Effects of Pulsed Electric Fields on Ovalbumin Solutions and Dialyzed Egg White

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Ovalbumin solutions (2%, pH 7.0, 200 ohm·cm) and dialyzed fresh egg white (pH 9.2, 200−250 ohm·cm) were subjected to 50−400 exponential decay pulses with an electric field strength of 27−33 kV/cm. The pulse width was ca. 0.3 μs (at a capacitance of 20 nF) or 0.9 μs (at 80 nF), and the corresponding dissipated energy was 0.7 or 2.3 J/(pulse·mL) of solution. The sample temperature was maintained below 29 °C. While the four sulfhydryl groups of native ovalbumin did not react with DTNB, they became reactive immediately after pulse processing, indicating either partial protein unfolding or enhanced SH ionization. The extent of SH reactivity increased with dissipated energy, 3.7 SH groups becoming reactive after 100 or 200 pulses at 31.5 kV/cm and 80 nF. However, SH reactivity was reversible, since only 0.79 or 0.2 SH group was found to remain reactive 30 min or 8 h after pulse processing. The fourth derivatives of UV spectra of ovalbumin were determined, before and 15−30 min after pulse processing, to assess possible polarity and conformation changes in the environment of tyrosine and tryptophan. No differences were observed. Thermal gels prepared from fresh or dialyzed egg white had markedly different mechanical and water retention characteristics. Pulse processing of dialyzed egg white (200 pulses, 30 kV/cm, 80 nF) only slightly reduced its gelling properties. Thus electric pulses known to induce significant microbial inactivation did not cause notable changes in the proteins investigated.

Keywords: *Electric pulses; ovalbumin; egg white; sulfhydryl groups; derivatives of UV spectra; gelation*

INTRODUCTION

Ovalbumin is the major protein component of egg white, corresponding to more than 50% of the total protein. Ovalbumin is a monomeric phosphoglycoprotein of 45 kDa, containing 1 carbohydrate unit, 0−2 residues of phosphoserine, 1 disulfide bond, and 4 SH groups. The amino acid sequence of ovalbumin has been determined (Nisbet et al., 1981), and a 3-D configuration has been proposed by Stein et al. (1991) on the basis of X-ray crystallography at 1.95 Å resolution, indicating three β-sheets and nine α-helices. The denaturation temperature of ovalbumin is close to 84 °C. Ovalbumin is the main constituent responsible for the gelling properties of egg white (Mine, 1995).

Currently approved conditions for heat pasteurization of liquid egg white (55.6 °C for 6.2 min or 56.7 °C for 3.5 min) may be insufficient to inactivate pathogenic organisms such as *Salmonella* spp. and *Listeria monocytogenes* (Muriana, 1997; Palumbo et al., 1995, 1996). Higher temperatures cause flavor changes and seriously impair protein functional properties. The latter may be further reduced upon subsequent spray-drying and storage in the dry (or frozen) state. Thus new processing methods are being developed, only some of them having received regulatory approval.

Some of these methods, such as increasing the pH of egg white, or adding H_2O_2, aim at improving the thermal inactivation of pathogenic or spoilage organisms (Palumbo et al., 1996). In contrast, the addition of nisin, NaCl (6−10%), or sucrose (>30%) help to control microbial growth during refrigerated storage. Other compounds (e.g., L-cysteine) can also be added to protect the protein constituents against thermal denaturation and, therefore, enable pasteurization at somewhat higher temperatures. In the case of liquid whole egg, which is less sensitive to heat than liquid egg white, ultrapasteurization at 68−70 °C for 90 s (followed by cooling, aseptic packaging, and refrigerated storage) (ovotherm process) can be used to give products with a refrigerated shelf life of 10−15 weeks instead of 7 days for products pasteurized at conventional temperatures (60−62 °C; 3−4 min) (Swartzel et al., 1990a,b, 1991; Martinez et al., 1994). Some changes in protein constituents were noted (Martinez et al., 1994; Dawson and Martinez-Dawson, 1998).

It is also possible to heat liquid egg using alternating electrical waves (Reznik, 1996, 1997; Katz, 1997). Because the frequency is low (50−99 kHz), protein coagulation is minimized.

However, nonthermal processes are more likely to preserve the functional properties of egg proteins. Irradiation with electrons at a dose of about 3 kGy efficiently reduced *Salmonella typhimurium* inoculated into liquid egg white, permitting satisfactory storage at 4 °C for up to 90 days (Wong et al., 1996). Functional properties were better than for control heat-pasteurized egg white (57 °C, 3.5 min). γ irradiation (1−4 kGy) of frozen liquid egg white did not affect scanning calorimetric profiles or electrophoretic patterns of protein constituents. Protein functional properties were also maintained (Ma et al., 1993). Another potential method

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is high-pressure processing. Liquid whole egg spiked with *Escherichia coli* was treated at 300−450 MPa and −15 to 50 °C. The maximum inactivation (∼7 log cycles) was obtained at 50 °C (Ponce et al., 1998a). In a similar study with *Listeria innocua*, an inactivation ratio of 5 log cycles was reached upon pressurization at 20 °C (Ponce et al., 1998b). Honma and Haga (1991) had previously established the limits of high-pressure pasteurization of egg white and egg yolk with respect to protein denaturation.

The continuous processing of egg white with high-voltage pulsed electric fields has been advocated (Dunn and Pearlman, 1987; Bushnell et al., 1993, 1996). The continuous processing of whole liquid egg inoculated with *E. coli* was reported by Martin-Belloso et al. (1997). One hundred exponential decay pulses of 4 μs each at a field strength of 26 kV/cm and at 37 °C induced a microbial inactivation of almost 6 log cycles. Inactivation increased with the number of pulses and with pulse width (4 rather than 2 μs). No protein coagulation was observed. Calderon-Miranda et al. (1999) obtained a 3.5 log cycle inactivation of *L. innocua* (ATCC 51742) by continuous processing of liquid whole egg with 32 exponential decay pulses (2 μs pulse width, 3.5 Hz pulse repeat frequency, 200 nF capacitor) at 50 kV/cm and 36 °C. The combination of electric pulses followed by exposure to nisin exhibited an additive effect in the overall inactivation of *L. innocua*. Synergistic effects were observed when electric field strength, number of pulses, and nisin concentration increased. Recently, Jeantet et al. (1999) batch processed dia-ultrafiltered egg white (conductivity of 1.5−1.8 × 10^{-3} S/cm) with 2−8 exponential decay pulses (∼10 μs pulse width, 100−900 Hz pulse frequency) at 20−35 kV/cm, 4−30 °C, and pH 7−9. Prior inoculation (10^3−10^7 CFU/mL) of egg white with *Salmonella enteritidis* revealed a maximum inactivation ratio of 3.5 log cycles. The surface hydrophobicity of egg white did not increase, an indication for the absence of protein unfolding.

While it is well demonstrated that pulsed electric fields of adequate voltage inactivate efficiently the vegetative forms of microorganisms (Knorr et al., 1994; Qin et al., 1995, 1996; Barbosa-Cánovas, 1996b, 1998; Vega-Mercado et al., 1997; Wouters and Smelt, 1997; PureBright, 1998; Barsotti and Cheftel, 1999; Wouters et al., 1999), much less is known concerning the effects of pulsed fields on proteins and other food constituents (Neumann, 1986; De Jong and Van Heesch 1998; Barsotti and Cheftel, 1999). Studies on enzyme inactivation by pulse processing of enzyme solutions or liquid foods reveal varying results, perhaps partly due to differences in electric systems and pulse types (Castro, 1994; Vega-Mercado et al., 1995; Barbosa-Cánovas et al., 1996a, 1998; Ho et al., 1997; Barsotti and Cheftel, 1999). Recently, Yeom et al. (1999) investigated the inactivation of a heat-resistant enzyme, papain, by continuous processing at 10−35 °C, using square wave pulses (4 μs pulse width, 1500 Hz pulse frequency), delivered to four collinear treatment chambers placed in series. Inactivation was not significant immediately after pulse processing but increased markedly (perhaps partly through self-proteolysis) after 24 h storage of the treated enzyme solution at 4 °C. Increasing the number of pulses (from 200 to 500) and the electric field strength (from 20 to 50 kV/cm) enhanced inactivation. Inactivation did not depend on the oxidation of the papain active site, a cysteine residue, but was related to some loss of α-helix structure, as determined by circular dichroism.

The present study deals with the effects of high-voltage pulsed electric fields on solutions of ovalbumin. Possible protein unfolding was investigated. It was also attempted to process liquid egg white with the same electric pulses and study the resulting changes in protein functional properties such as solubility and gelling ability.

MATERIALS AND METHODS

Materials. Ovalbumin was from Sigma (St. Louis, MO 63178) (A-5378 lot 37H7015, grade III, 98% pure). For electric pulse treatment, a 2% (w/v) ovalbumin solution was prepared in 33 mM sodium−potassium phosphate buffer, pH 7. The exact protein concentration, estimated from the absorbance at 280 nm with $E^{1\%}_{280} = 0.712$ (Glazer et al.,1963), was 1.88%. The resistivity of the solution was approximately 200 ohm·cm at 25 °C. A solution in 10 mM sodium−potassium phosphate buffer was also tried; its resistivity was about 600 ohm·cm, but electrical arcing was occasionally observed during electric pulse treatment at fields higher than 30 kV/cm.

It should be noted that acceptable sample resistivity for pulse processing with the high voltage system used is between 200 and 600 ohm·cm. It is not possible to reach high voltage across the electrodes when the food sample is too conductive (resistivity below 200 ohm·cm). When resistivity is above 500 ohm·cm, a large proportion of the current, and of the pulse energy, passes through a protection resistance (30 ohm) placed in parallel with the treatment chamber.

Egg white was obtained from freshly laid eggs purchased at a local supermarket. Egg white was carefully separated from the yolk and chalaza and homogenized by gentle stirring. Since its resistivity was low, i.e., close to 95 ohm·cm, it was dialyzed to lower the ionic strength. To this end, 20 mL of egg white samples was dialyzed against 4 L of 20, 33, or 50 mM sodium phosphate buffer, pH 7, at 4 °C for 16 h with gentle stirring, but some protein flocculation was observed. After experimentation with various conditions, dialysis at 4 °C for 1 h against ultrapure water was selected since it increased resistivity to 200−250 ohm·cm at 25 °C without any protein precipitation. The pH was 9.0−9.2 before and 9.1−9.2 after dialysis. Protein concentration in egg white samples was determined with bicinchoninic acid and found to be 9.5% w/w for fresh egg white and 8.9% w/w for dialyzed egg white.

Ellman's reagent, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (D-8130, lot 38H0407), sodium docecyl sulfate (SDS) (L-4509, lot 36H0054), glycine (G-7126, lot 61H0250), ethylenediaminetetraacetic acid (EDTA) (ED-2SS, lot 86F0380), tris(hydroxymethyl)aminomethane (TRIS) (T1503, lot 45H5734), bicinchoninic acid (B-9643, lot 75H5060), and the ethyl esters of tyrosine (T-4879, lot 45H1549), tryptophan (T-8755, lot 45H1548), and phenylalanine (P-2751, lot 124F0086) were from Sigma. Urea (U-160, lot 368047) was from Labosi (F-75015 Paris).

Treatment by Pulsed Electric Fields. The system used was from CENTRALP (F-38070 St. Quentin Fallavier). It consists of a high-voltage power source (25 kV), a capacitor bank, a fast commuting switch, a treatment chamber with two flat circular electrodes, and an oscilloscope with voltage and current probes. The power source delivers a constant current of 300 mA to the capacitors with a maximum power of 2500 W. The voltage can be fixed between 0 and 25 kV. The capacitor bank consists of 4 bar of 12 single ceramic elements of 1.67 nF each. The total capacity may vary from 20 to 80 nF. The MOS-FET switch enables to commute up to 25 kV and 2 kA, the commuting time being about 5 ns. Exponential decay pulses are produced with a width of 0.3−1.5 μs with a maximum repeat frequency of 100 Hz. The parallel electrodes are made of stainless steel, the cathode being at the top and the anode at the bottom, in contact with a large grounded copper plate. The treatment chamber used in the present study is a cylindric epoxy resin cylinder with a 38 mm internal

diameter and a height of 5 mm fixed on the anode with plastic screws. Thus the treatment chamber holds 5.7 mL of sample. Voltage and current are measured with a Model TDS 380 Tektronix oscilloscope. One channel measures $^1/_{1000}$th of the voltage across the electrodes, and another one measures voltage across a resistance of 5 mohm, the value being converted to current intensity across the electrodes by multiplying by 200. A protection resistance (30 ohm) was connected in parallel with the treatment chamber, because the MOSFET switch can be damaged if individual high-voltage pulses exceed 7 μs.

Ovalbumin solutions and fresh egg white were subjected to various processing conditions, with a maximal electric field strength of 27−33 kV/cm, varying the number of pulses (50−400 pulses in series of 20 or 50 at a pulse repeat frequency of 1.1 Hz) and also the number of capacitors (C = 20 or 80 nF, modifying therefore the energy per pulse). Intervals of 1−2 min were allowed between series of 20 or 50 pulses to permit heat removal from the treatment chamber through the bottom electrode and the copper plate immediately below. It was checked using a thermocouple to ensure that, within 1 min from the last series of pulses, the sample temperature never exceeded 25 °C (ovalbumin solutions) or 27−28 °C (egg white). It was observed that the pH of the samples remained constant and equal to the initial pH (7.0 for ovalbumin solutions and 9.0−9.2 for egg white) after electric processing.

At 80 nF capacitance and 31.5 kV/cm, the energy dissipated in the sample per pulse can be calculated

$$w_{\text{pulse}} = \int_0^t u(t)\, i(t)\, \mathrm{d}t$$

to be about 13 J. In the equation $u(t)$ represents the voltage across the electrodes at time = t, and $i(t)$ represents the current intensity through the sample at time = t. Due to the presence of the 30 ohm protection resistance in parallel with the sample, only 65−75% of the total current intensity passed through the sample in ovalbumin and egg white experiments.

Analysis of DTNB-Reactive Sulfhydryl Groups. Reactive SH groups were analyzed within 2 min of electric pulse processing, using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), according to Ellman (1959) with the modifications of Shimada and Cheftel (1988). Ellman's reagent solution (0.03 mL) (4 mg of DTNB/mL of standard buffer) was added to a 3 mL aliquot of ovalbumin solution (0.05% w/v, diluted 40 times with standard buffer from the original protein solution at 2% w/v). The standard buffer (pH 8.0) was 86 mM TRIS, 90 mM glycine, and 4 mM EDTA. The solution was rapidly mixed, and absorbance was read at 412 nm, using the standard buffer as a blank. Absorbance was followed for up to 2 h at 25 °C to study the reaction kinetics. A molar extinction coefficient of 1.36×10^4 M^{-1} cm^{-1} was used to calculate moles of reactive SH groups per mole of ovalbumin (Ellman, 1959). For each series of experiments, total SH groups were also determined using instead of the standard buffer a solution consisting of the standard buffer made 8 M in urea and 0.5% w/v in sodium dodecyl sulfate. These denaturants unfold the protein so that all SH groups become reactive.

UV Absorbance of Aromatic Amino Acids in the Fourth Derivative Mode. This method enhances the generally poor resolution of zero-order UV absorbance spectra (Lange et al., 1996) and detects modifications in protein conformation by measuring polarity changes in the microenvironment of aromatic amino acids (Mombelli et al., 1997).

UV spectra of ovalbumin solutions (1.4 mg of ovalbumin/mL) in 33 mM sodium−potassium phosphate buffer, pH 7, were recorded in the double beam mode with a Varian Cary 100 spectrophotometer (Mulgrave, Victoria 3170, Australia) from 240 to 305 nm, in a 10 mm quartz cuvette, at room temperature. Correction for baseline was done with the buffer. The monochromator proceeded in steps of 0.1 nm with a data acquisition time of 0.5 s. The spectral bandwidth was 1 nm. The derivation procedure was based on the method published by Lange et al. (1996), where the original spectrum is shifted over a small distance, $\Delta\lambda$ = 2.6 nm (derivative window), toward longer wavelengths. The difference between the shifted and the original spectrum is used for calculation of the first derivative. Derivatives of higher order are obtained by repeating this procedure. Smoothing of the fourth derivative curve was done according to Savitzky and Golay (1964), using the software (CaryWin 2.0) from the spectrophotometer and taking an interval of 0.2 nm. A maximum of the fourth derivative of the UV spectrum corresponds to a maximum of the original spectrum. UV spectra of solutions of ethyl esters of aromatic amino acids in the same phosphate buffer were also recorded. Bands for tyrosine, tryptophan, or phenylalanine ethyl esters corresponded to the 275−286, 282−292, or 250−270 nm sections of the UV spectrum, respectively. However, these bands are normally "red shifted" for amino acid residues within the more hydrophobic environment of native proteins. The fourth derivative curve of native ovalbumin in the pH 7 buffer indicates the following specific band(s) for each aromatic amino acid residue (as shown later in Figure 3): maximum at 280.4 nm and minimum at 282.8 nm for tyrosine; minimum at 289.2 nm and maximum at 292 nm for tryptophan; maxima at 253.4, 259.2, 265.2, and 269.4 for phenylalanine. An intermediate maximum at 286.4 nm corresponds to an overlapping of bands from tyrosine and tryptophan and is therefore not taken into account.

Thermal Gelation of Egg White. The same batch of fresh egg white was dialyzed as previously indicated and then processed by electric pulses. Ten milliliters of fresh, dialyzed, or dialyzed plus pulse processed egg white was placed in glass tubes (internal diameter = 13 mm; height = 25 mm) closed at both ends by a silicone stopper. About 2 h after electric pulse processing, the tubes were immersed for 25 min in a circulating stirred water bath at 90 °C and then immediately cooled in cold water and kept for 24 h at 4 °C.

Texture Determination of Egg White Gels. Before texture analysis, heat-induced gels were equilibrated at room temperature for 1 h and then taken out of the tubes. Gel slices (diameter = 13 mm; height = 15 mm) were carefully prepared with a razor blade. The mechanical characteristics of gels were measured with a TA-XT2 texture analyzer (Stable Microsystems, Surrey, England) by compression, with a flat probe (diameter = 20 mm) at a speed of 12 mm/min to a distance of 2 mm. Gel rigidity was defined as the stress F_0/S (g/cm^2) measured at 2 mm of compression, where F_0 was the force exerted on the probe immediately after compression of the sample and S was the cross section area of the gel sample. The compression was then maintained for 10 min, and the force (F_t) exerted on the probe was measured versus time. The elasticity index and the relaxation time were calculated according to Peleg (1979) from the normalized model

$$F_0 t/(F_0 - F_t) = 1/ab + t/a$$

where $(1 - a)$ is the elasticity index and $1/b$ is the relaxation time (in seconds). The elasticity index is a measure of the stress remaining in the gel after the relaxation process. In the model used, the elasticity index would be equal to 0 for a liquid and to 1 for a solid. Gel rigidity, elasticity index, and relaxation time were taken as the means of five measurements on distinct samples.

Water Holding Capacity of Gels. Approximately 1 g of gel equilibrated to room temperature was placed on a polyethylene net membrane (20 μm pores; Pharmacia, Sweden) maintained in the middle position of a 50 mL centrifuge tube. Water loss was determined in triplicate by weighing after centrifugation at 1086g for 5 min (Sorvall RG 5B centrifuge, SS-34 rotor). The water holding capacity was expressed as grams of water remaining in the centrifuged gel per 100 g of water in the initial gel. For the determination of dry solids, about 1 g of egg white gel was cut into small pieces, placed in aluminum pans, and kept in an oven at 104 °C for 24 h.

RESULTS AND DISCUSSION

Effects of Electric Pulse Processing of Ovalbumin Solutions on the Reactivity of Sulfhydryl

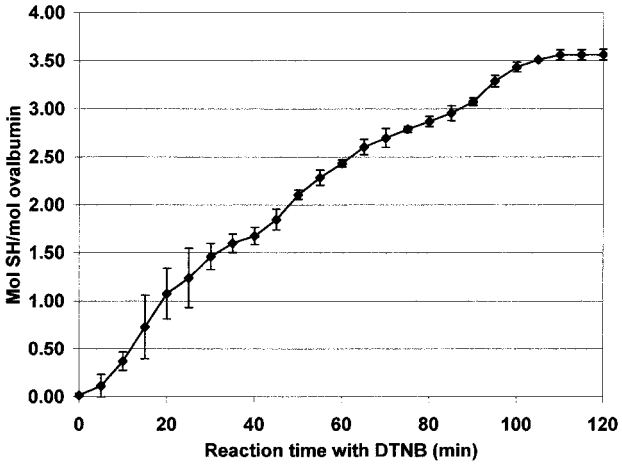

Figure 1. Effects of electric pulse processing of a 2% (w/v) ovalbumin solution on the kinetics of reaction of SH groups with DTNB. Electric conditions: $E_{max} = 31.5 \pm 1.0$ kV/cm; 80 nF capacitance; 200 pulses in series of 20 at a repeat frequency of 1.1 Hz with 1−2 min intervals between series of pulses; maximum sample temperature = 25 °C. Reaction with DTNB initiated 2 min after the end of pulse processing. Mean and standard deviation of three independent experiments.

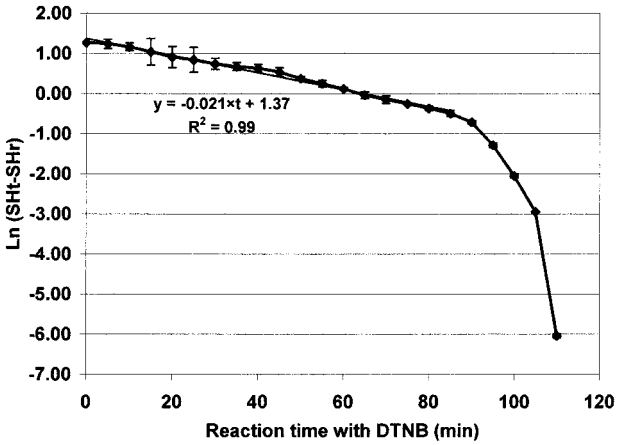

Figure 2. Pseudo-first-order kinetic plot of non-DTNB-reacted SH groups of ovalbumin solutions previously subjected to electric pulse processing (SHt = maximum SH groups reacted; SHr = SH groups reacted at time *t*). Conditions: 2% (w/v) ovalbumin solutions; $E_{max} = 31.5 \pm 1.0$ kV/cm; 80 nF capacitance; 200 pulses in series of 20 at a repeat frequency of 1.1 Hz with 1−2 min intervals between series of pulses; maximum sample temperature = 25 °C. Reaction with DTNB initiated 2 min after the end of pulse processing. Mean and standard deviation of three independent experiments.

Groups. The four sulfhydryl groups of native ovalbumin did not react with DTNB in the absence of protein denaturants. When denaturing agents were added at a final concentration of 8 M urea and 0.5% (w/v) SDS, most SH groups reacted (3.8 mol of SH/mol of ovalbumin) with DTNB after a reaction time of about 10 min. These results are in agreement with those reported by Doi and Kitakabake (1997).

When ovalbumin solutions (2% w/v in 33 mM sodium−potassium phosphate buffer, pH 7) were subjected to pulsed electric fields (200 pulses by series of 20 pulses at a repeat frequency of 1.1 Hz, $C = 80$ nF, and $E_{max} = 31.5 \pm 1.0$ kV/cm), SH groups became reactive to DTNB in the pH 8.0 buffer in the absence of denaturants. The reaction was slower than with ovalbumin in the presence of denaturants, but over a period of 100−120 min, 3.6 of the 4 SH groups of ovalbumin were found to react (Figure 1). Average values and standard deviations were calculated from three or four independent experiments. These results suggest that electric pulse processing induced at least partial unfolding of the protein structure, thus exposing all four sulfhydryl groups to the surface. Alternatively, pulse processing could have enhanced ionization of SH groups into S^-, the ionized form being more reactive. However, when the electrically processed ovalbumin solution was maintained at 4 °C for various times before reaction with DTNB, sulfhydryl groups were observed to become less reactive. A total of 3.6, 0.79, 0.52, 0.20, and 0.16 DTNB-reactive SH groups per mole of ovalbumin were detected after 2 min, 30 min, 4 h, 8 h, and 24 h, respectively. This suggests that the partial protein unfolding, or the enhanced ionization of SH groups, was transient and quickly reversible.

Although the reaction between the SH groups of proteins and DTNB is known to follow second-order kinetics, pseudo-first-order kinetics is observed when DTNB is in excess (Okabe et al., 1970). In the present experiments a 10-fold molar excess of DTNB was used. The equation for the pseudo-first-order reaction is $\mathrm{Ln}(SHt - SHr) = -K_{obs}t + \mathrm{Ln}(SHt)$, where SHt is the total number of SH groups derived from the maximum value of Figure 1 (3.59 ± 0.05 mol of SH/ mol ovalbumin), SHr the number of SH groups having reacted at time *t*, and K_{obs} the reaction rate constant. Figure 2 shows the pseudo-first-order SH−DTNB reaction for ovalbumin after electric pulse processing (200 pulses fired by series of 20 pulses at a repeat frequency of 1.1 Hz, $E_{max} = 31.5 \pm 1.0$ kV/cm, $C = 80$ nF, maximum sample temperature = 24 °C).

Several other experiments were carried out with the same 2% (w/v) ovalbumin solutions processed under different electrical conditions (50−400 pulses, with a capacitance $C = 20$ or 80 nF). Conditions and results are reported in Table 1. They clearly indicate that not all electric pulse conditions were able to increase the reactivity of the SH groups of ovalbumin. For a given electric field strength of 31.5 ± 1.0 kV/cm, both the number of pulses and the total capacitance (i.e., the energy per pulse) significantly influenced SH group reactivity toward DTNB, as determined by the maximum number of SH groups (mol of SH/ mol of ovalbumin) that reacted during the 2 h reaction time with DTNB. When this maximum number is plotted against the total dissipated energy per milliliter of sample, there appears to be a threshold of energy (∼120 J/ mL, at 80 nF) for SH groups to become reactive. Above this threshold, the maximum number of reactive SH groups increases with the total energy dissipated in the sample (Table 1). However, in the case of experiments performed at 20 nF, the energy per pulse [ca. 0.7 J/(pulse•mL)] apparently remained too low to induce a high number of reactive SH groups, even at a total dissipated energy of 258 J/mL of sample (Table 1). In all cases, it was checked that when the processed samples were kept at 4 °C for 24 h before adding DTNB, sulfhydryl groups reversibly became nonreactive to DTNB (0.05−0.16 mol of SH/mol of ovalbumin). These results appear to indicate that high-voltage electric pulse processing did not induce permanent modifications of ovalbumin.

It should be also noted that electric pulse processing did not cause any visible protein aggregation since no flocculation or precipitation was observed. It is possible that electric pulse processing of ovalbumin solutions at protein concentrations above 2% w/v could induce suf-

Table 1. Electric Pulse Processing of Ovalbumin Solutions:[a] Processing Conditions[b] and Effects on DTNB-Reactive SH Groups[c]

capacitance (nF)	no. of pulses	max electric field across electrodes (kV/cm)	max current across electrodes (kA)	energy dissipated per pulse and per mL of sample [J/(pulse·mL)]	total energy dissipated per mL of sample (J/mL)	max no. of DTNB-reactive SH groups[c] (mol of SH/mol of ovalbumin)
20	75	28.8 (1.8)	1.05 (0.09)	0.70 (0.04)	54.7 (3)	0.15 (0.05)
20	200				143 (4)	1.0 (0.1)
20	400				258 (26)	1.3 (0.1)
80	50	31.5 (1.0)	1.26 (0.03)	2.29 (0.10)	119 (14)	0.16 (0.06)
80	75				162 (8)	1.7 (0.1)
80	100				234 (12)	3.8 (0.1)
80	200				465 (22)	3.6 (0.05)

[a] 2% (w/v) ovalbumin solution in 33 mM sodium−potassium phosphate buffer, pH 7; resistivity ~200 ohm·cm. [b] Stainless steel parallel plate electrodes, $d = 5$ mm, surface = 11 cm^2. Volume of sample in treatment chamber = 5.7 mL. Exponential decay pulses, fired by series of 20 or 50 at a pulse repeat frequency of 1.1 Hz, with 1−2 min intervals between series. Pulse width ~0.3 μs at 20 nF and ~0.9 μs at 80 nF. Maximum temperature of sample = 25 °C. [c] Reaction with DTNB initiated 2 min after the end of pulse processing. Reaction time = 120 min at ~25 °C. Average value (and standard deviation) of three independent experiments carried out on different days.

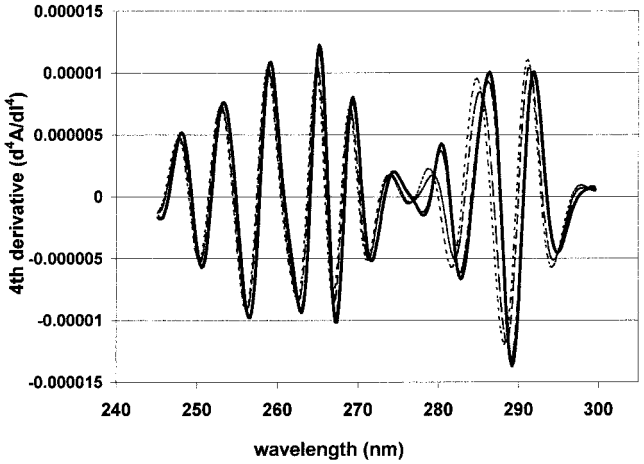

Figure 3. Fourth derivatives of the UV spectrum of ovalbumin before (−) and 10 min after thermal processing at 70 (- − -), 80 (- - -), or 90 °C (· · ·) for 10 min. The ovalbumin concentration for thermal processing was 2% (w/v) in 33 mM sodium−potassium buffer, pH = 7. The ovalbumin concentration for spectrum scanning was 1.4 mg/mL after dilution with the same buffer. The spectrum was obtained on a Varian Cary 100 at 12 nm/min at a bandwidth of 1 nm and data interval of 0.1 nm.

ficient protein unfolding or SH group ionization to cause the formation of soluble and insoluble aggregates.

Effects of Heating or Electric Pulse Processing of Ovalbumin on the Fourth Derivative of UV Spectra. Since the transient increase detected in SH group reactivity after electric pulse processing of ovalbumin could be due to partial protein unfolding, it was of interest to use another method for confirmation.

The fourth derivative of the UV spectrum is a very sensitive method to detect modifications in the environment of tyrosine, tryptophan, and phenylalanine residues of a protein in solution. Fourth derivative changes usually induce both wavelength and amplitude shifts. Such changes can be seen in Figure 3 for solutions of ovalbumin (2% w/v in 33 mM sodium−potassium phosphate buffer, pH 7.0) heated at 50, 60, 70, 80, or 90 °C for 10 min, cooled, then diluted to 1.5 mg/mL in the same buffer, and scanned for UV spectrum and fourth derivative within 10 min of heating. None of the heated solutions developed visible turbidity. No major change in the spectrum relative to that of native ovalbumin was detected below 70 °C (data not shown on Figure 3), while at higher temperatures a "blue" shift (to shorter wavelengths) was observed in all bands but mainly for tyrosine. For tyrosine residues, the shift was from 280.4 nm to 280.2, 279.3, or 278.8 nm, when the protein was heated at 70, 80, or 90 °C, respectively. For tryptophan residues, the shift was from 292 nm to 291.8, 291.4, or 291.2 nm (at 70, 80, or 90 °C, respectively). In the phenylalanine region, the shift was from 265.2 nm to 265.0 (at 80 °C) or to 264.8 nm (at 90 °C). Such blue shifts correspond to a less apolar environment around these amino acids. Average shift values and standard deviations (±0.02 nm, in all cases) were calculated from three UV spectra on each heated ovalbumin solution. The tyrosine shift occurred mainly between 70 and 80 °C. The wavelength shifts are accompanied by changes in amplitude: marked decrease for tyrosine, lesser decrease for phenylalanine, or increase for tryptophan, corresponding to a broadening or sharpening of the amino acid bands on the initial UV spectrum (not shown). Changes in amplitude at selected wavelengths are generally used to calculate the extent of protein unfolding induced by heat processing (Mombelli et al., 1997).

Experiments were also carried out with ovalbumin solutions (2% w/v in 33 mM sodium−potassium phosphate buffer, pH 7.0) processed by electric pulses (200 pulses by series of 20, at a repeat frequency of 1.1 Hz, with 1−2 min intervals between series of pulses, E_{max} = 31 kV/cm, C = 80 nF, maximum sample temperature 24 °C), then diluted to 1.4 mg/mL in the same buffer, and scanned for UV spectrum and fourth derivative within 15 and 30 min of electrical processing. Results were obtained from two independent experiments (three spectra on each solution) after 30 min and one experiment after 15 min. The fourth derivatives of control (native) ovalbumin and of electrically processed ovalbumin were identical (as in Figure 3, before thermal processing).

It is difficult to explain how the four SH groups of ovalbumin, initially nonreactive to DTNB, became exposed and reactive after sufficient electric pulse processing (Figures 1 and 2; Table 1) without any change in protein configuration near tyrosine, tryptophan, or phenylalanine residues. One possible explanation is that electric pulse processing increased the exposition of SH groups or their ionization into S^-, thus making them more reactive toward DTNB, without protein unfolding in the environment of aromatic amino acids. Another possibility is that partial unfolding induced by electric processing was too rapidly reversible to be detected on the UV spectrum (scanned 15 and 30

Table 2. Composition and Mechanical Characteristics[a] of Heat-Induced Gels[b] from Fresh,[c] Dialyzed,[d] and Dialyzed and Then Electric Pulse Processed Egg White[e]

egg white sample used for gel preparation	dry solids (% w/w)[g]	water holding capacity (% w/w)[f,g]	rigidity (g/cm^2)[g]	elasticity index[g]	relaxation time (s)[g]
fresh egg white	12.2 (0.2)[a]	85 (3)[a]	57 (10)[a]	0.48 (0.01)[a]	86 (5)[a]
dialyzed egg white	11.3 (0.3)[b]	90 (1)[b]	29 (5)[b]	0.58 (0.01)[b]	86 (11)[a]
dialyzed and then electric pulse processed egg white	11.2 (0.1)[b]	89 (2)[b]	20 (7)[c]	0.57 (0.02)[b]	92 (3)[a]

[a] Measured on a TA-XT2 texture analyzer by compression of gel slices (diameter = 13 mm; height =15 mm) at a speed of 12 mm/min to a distance of 2 mm. Average values (and standard deviation) from five gels prepared independently from each of the three egg white samples. [b] Samples heated in glass tubes (internal diameter = 13 mm; height 25 mm) at 90 °C for 25 min, then cooled, and kept at 4 °C for 24 h before texture measurements. [c] Egg white separated from the yolk and chalaza and then stirred for homogeneity. Resistivity 95 ± 8 ohm·cm; pH = 9.0−9.2. [d] Corresponding egg white dialyzed at 4 °C for 1 h in ultrapure water and then stirred for homogeneity. Resistivity ∼210−250 ohm·cm; pH 9.1−9.2. [e] Previous dialyzed egg white subjected to 200 electric pulses by series of 32, at a repeat frequency of 1.1 Hz; $C = 80$ nF; $E_{max} = 30$ kV/cm; initial temperature = 27 °C; final temperature = 28.5 °C. Heat-induced gels prepared 2 h after electric pulse processing. [f] Water holding capacity expressed in grams of H_2O in centrifuged gel per 100 g of H_2O of initial gel. [g] Different letters in the same column indicate significant differences ($p = 0.05$).

min after electric processing). It has indeed been shown that enhanced reactivity of SH groups to DTNB as detected 2 min after pulse processing had almost completely reverted to nonreactivity after 30 min.

Ovalbumin contains 10 tyrosine, 3 tryptophan, and 20 phenylalanine residues (Nisbet et al., 1981). In the amino acid sequence, 1 tyrosine and 8 phenylalanine residues are located close to the 4 residues of cysteine, while a few other tyrosine and phenylalanine residues appear to rest in the near steric environment of the SH groups (Stein et al., 1991). These SH groups also appear to be relatively close to the protein surface, so that enhanced ionization to S^- may be more important than protein unfolding for reactivity to DTNB.

Effects of Electric Pulse Processing on the Gelling Properties of Egg White. The electrical resistivity of fresh egg white was found to be 95 ± 8 ohm·cm, and since low sample resistivity prevents reaching high voltage across the electrodes, egg white was dialyzed prior to electric pulse processing. As indicated in Materials and Methods, dialysis against ultrapure water for 1 h was preferred because it increased electrical resistivity up to a value (200−250 ohm·cm) adequate for pulse processing without causing any protein precipitation or flocculation. It should be recalled that dialysis removes a large part of the mineral salts and glucose present in egg white. It was checked that electric pulse processing of dialyzed egg white did not induce any protein precipitation.

Gelling properties were determined by measuring rigidity, elasticity index, relaxation time, and water holding capacity of heat-induced gels from fresh, dialyzed, and dialyzed and then pulse processed egg whites. Average values and standard deviations were calculated from five thermal gels prepared independently for each of the three egg white samples. Results are shown in Table 2, together with the dry solid content of gels. No spontaneous exudation was observed in any of the gels. Besides, it should be pointed out that thermal gels of electric pulse processed (dialyzed) egg white were prepared 2 h after pulse processing.

Marked differences ($p = 0.01$) were observed in the rigidity and elasticity index of heat-induced gels from fresh egg white and from the corresponding dialyzed egg white (Table 2). The higher rigidity and lower elasticity of gels from nondialyzed egg white can probably be accounted for by a greater extent of protein aggregation in the presence of mineral ions. The slightly higher ($p = 0.05$) water holding capacity of gels from dialyzed egg white could likewise be due to a finer gel network. However, the lower dry solid content (and protein content) of gels from dialyzed egg white is probably also partly responsible for these modified characteristics.

The differences in mechanical properties and water holding capacity between gels from the dialyzed egg white and from the corresponding electric pulse processed (dialyzed) egg white are much less or not at all significant (Table 2). A significant difference ($p = 0.05$) was noted only for gel rigidity. Thus it can be said that high-voltage pulses did not induce marked changes in the gelling properties of dialyzed egg white.

These results are in agreement with the fact that ovalbumin, the major protein component responsible for the gelling properties of egg white, was not significantly modified by high-voltage electric pulses, as judged 10−30 min after pulse processing (see the two previous sections of Results and Discussion).

CONCLUSIONS

The present study indicates that electric pulses of high-field strength (∼31.5 kV/cm) generally known to induce efficient microbial inactivation can be applied to solutions of ovalbumin (1.9 g of protein/100 mL) without causing notable or permanent modifications in protein conformation. The same electric pulses can also be applied to dialyzed egg white (8.9 g of protein/100 mL) without any protein precipitation or marked alterations of gelling properties.

Prior dialysis of egg white was necessary to reduce electric conductivity and reach pulses of high-field strength. Such dialysis did not reduce protein solubility but was clearly detrimental to the gelling properties of egg white.

Further experiments should therefore be carried out to assess microbial inactivation in inoculated fresh or dialyzed egg white treated by electric field pulses of various field strengths.

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