JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Pulsed-Light Treatment on Milk Proteins and Lipids

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Pulsed-light treatment offers the food industry a new technology for food preservation. It allows the inactivation of numerous micro-organisms including most infectious foodborne pathogens. In addition to microbial destruction, one can also question whether pulsed-light treatment induced conformational changes in food components. To investigate this question, the influence of pulsed-light treatment on protein components of milk was evaluated by using UV spectroscopy, spectrofluorometry, electrophoresis, and determination of amino acid composition. Pulsed-light treatment resulted in an increase of UV absorbance at 280 nm. The intrinsic tryptophan fluorescence of β-lactoglobulin (BLG) showed a 7 nm red shift after 10 pulses. SDS-PAGE showed the formation of dimers after treatment of BLG by 5 pulses and more. No significant changes in the amino acid composition of proteins and lipid oxidation were observed after pulsed-light treatment. The obtained results indicated changes in the polarity of the tryptophanyl residue microenvironment of BLG solutions or changes in the tryptophan indole structure and some aggregation of studied proteins. Hence, pulsed-light treatment did not lead to very significant changes in protein components; consequently, it could be applied to process protein foods for their better preservation.

KEYWORDS: Pulsed-light process; milk proteins; composition; food application

INTRODUCTION

Food contamination is of major concern for food industries because of the foodborne illnesses that can result from it. Several technologies aiming at reducing or eliminating the microbiological risks associated with contaminated foods have been already evaluated. A variety of food preservation methods are available, including heating, dehydration, freezing, and adding preservatives. Although these technologies can produce safe foods, heating and freezing may contribute to the degradation of some components, which can impair food quality. To prevent undesirable heat effects on foods, major efforts have been made to develop nonthermal technologies, which can produce safe but minimally processed foods with satisfactory nutritional and organoleptic qualities (*1*). Among these emerging technologies, the most promising include high-pressure treatment, the use of pulsed-electric fields, and the application of pulsed light (*2*). The engineering aspects of pulsed-light applications in the context of food processing, safety, and quality are currently being studied extensively (*3*–*5*).

Intense light pulsing is a technique performed to decontaminate food surfaces by killing micro-organisms, using short pulses of intense flashes of broad spectrum light ranging from 200 nm (UV) to 1000 nm (near-infrared region). Pulsed light is produced using technologies that multiply the flash power many-fold. Power is magnified by storing electrical energy in a capacitor over relatively long times (fractions of second) and realizing it in a xenon lamp in a short time (10^{-6} or 10^{-3} s). The limited energy cost of pulsed light and its great flexibility and speed are some of the major interests of the technique. It has potential applications for the treatment of foods, packages, medical devices, and packaging and processing equipment for the food, medical, and pharmaceutical industries and water and air (*6*). The mode of action of pulsed-light process is due to the effect of the short pulse width, the high peak power, and the broad

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10.1021/jf0729964 CCC: $40.75
Published on Web 02/22/2008

spectrum of the flash (*7, 8*). Light pulses may be used to reduce or eliminate the need for chemical disinfectants and preservatives. Other advantages of pulsed light are the lack of residual compounds and the absence of applied chemicals that can cause ecological problems and/or are potentially harmful to humans. This method is capable of reducing the microbial populations, vegetative cells, and spores on the food surfaces, with remarkable rapidity and effectiveness. Although its capacity to inactivate micro-organisms in vitro or in food products has been extensively studied (*4, 8–14*), there are no literature data on the effects of pulsed-light treatment on food and food ingredients of interest for the food industry. The functional properties of food proteins are those physicochemical properties that affect the behavior of proteins in food systems during preparation, processing, storage, or consumption. These characteristics influence the quality and organoleptic attributes of foods. The aim of this study was to study whether pulsed-light treatment has an impact (i) on the conformational structure and composition of milk proteins studied by UV spectroscopy, spectrofluorometry, electrophoresis, and amino acids composition and (ii) on lipid oxidation and, consequently, to determine if such a technology is accurate for potential food applications.

MATERIALS AND METHODS

Sample Preparation. β-Lactoglobulin (BLG) and α-lactalbumin (ALA) were prepared according to the method of Mailliart and Ribadeau Dumas (*15*). Sodium caseinate and delactosed whey proteins were obtained from Lactalis (Laval, France). Skim milk powder was from Merck (Darmstadt, Germany). UHT delactosed skim milk ("Matin léger") was from Candia. UHT whole milk was obtained from a local supermarket. Milk proteins (BLG, sodium caseinate, whey proteins) were dissolved in distilled water (4 mg/mL). Skim milk was reconstituted by dissolving milk powder in distilled water (10%, w/v).

Before the study of the effect of pulsed-light treatment on peptides, a solution of sodium caseinate (8 mg/mL 25 mM Tris-HCl buffer, pH 7.0) and UHT delactosed skim milk was hydrolyzed. Hydrolysis was performed by adding 0.25 and 1 mL of TPCK-trypsin (EC 3.4.21.4; 10000 BAEE U/mg) and proteinase K (EC 3.4.21.64; 30 U/mg) (Sigma-Aldrich, Saint Quentin Fallavier, France; initial concentration = 10 mg/mL), respectively, to 30 mL of sodium caseinate solution and UHT delactosed skim milk. Hydrolysis was performed for 17 h at 37 °C. Hydrolysates were stocked at −20 °C before pulsed-light treatment.

Pulsed Light Treatment. The pulsed-light generator developed by the society Claranor (La Colombière, France) was used in this study. The pulsed-light system is composed of a power supply unit and a flash lamp. In this apparatus, the treatment chamber of the samples is made in a rectangular parallelepiped unit (width = 60 cm, height = 45 cm, depth = 25 cm,) containing one cylindrical xenon lamp and an air cooling system. This system produces a broad spectrum of pulsed light in the range of 180–1100 nm. Each flash is of a very short duration of 0.3 ms.

Samples of protein solutions, milk, and hydrolysates were treated with pulsed light at a distance of 4 cm from the xenon lamp, at an energy of 2.2 J/cm^2, and with 1, 3, 5, 7, and 10 pulses. After exposure to pulsed light, treated and untreated samples (controls) were studied.

Analysis of Proteins Treated by Pulsed Light. *UV Spectra.* After the pulsed-light treatment, the UV spectra of treated and untreated (control) samples were measured using a spectrophotometer (Cary 13, Varian) at room temperature from 220 to 400 nm.

Intrinsic Fluorescence Spectra. Conformational changes of protein solutions (BLG, ALA, and sodium caseinate) were monitored by intrinsic tryptophan fluorescence spectra. Skim milk was analyzed by frontal fluorescence.

Intrinsic fluorescence was assayed using an excitation wavelength of 295 nm and measuring emission wavelength between 305 and 390 nm. Fluorescence study was performed on a Hitachi F-4500 spectrofluorometer, and fluorescence intensity was expressed as arbitrary units (au).

Tricine−Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (Tricine-SDS-PAGE). To determine the effect of pulsed light on proteins, Tricine-SDS-PAGE was carried out on 10% acrylamide gels as described by Schägger and von Jagow (*16*). The protein samples were analyzed using a Mini-Protean II Gel Electrophoresis (Bio-Rad, Hercules, CA). The samples were boiled for 5 min before application. Electrophoresis was performed at an intensity of 10 mA in the stacking gel and at 20 mA in the running gel. The gels were stained with Coomassie Blue (R250). SDS-PAGE were performed in the presence or absence of 2-mercaptoethanol (ME). A low molecular mass protein marker (Bio-Rad) was used as standard.

Amino Acid Analysis. The proteins, before and after pulsed-light treatment, were hydrolyzed under vacuum in the presence of constant-boiling 6 N HCl for 24 h at 110 °C in a Pico-Tag station (Waters) as described by Bidlingmeyer et al. (*17*). After acid hydrolysis, the amino acids were derivatized with phenyl isothiocyanate (PITC) and quantified by reversed phase high-performance liquid chromatography (RP-HPLC) on a Pico-Tag C_{18} column (3.9 mm × 15 cm) as previously described (*18*).

Analysis of Treated Hydrolysates by Pulsed Light. After pulsed-light treatment, samples were analyzed by RP-HPLC on a Jupiter Proteo 90 A column (Phenomenex, Interchim, Montluçon, France) with an HPLC apparatus (Alliance system, Waters, Milford, MA). The column was equilibrated with solvent A [H_2O, trifluoroacetic acid (TFA) 0.11%]. After injection of 20 μL of sample, peptides were eluted by a 0–100% solvent B gradient (20% H_2O, 80% acetonitrile, 0.09% TFA, v/v/v). Detection of peptides was performed at 220 nm (spectrophotometer model 996, Waters).

Analysis of Lipid Components after Treatment of Milk by Pulsed Light. To determine the effect of pulsed light on lipid oxidation and the possible interaction between lipids and proteins, whole milk was analyzed by frontal fluorescence using a Hitachi F-4500 spectrofluorometer before and after pulsed-light treatment. Two spectra of excitation–emission have been performed on each sample of whole milk treated or not with pulsed light.

The first spectrum was obtained by measuring the emission from 280 to 450 nm, every 2 nm, with an excitation from 260 to 350 nm, every 10 nm, at a speed of 2400 nm/min. Split widths were of 5 nm for excitation and 2.5 nm for emission, with a voltage of 700 V.

The second spectrum of excitation–emission was obtained by measuring the emission spectra between 380 and 600 nm, every 5 nm, with an excitation from 300 to 470 nm, every 10 nm, at a speed of 2400 nm/min. Split widths were of 10 nm for excitation and 5 nm for emission, with a voltage of 950 V.

RESULTS AND DISCUSSION

Pulsed light is a novel food preservation method effective for microbial inactivation. However, to apply such a process, it is essential to determine the potential effects of pulsed-light treatment on food components. In all experiments, UV absorbance, intrinsic tryptophan fluorescence, aggregation, and amino acid composition of milk proteins after pulsed-light treatment at an energy of 2.2 J/cm^2 were determined.

Effect of Pulsed-Light Treatment on Proteins. *UV Spectra.* UV spectra of pulsed-light-treated proteins (BLG, ALA, and sodium caseinate) were studied after pulsed-light processing. Changes in UV spectra of studied proteins treated with different numbers of pulses at 2.2 J/cm^2 of energy are shown in **Figure 1**. The maximum absorbance at 280 nm (A_{280}) of BLG (**Figure 1A**) and ALA (**Figure 1C**) increased gradually with a rise in intensity of pulsed-light treatment, mainly resulting from an aggregation of proteins. In the case of sodium caseinate (**Figure 1B**), a slight change in maximum A_{280} was observed. These results are in agreement with those obtained for soybean glycinin treated by high pressure for which the spectra intensities of soybean glycinin increased with the increase of applied pressure (*19*).

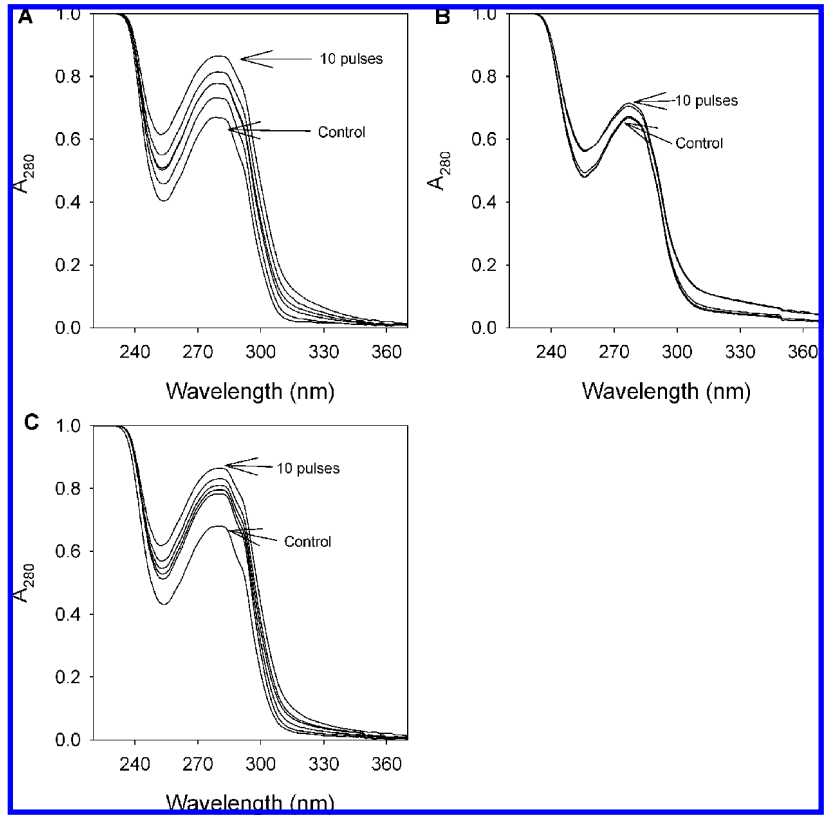

Figure 1. UV spectra of (**A**) β-lactoglobulin, (**B**) sodium caseinate, and (**C**) α-lactalbumin before and after treatment until 10 pulses with pulsed light at a distance of 4 cm from the xenon lamp and at an energy of 2.2 J/cm^2.

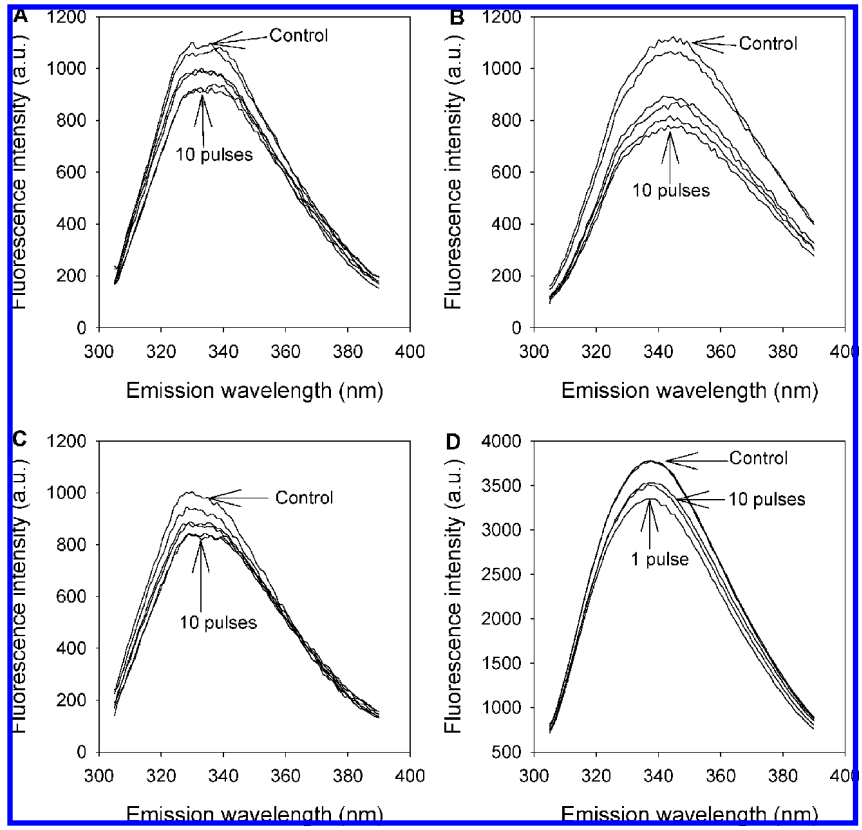

Figure 2. Fluorescence spectra of (**A**) β-lactoglobulin, (**B**) sodium caseinate, (**C**) α-lactalbumin, and (**D**) skim milk before and after treatment until 10 pulses with pulsed light at a distance of 4 cm from the xenon lamp and at an energy of 2.2 J/cm^2.

Spectrofluorometry Study. The recorded protein fluorescence emission maximum is characteristic of tryptophanyl residues placed in a relatively hydrophobic environment. **Figure 2** illustrates the intrinsic tryptophan fluorescence spectra of untreated (controls) and treated proteins by various numbers of pulses (1, 3, 5, 7, and 10).

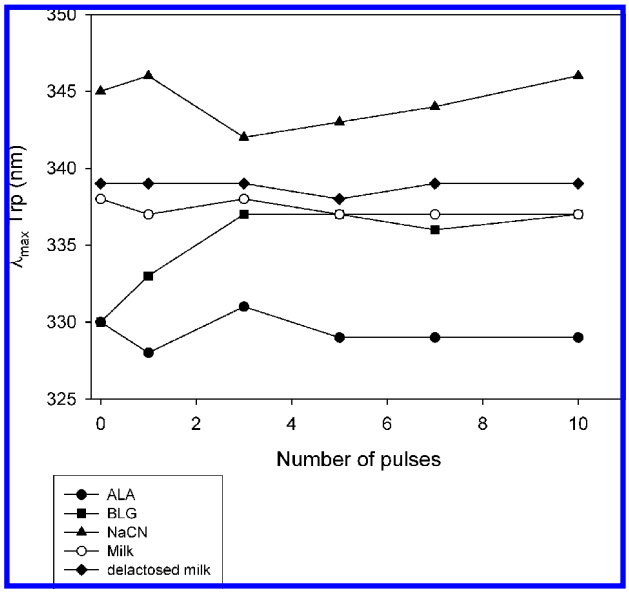

Figure 3. Variation of maximum emission wavelength of tryptophan (λ_{max} Trp) of milk protein solutions after pulsed-light treatment (●, α-lactalbumin; ■, β-lactoglobulin; ▲, sodium caseinate; ○, skim milk; ◆, delactosed milk). Results are the mean of three determinations; the error did not exceed the size of the data symbols.

In the case of BLG, the fluorescence intensity showed a small decrease and a red shift was observed after pulsed-light treatment, which indicates changes in the polarity of the microenvironment of tryptophanyl residues of the protein from a less polar to a more polar environment (**Figure 2A**). Pulsed-light treatment by 1 pulse resulted in a 3 nm red shift of the maximum emission wavelength (from 330 to 333 nm). After treatment with up to 10 pulses, the maximum emission wavelength shifted from 333 to 337 nm (**Figure 3**). This 7 nm red shift of BLG after pulsed-light treatment is similar to the results of high hydrostatic pressure treatments of BLG, showing 4 nm (*20*) and 13 nm (*21*) red shifts. Such red shifts indicate a change in the polarity of the tryptophan environment, from a less hydrophilic environment to a more hydrophilic environment. It may be a sign of light-induced folding changes or modification of the tryptophan indoles.

In the case of ALA (**Figure 2C**), no effect was observed up to 3 pulses. After treatment with 4 and 5 pulses, a decrease of maximum intensity of fluorescence as well as a 3 nm red shift (from 326 to 329 nm) was observed.

A decrease in fluorescence intensity was observed also in the case of sodium caseinate (**Figure 2B**) but without a shift of the maximum emission wavelength (**Figure 3**). The analysis of skim milk by frontal fluorescence showed no difference between treated and untreated samples (**Figure 2D**). The same result was obtained with delactosed milk (data not shown). The results of both UV spectroscopy and fluorescence indicate an alteration of milk proteins spectra after treatment by pulsed light. These modifications are mainly due to protein aggregation. However, tryptophanyl residues may be also affected by this treatment. Puppo et al. (*22*) and Li et al. (*23*) observed an aggregation of soybean proteins treated by high pressure and pulsed electric fields, respectively.

SDS-PAGE. The electrophoretic profiles of protein samples treated with different numbers of pulses (1, 3, 5, 7, and 10) were determined. Protein samples were analyzed in the absence or presence of ME, which allowed evaluation of the contribution of disulfide-stabilized aggregates of both untreated and treated protein solutions by pulsed light (**Figure 4**).

After a pulsed-light treatment of BLG with 1 and 3 pulses, no difference among treated and untreated samples can be observed. However, after 5, 7, and 10 pulses treatments, formation of dimers was observed corresponding to a band of ~36 kDa (**Figure 4A**). In the presence of ME, there was no difference among treated and untreated proteins (**Figure 4B**). The bands of ~36 kDa disappeared in the presence of ME, demonstrating that the aggregation was due to the formation of disulfide bonds. Funtenberger et al. (*24, 25*) showed that aggregation of BLG induced by high pressure is correlated to formation of S−S bonds through SH/S−S interchange reactions and formation of hydrophobic interactions. Liu et al. (*20*) also reported the presence of dimers of BLG after high hydrostatic pressure treatment, which may indicate the presence of a small quantity of nonreducible dimers after high hydrostatic pressure and pulsed-light treatments. It has been reported that BLG, the main protein of whey proteins, is more sensitive to high-pressure treatment (*26, 27*). These studies indicated that pressure treatment has a notable effect on conformational and aggregation properties of proteins.

In contrast, no difference could be observed in the case of sodium caseinate and whey proteins before and after pulsed-light treatment (**Figure 4C**,**D** and **E**,**F**). No modification appeared either after treatment of ALA (data not shown). It has been reported that most of the functional properties of caseins or sodium caseinate are largely unaffected by heating or high-pressure treatment (*28*). In addition, under conditions where BLG-stabilized emulsions become extensively aggregated, equivalent emulsions stabilized by sodium caseinate remain unchanged after high hydrostatic pressure treatment (*29*).

Amino Acid Composition. The amino acid compositions of untreated and treated proteins are shown in **Table 1**. No significant quantitative changes were observed even after 10 pulses. The amount of tryptophan, which is acid-labile, could not be determined by this method; however, it was shown by spectrofluorometry and UV spectroscopy methods that tryptophan was detected. Similarly, the total content of amino acids did not undergo modification after treatment of a vegetable beverage by pulsed electric fields (*30*).

Analysis of skim milk by UV spectroscopy, fluorescence, and SDS-PAGE and by determination of amino acid composition did not show any difference after treatment by pulsed light. Milk is naturally rich in reducing sugars (49 g/L) and proteins (33 g/L), which makes it a food sensitive to Maillard reaction (*31, 32*). In milk, this reaction is undesirable because it decreases its nutritional value. Analysis of the amino acid composition of lactosed milk (**Table 2**) before and after treatment by pulsed light does not show any modification, particularly in lysyl residues content [main sites of Maillard reaction (*33*)]. Maillard reaction was not observed either after treatment of milk by high pressure (*34*).

Pulsed-Light Effect on Peptide Hydrolysates. RP-HPLC profiles of hydrolysates of sodium caseinate (**Figure 5**) and of skim milk (data not shown) untreated and treated by pulsed light with 1, 5, or 10 pulses showed that there was no significant difference between profiles of untreated and pulsed-light-treated hydrolysate samples. These results demonstrate that pulsed light has no effect on small peptide fragments.

Effect of Pulsed Light on Lipids. Whole milk was analyzed by frontal fluorescence. A main peak corresponding to an excitation maximum at 280 nm and an emission maximum at about 334 nm was observed. The extraction of emission spectra after excitation at 280 nm (corresponding to excitation spectra

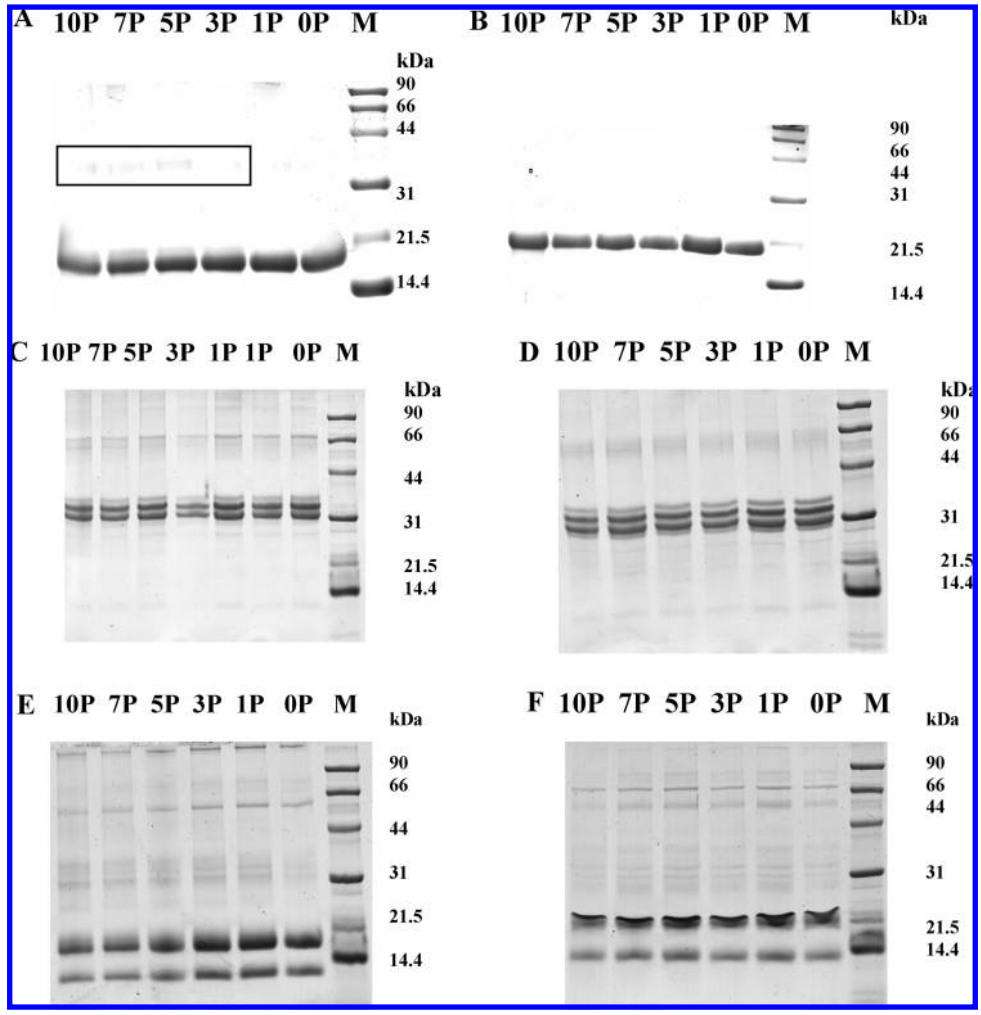

Figure 4. SDS-PAGE profiles of untreated and pulsed-light-treated samples with 0, 1, 3, 5, 7, and 10 pulses (0P−10P): β-lactoglobulin without (**A**) and with (**B**) 2-mercaptoethanol; sodium caseinate without (**C**) and with (**D**) 2-mercaptoethanol; whey proteins without (**E**) and with (**F**) 2-mercaptoethanol. M, low molecular mass markers, phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean tryptic inhibitor (21.5 kDa), and lysozyme (14.4 kDa). Bands corresponding to dimer formation are boxed in (**A**).

Table 1. Comparison of Amino Acid Compositions of Milk Proteins before and after Pulsed-Light Treatment

	β-lactoglobulin			sodium caseinate			α-lactalbumin		
	control	1 pulse	10 pulses	control	1 pulse	10 pulses	control	1 pulse	10 pulses
Asx (Asn + Asp)	9.71	9.28	9.62	6.56	7.12	6.77	10.2	10.6	10.4
Glx (Gln + Glu)	15.2	14.9	15.3	19.3	19.2	18.8	14.7	14.7	14.8
Ser	4.2	4.24	4.28	6.69	6.52	6.63	5.24	5.32	5.3
Gly	2.5	2.53	2.43	3.13	3.03	3.11	3.09	2.97	3.14
His	1.39	1.62	1.52	2.41	2.41	2.28	1.88	1.43	1.86
Arg	1.88	2.02	1.9	2.43	2.63	2.64	2.02	1.83	2.01
Thr	4.98	4.87	4.92	4.19	4.39	4.59	5.23	5.27	5.25
Ala	5.21	5.03	5.18	4.06	4.13	4.23	6.97	7.09	6.89
Pro	5.21	5.03	5.18	10.9	10.7	11	5.17	5.43	5.38
Tyr	2.1	2.37	2.14	3.44	3.47	3.11	2.51	2.35	2.4
Val	6.17	6.19	6.2	7.43	6.95	7.08	6.25	6.14	6.2
Met	2.49	2.5	2.47	2.52	2.39	2.42	2.06	2.06	2.05
Cys	1.64	2.04	1.39	0	0	0	2.13	2.1	1.78
Ile	5.64	5.86	5.71	5.18	5.12	5.25	5.83	5.68	5.82
Leu	14.2	14.2	14.3	9.55	9.35	9.6	13	13.1	13.1
Phe	2.74	2.75	2.76	4.28	4.14	4.2	3.1	3.1	3.12
Lys	11.2	11.2	11.3	8.01	8.47	8.3	10.6	10.8	10.6
Trp	ND[a]	ND	ND	ND	ND	ND	ND	ND	ND

[a] Not determined.

of proteins) did not show any difference between untreated (control) and pulsed-light-treated samples of whole milk. These results show the absence of oxidation reactions of proteins by lipids (**Figure 6**).

The presence of vitamin A in milk can be detected by fluorescence. After an excitation of vitamin A near 320 nm, an emission spectrum showing a maximum absorption at 410 nm can be observed. Analysis of spectra (**Figure 7**) shows a

Table 2. Comparison of Amino Acid Compositions of Skim Milk before and after Pulsed-Light Treatment

	control	10 pulses
Asx (Asn + Asp)	6.85	6.65
Glx (Gln + Glu)	18.80	18.00
Ser	7.32	7.28
Gly	3.70	4.15
His	3.18	2.76
Arg	1.99	1.76
Thr	5.45	5.72
Ala	5.23	5.21
Pro	12.24	12.57
Tyr	0.73	0.68
Val	8.02	8.06
Met	1.72	1.96
Cys	0.17	0.29
Ile	5.79	5.90
Leu	10.86	11.40
Phe	4.02	4.27
Lys	3.92	3.33
Trp	ND[a]	ND

[a] Not determined.

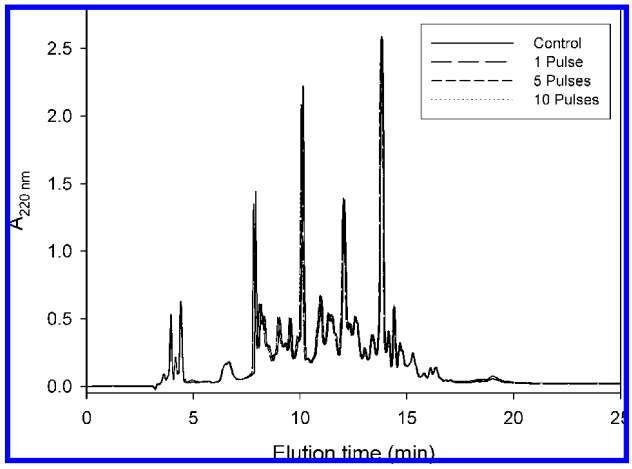

Figure 5. RP-HPLC profile of sodium caseinate hydrolyzed for 17 h at 37 °C with a mixture of trypsin and proteinase K before and after pulsed-light treatment. RP-HPLC was performed on a Jupiter Proteo 90 A column. Column was equilibrated with solvent A [H_2O, trifluoroacetic acid (TFA) 0.11%]. After injection of 20 μL of sample, peptides were eluted by a 0–100% solvent B gradient (20% H_2O, 80% acetonitrile, 0.09% TFA, v/v/v). Detection of peptides was performed at 220 nm.

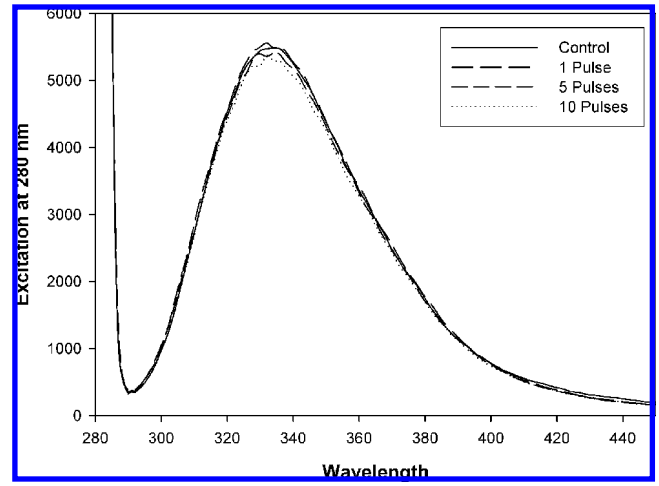

Figure 6. Fluorescence emission spectra of milk proteins after excitation at 280 nm.

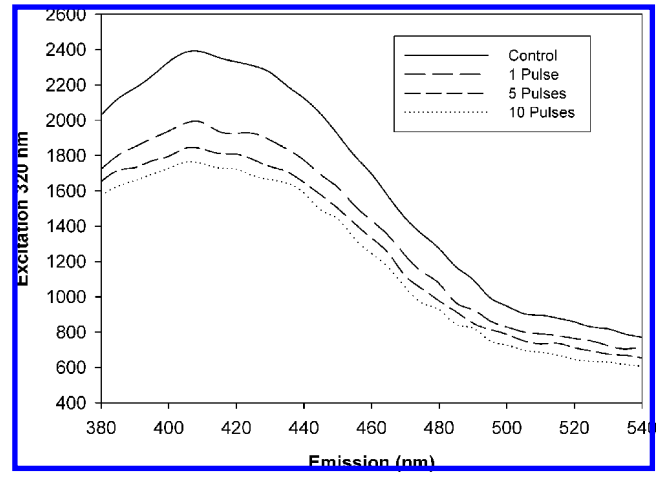

Figure 7. Fluorescence emission spectra of vitamin A contained in milk.

decrease of maximum intensity at 410 nm after treatment by pulsed light, demonstrating that vitamin A should be partially oxidized and/or partially decomposed by applied light. Considering the known photosensitivity of retinol, this is highly likely.

The spectra obtained by using an excitation wavelength from 380 to 600 nm and observing an emission wavelength from 300 to 470 nm for untreated and pulsed-light-treated (10 pulses) whole milk do not present any significant difference (data not shown). In the case of milk oxidation by light (*35*), a peak is observed presenting a maximum of emission at 420 nm for a maximum of excitation at about 360 nm. This fluorescence is due to the formation of oxidation products by reactions between amino groups and aldehydes formed during the oxidation. In our study, such a peak was not observed. Therefore, pulsed-light treatment, at applied doses, does not cause the oxidation of fatty acids. Similarly, Dunn (*3*) and Qin et al. (*36*), carrying out studies of physicochemical characteristics and shelf life of milk and milk derivatives after pulsed electric field treatment, did not observe changes in the fat content. Moreover, the fat percentage did not vary with time as a result of pulsed electric field treatment of a Spanish vegetable beverage, and therefore this treatment did not cause oxidation of fats (*30*). In contrast, high-pressure treatment induced increased rates of lipid oxidation in chicken and beef muscle (*37*). This is similar to the observation made by Sequeira-Munoz et al. (*38*), who found that the formation of oxidation products increased significantly with pressure and also with pressurization time versus the untreated samples for all pressure levels tested. In another study, it has also been shown that the high-pressure technology can induce the formation of complexes between proteins and polysaccharides (*39*). Such effects on the constituents of food products lead to a modification of their functional properties.

In summary, pulsed-light treatment resulted in a slight increase of UV absorbance at 280 nm and a decrease in intrinsic tryptophan fluorescence intensity for all protein samples studied, with a 7 nm red shift in the case of BLG after a 10-pulse treatment. This indicates changes in the polarity of the tryptophanyl residue microenvironment and the possibility of protein aggregation. No significant change in amino acid composition was observed after pulsed-light treatment. It can be concluded that during pulsed-light treatments there was no significant conformational change in milk proteins despite an aggregation by disulfide bonds, and there was no appearance of oxidation products. Thus, pulsed-light-treated milk proteins may display many if not all of its functionalities. These observations confirm

the usefulness of the pulsed-light process in food decontamination. Further studies on proteins and other food components are required to understand the full potential of this technique in complex food systems, as well as to optimize the operating conditions for food processing. This may also lead to the development of high-value food products enriching the marketplace.

ABBREVIATIONS USED

ALA, α-lactalbumin; BLG, β-lactoglobulin; ME, 2-mercaptoethanol; PITC, phenylisothiocyanate; SDS–PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid.

ACKNOWLEDGMENT

We thank Yvan Choiset (INRA, BIA-FIPL, Nantes) for technical assistance and Dr. Claude Genot (INRA, BIA-ISD, Nantes) for advice in spectrofluorometry studies.

LITERATURE CITED

(1) Barbosa-Cánovas, G. V.; Gongora-Nieto, M. M.; Swanson, B. G. Nonthermal electrical methods in food preservation. *Food Sci. Int.* **1998**, *4*, 363–370.
(2) Anderson, J. G.; Rowan, N. J.; MacGregor, S. J.; Fouracre, R. A.; Farish, O. Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light. *IEEE Trans. Plasma Sci.* **2000**, *28*, 83–88.
(3) Dunn, J. E. Pulsed light and pulsed electric field for foods and eggs. *Poult. Sci.* **1995**, *75*, 1133–1136.
(4) Rowan, N. J.; MacGregor, S. J.; Anderson, J. G.; Fouracre, R. A.; Mcllvaney, L.; Farish, O. Pulsed-light inactivation of food-related microorganisms. *Appl. Environ. Microbiol.* **1999**, *65*, 1312–1315.
(5) Wekhof, A. Disinfection with flash lamp. *PDA J. Pharm. Sci. Technol.* **2000**, *54*, 264–276.
(6) Dunn, J.; Burgess, D.; Leo, F. Investigation of pulsed light for terminal sterilization of WFI filled blow/fill/seal polyethylene containers. *PDA J. Pharm. Sci. Technol.* **1997**, *51*, 111–115.
(7) Dunn, J.; Ott, T.; Clark, W. Pulsed-light treatment of food and packaging. *Food Technol.* **1995**, *49*, 95–98.
(8) Takeshita, K.; Shibato, J.; Sameshima, T.; Fukunaga, S.; Isobe, S.; Arihara, K.; Itoh, M. Damage of yeast cells induced by pulsed light irradiation. *Int. J. Food Microbiol.* **2003**, *85*, 151–158.
(9) Smith, W. L.; Lagunas-Solar, M. C.; Cullor, J. S. Use of pulsed ultraviolet laser light for the cold pasteurisation of bovine milk. *J. Food Prot.* **2002**, *65*, 1480–1482.
(10) Gómez-López, V. M.; Devlieghere, F.; Bonduelle, V.; Debevere, J. Intense light pulses decontamination of minimally processed vegetables and their shelf-life. Int. *J. Food Microbiol.* **2005**, *103*, 79–89.
(11) Feuilloley, M. G. J.; Bourdet, G.; Orange, N. Effect of white pulsed light on *Pseudomonas aeruginosa* culturability and its endotoxin when present in ampoules for injection. *Eur. J. Parenteral Pharm. Sci.* **2006**, *11*, 37–43.
(12) Lagunar-Solar, M. C.; Piña, C.; MacDonal, J. D.; Bolkan, L. Development of pulsed UV light processes for surface fungal disinfection of fresh fruits. *J. Food Prot.* **2006**, *69*, 376–384.
(13) Kaack, K. L.; Lyager, B. Treatment of slices from carrot (*Daucus carota*) using high intensity white pulsed light. *Eur. Food Res. Technol.* **2007**, *224*, 561–566.
(14) Turtoi, M.; Nicolau, A. Intense light pulse treatment as alternative method for mould spores destruction on paper−polyethylene packaging material. *J. Food Eng.* **2007**, *83*, 47–53.
(15) Mailliart, P.; Ribadeau Dumas, B. Preparation of β-lactoglobulin and β-lactoglobulin-free proteins from whey retentate by NaCl salting out at low pH. *J. Food Sci.* **1988**, *53*, 743–752.
(16) Schägger, H.; von Jagow, G. Tricine−sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **1987**, *166*, 368–379.
(17) Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L. Rapid analysis of amino acids using pre-column derivatization. *J. Chromatogr., A* **1984**, *336*, 93–104.
(18) Dalgalarrondo, M.; Chobert, J.-M.; Dufour, E.; Bertrand-Harb, C.; Dumont, J.-P.; Haertlé, T. Characterization of bovine-β-lactoglobulin B tryptic peptides by reversed-phase liquid chromatography. *Milchwissenschaft* **1990**, *45*, 212–216.
(19) Zhang, H.; Li, L.; Tatsumi, E.; Kotwal, S. Influence of high pressure on conformational changes of soybean glycinin. *Innovative Food Sci. Emerging Technol.* **2003**, *4*, 269–275.
(20) Liu, X.; Powers, J. R.; Sawanson, B. G.; Hill, H. H.; Clark, S. Modification of whey protein concentrate hydrophobicity by high hydrostatic pressure. *Innovative Food Sci. Emerging Technol.* **2005**, *6*, 310–317.
(21) Yang, J.; Dunker, A. K.; Powers, J. R.; Clark, S.; Swanson, B. G. β-Lactoglobulin molten globule induced by high pressure. *J. Agric. Food Chem.* **2001**, *49*, 3236–3243.
(22) Puppo, M. C.; Chapleau, N.; Speroni, F.; Lamballerie-Anton, M. D.; Michel, F.; Añon, C.; Anton, M. Physicochemical modification of high-pressure-treated soybean protein isolates. *J. Agric. Food Chem.* **2004**, *52*, 1564–1572.
(23) Li, Y.; Chen, Z.; Mo, H. Effects of pulsed electric fields on physicochemical properties of soybean protein isolates. *Lebensm. Wiss. Technol.* **2007**, *40*, 1167–1175.
(24) Funtenberger, S.; Dumay, E.; Cheftel, J.-C. Pressure-induced aggregation of β-lactoglobulin in pH 7.0 buffers. *Lebensm. Wiss. Technol.* **1995**, *28*, 410–418.
(25) Funtenberger, S.; Dumay, E.; Cheftel, J.-C. High-pressure promotes β-lactoglobulin aggregation through SH/S−S interchange reactions. *J. Agric. Food Chem.* **1997**, *45*, 912–921.
(26) Dumay, E. M.; Kalichevsky, M. T.; Cheftel, J.-C. High-pressure unfolding and aggregation of β-lactoglobulin and the baroprotective effects of sucrose. *J. Agric. Food Chem.* **1994**, *42*, 1861–1868.
(27) Galazka, V. B.; Sumner, I. G.; Ledward, D. A. Changes in protein−protein and protein−polysaccharide in interactions induced by high pressure. *Food Chem.* **1996**, *57*, 393–398.
(28) Galazka, V. B.; Dickinson, E.; Ledward, D. A. Influence of high pressure processing on protein solutions and emulsions. *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 182–187.
(29) Dickinson, E.; James, J. D. Influence of competitive adsorption on flocculation and rheology of high-pressure-treated milk protein-stabilized emulsions. *J. Agric. Food Chem.* **1999**, *47*, 25–30.
(30) Cortés, C.; Esteve, M. J.; Frígola, A.; Torregrosa, F. Quality characteristics of horchata (a Spanish vegetable beverage) treated with pulsed electric fields during shelf-life. *Food Chem.* **2005**, *91*, 319–325.
(31) Turner, L. G.; Swaisgood, H. E.; Hansen, A. P. Interaction of lactose and proteins of skim milk during ultra-high temperature processing. *J. Dairy Sci.* **1978**, *61*, 384–392.
(32) Evangelitis, F.; Calgagno, C.; Nardi, S.; Zunin, P. Deterioration of protein fraction by Maillard reaction in dietectic milks. *J. Dairy Res.* **1999**, *66*, 237–243.
(33) Hurrell, R. F.; Carpenter, K. J. Maillard reactions in foods. In *Physical, Chemical, and Biological Changes in Food Caused by Thermal Processing*; Hoyem, T., Kvale, O., Eds.; Applied Science Publishers: London, U.K., 1977; pp 168–184.
(34) López Fandiño, R.; Carrascosa, A. V.; Olano, A. The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk. *J. Dairy Sci.* **1996**, *79*, 929–1126.
(35) Andersen, C. M.; Vishart, M.; Kolm, V. K. Application of fluorescence spectroscopy in the evaluation of light-induced oxidation in cheese. *J. Agric. Food Chem.* **2005**, *53*, 9985–9992.
(36) Qin, B. L.; Pothakamury, U. R.; Vega-Mercado, H.; Martín-Belloso, O. M.; Barbosa-Cánovas, G. V.; Swanson, B. G. Food pasteurization using high-intensity pulsed electric fields. *Food Technol.* **1995**, *12*, 55–60.

(37) Ma, H. J.; Ledward, D. A.; Zamri, A. I.; Frazier, R. A.; Zhou, G. H. Effects of high pressure/thermal treatment on lipid oxidation in beef and chicken muscle. *Food Chem.* **2007**, *104*, 1575–1579.

(38) Sequeira-Munoz, A.; Chevalier, D.; Le Bail, A.; Ramaswamy, H. S.; Simpson, B. K. Physicochemical changes induced in carp (*Cyprinus carpio*) fillets by high pressure processing at low temperature. *Innovative Food Sci. Emerging Technol.* **2006**, *7*, 13–18.

(39) Paquin, P. Technological properties of high pressure homogenizers: the effect of fat globules, milk proteins, and polysaccharides. *Int. Dairy J.* **1999**, *9*, 329–335.

Received for review October 11, 2007. Revised manuscript received January 14, 2008. Accepted January 19, 2008. We acknowledge "Sécuripôle" for financial support.

JF0729964