 0.05$).

exclusion chromatography. The protein solutions were eluted with or without prior separation of the soluble aggregates with acidification. Figure 3 illustrates the differences in the elution chromatograms for 1% WPI solutions kept at neutral pH (panel A) or after treatment with sodium acetate buffer at pH 4.7 (panel B). Figure 3A shows some nonnative monomers as well as small aggregates of whey proteins together with a native β-lactoglobulin peak eluting at 10.6 mL and a native α-lactalbumin peak eluting at 12.5 mL. After acid precipitation, the supernatants that were analyzed contained only the native protein fractions (Figure 3B), in agreement with previous research on heat-induced whey protein aggregation.[22]

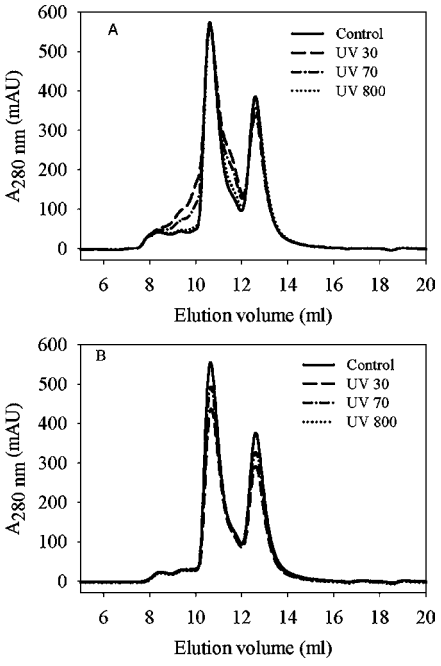

Figure 3. Size-exclusion chromatography profiles of WPI 1% solution obtained at 280 nm for control and UV-treated samples (A) as is and (B) treated with sodium acetate buffer, pH 4.7. The UV irradiation dosage was varied by changing the flow rate of solution. Numbers in the legend represent the flow rate in milliliters per minute. The chromatograms are representative runs.

As UV dosage increased, the amount of aggregates eluting before 10 mL increased, clearly demonstrating UV-induced aggregation of the whey proteins. Furthermore, the shoulder present between the two native protein peaks also increased with the extent of UV treatment (Figure 3A). This intermediate shoulder may be an indication of the formation of nonnative monomers of β-lactoglobulin or α-lactalbumin, which precipitated after treatment with acetate buffer (Figure 3B). These results were in agreement with previous work that reported the formation of α-lactalbumin dimers after UV treatment.[9] In agreement with the results shown above, the elution profiles of 5% WPI solutions treated by UV light were similar to those of control samples either before or after treatment with acidic buffer (data not shown).

To determine if there were changes in the amount of native monomer proteins with UV treatment, the total area of the peaks was quantified after elution of protein solutions acidified or not with pH 4.7 buffer. Figure 4 illustrated the fraction of the acid-soluble proteins (determined as the ratio of the area of proteins soluble at pH 4.7 to the area of proteins nontreated with sodium acetate buffer, measured at 280 nm) as it was affected by UV light dosage. In the case of 5% WPI solutions, there was no significant decrease in the amount of native proteins eluted as a function of UV treatment. These results were in agreement with the lack of significant changes in the surface hydrophobicity and concentration of thiol groups of 5% WPI mentioned previously. On the other hand, Figure 4 indicated that, in the case of 1% WPI, the solution pumped through the reactor at a flow rate of 30 mLmin^{-1} lost 29% of the native whey protein. As the flow rate increased (i.e., the length of UV exposure decreased), the fraction of denatured protein decreased until, for UV 800 sample, it became comparable to that of control.

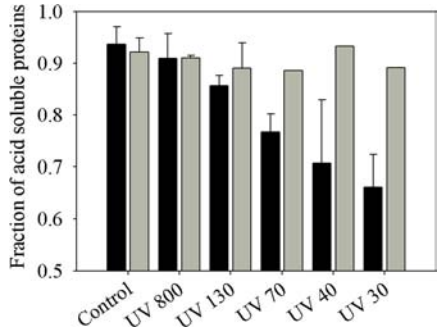

Figure 4. Acid-soluble protein fraction of whey protein isolate (WPI) solutions as a function of UV irradiation treatment. Black bars represent 1% WPI and gray bars represent 5% WPI solutions.

Modifications of proteins exposed to UV irradiation result mostly from the absorption of light from aromatic residues tryptophan, tyrosine, and phenylalanine. At 254 nm, the relative absorption coefficients of tryptophan, tyrosine, and phenylalanine are 1, 0.11, and 0.05, respectively,[23] which indicates that

tryptophan has higher probability to undergo changes due to irradiation than the other aromatic residues. Among the products of photoxidation of these residues, *N*-formylkynurenine and dityrosine (oxidation products of tryptophan and tyrosine, respectively) show an absorption maximum at 321 nm.[8,24] Therefore, indication of the presence of these compounds can be obtained following the absorbance at 321 nm in size-exclusion chromatography determinations. Figure 5

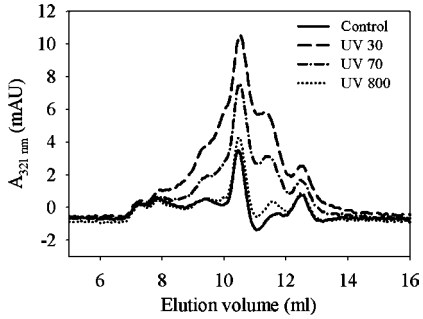

Figure 5. Size-exclusion chromatography profiles of 1% WPI solution obtained at 321 nm for the detection of *N*-formylkynurenine and dityrosine, products of oxidation of tryptophan and tyrosine, respectively. The UV irradiation dosage varied by changing the flow rate of solution through the reactor. The legend represents the flow rate in milliliters per minute. The chromatograms are representative runs.

illustrates the chromatographic elution peak for 1% WPI solutions obtained at 321 nm. The absorbance at 321 nm greatly increased with UV exposure, clearly confirming the formation of oxidation products of tryptophan and tyrosine. Similar profiles were also obtained for 5% WPI solutions, albeit lower in magnitude compared to the WPI 1% solution, suggesting that the oxidation of tryptophan and tyrosine occurred in 5% WPI solutions as well, explaining at least in part the decrease in intensity of the intrinsic fluorescence spectrum for these solutions (Figure 1B).

Another approach to the evaluation of structural changes in whey proteins is determination of the susceptibility of WPI proteins to pepsin hydrolysis. For this reason, 1% and 5% WPI solutions treated at 30 and 70 mL·min^{-1} (i.e., the highest dosage of UV light) were hydrolyzed with pepsin and the elution profiles of the resulting solutions were compared with those of untreated control samples. Figure 6 shows the peptide profile eluted by reversed-phase HPLC of a 1% WPI

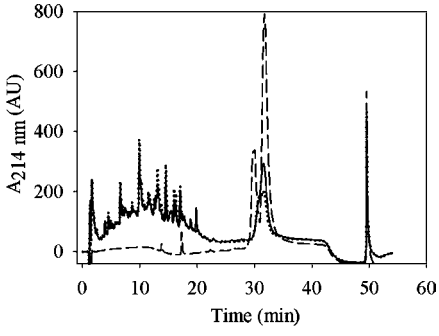

Figure 6. RP-HPLC profiles of WPI solutions (protein content 3 mg·mL^{-1}). (– – –) Control WPI not treated with UV or pepsin; (—) control that was treated with pepsin but not UV; (···) sample treated with UV at flow rate 30 mL·min^{-1}, followed by pepsin treatment.

unhydrolyzed sample, as well as untreated and UV 30-treated samples hydrolyzed with pepsin. The untreated, unhydrolyzed protein showed two main elution peaks, corresponding to α-lactalbumin and β-lactoglobulin, eluting at 29.8 and 31.7 min, respectively. Both whey proteins were hydrolyzed when the control solution was treated with pepsin, with α-lactalbumin being almost completely hydrolyzed (a small part of the peak overlapped with α-lactalbumin) while β-lactoglobulin, which is reported to be more resistant to the enzyme,[25] was further hydrolyzed after exposure to UV irradiation (Figure 6). Pepsin hydrolysis reduced the total area of both whey protein peaks by 64.3% ± 0.5% in the control 1% WPI solution and by 72.8% ± 1.4% in the UV 30 1% WPI solution. In the hydrolyzed samples, a large population of peptides appeared in the elution chromatogram (eluting in the first 25 min) with no distinct differences between the UV-treated and untreated samples. Similar results were obtained for the UV 70 sample, indicating a greater susceptibility of the UV-irradiated samples to pepsin hydrolysis. On the other hand, the reduction in the area of two main proteins in the 5% WPI sample was not significantly different from that of control samples. It was therefore concluded that, in 1% WPI solutions, protein unfolding assisted by disulfide bond cleavage made more peptide bonds accessible to pepsin and increased the susceptibility of whey proteins to pepsin hydrolysis.[25]

The effect of varying the mixing speed in the reactor was evaluated besides the influence of flow rate of the solution through the reactor. In this case, only 1% WPI solution with a flow rate of 30 mL·min^{-1} was employed since the longest exposure time exhibited the greatest changes in protein structure due to UV irradiation (see above). The WPI solution was stirred at 50 or 250 rpm while passing through the reactor. No significant differences were found in the magnitude of the red shift and intensity of tryptophan fluorescence spectra, total and accessible thiol group concentration, hydrophobicity, and degree of protein aggregation as well as pepsin hydrolysis in samples stirred either at 50 or 250 rpm when compared with the unstirred samples. These results indicated that, in the case of WPI solutions, the flow rate was the most important factor, as the solutions had low viscosity and stirring did not increase the extent of exposure to the UV light.

The present study demonstrated that denaturation of whey proteins occurred upon exposure to UV irradiation. Significant changes in the tertiary and quaternary structure of whey proteins, accessibility of thiol groups, and oxidation of tryptophan and tyrosine were observed to a greater extent for 1% WPI than 5% WPI solutions. This may be due to the higher turbidity and lower penetration of UV irradiation in the 5% WPI solution. In all cases, the extent of protein denaturation due to UV irradiation was low when compared to ultra-high-temperature (UHT) or high-temperature short-time (HTST) heating treatments.[12,22] Furthermore, in the case of whey protein solutions, the extent of mixing in the reactor did not show any effect on the efficacy of the treatment. As more data become available on the effect of UV irradiation on microbial inactivation, further research is needed to assess whether a desirable pasteurization can be achieved under conditions of low UV irradiation exposure that, simultaneously, minimizes the degree of whey protein denaturation.

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Funding

We acknowledge financial support from OMAFRA Food Research Program.

Notes

The authors declare no competing financial interest.

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