

Characterization of High-Pressure-Treated Egg Albumen

Stefania Iametti,^{†,‡} Elena Donnizzelli,^{†,‡} Paola Pittia,[§] Pier Paolo Rovere,^{||}
Nicoletta Squarcina,^{||} and Francesco Bonomi^{*,†,‡}

Dipartimento di Scienze Molecolari Agroalimentari and Centro Interuniversitario per lo Studio delle Macromolecole Informazionali, University of Milan, Via Celoria 2, I-20133 Milan, Italy;
Dipartimento di Scienze degli Alimenti, University of Udine, Via Marangon 31, Udine, Italy; and
SSICA, Via Tanara 31, Parma, Italy

Addition of NaCl or sucrose to egg albumen prior to high-pressure treatment (up to 10 min at 800 MPa) prevented insolubilization or gel formation after pressure treatment. As a consequence of protein unfolding, the treated albumen had increased viscosity but retained its foaming and heat-gelling properties. Susceptibility of egg albumen proteins to hydrolysis by trypsin increased dramatically after pressure treatment. The S-form of ovalbumin, the presence of which is an index of egg aging, was not found in any of the pressure-treated samples, which also did not display evidence for covalent protein aggregation. However, recognition of ovalbumin by an anti-ovalbumin antiserum was reduced to 40% of that of untreated sample.

Keywords: *Egg albumen; high pressure; foaming and heat-gelling properties; digestibility; food allergens*

INTRODUCTION

Chicken egg albumen represents an extensively used food ingredient, mostly because of its functional properties. The gelling, emulsifying, and foaming properties of fresh albumen are fundamental for making possible the production and assessment of the final properties (texture, flavor, etc.) of many food products. In contrast, most of the functional properties of egg albumen are lost or modified after even the mildest heat treatments (i.e., pasteurization) that are normally used for sanitation of egg components.

It was observed by some authors that liquid egg white partially coagulated when treated at pressure >500 MPa and that strong self-supporting gels were formed at pressure >600 MPa (Messens et al., 1997). The hardness and the elastic modulus of the gels increased with increasing pressure but remained significantly lower than those obtained by heat treatment (Okamoto et al., 1990). High-pressure treatment of eggs or of their components (yolk or albumen) represents a very interesting technology, because the treated samples retained their natural flavor and nutritional value (Hayashi et al., 1989; Mine, 1995).

Ovalbumin, which represents >50% (w/w) of egg white proteins, has a prominent role in determining functional properties of albumen. As described by many authors (Kato et al., 1987; Doi et al., 1991; Iametti et al., 1998), modifications induced by high-pressure treatment of isolated ovalbumin resulted in some structural changes that were correlated to the variation of some

functional properties. Of particular relevance to these issues are modifications affecting the four SH groups present in ovalbumin. The thiols are important in the formations of protein aggregates and in the stabilization of a gel structure upon heat treatment, which likely occurs through a disulfide-exchange mechanism (Mine, 1996). However, the effects of other components in egg albumen on treatment-induced molecular modifications of ovalbumin were not considered in these studies.

Among the most relevant effects of conformational modification of ovalbumin induced by a technological treatment are those involved in determining the susceptibility of treated material to trypsin action (Takahashi and Hirose, 1992). Native ovalbumin is reportedly resistant to digestion by trypsin, whereas heat-denatured or acid-treated ovalbumin displays an increased susceptibility to this protease (Ottesen and Wallevik, 1968). Ovalbumin is also one of the major food allergens (Matsuda and Nakamura, 1993), and an allergenic epitope of ovalbumin has been identified (Johnsen and Elasyed, 1990; Kahlert et al., 1992). Studies on treatment-dependent changes in the recognition of ovalbumin by specific antibodies were carried out on solutions of the pure protein and have indicated a pressure-induced decrease in the number of epitopic regions (Iametti et al., 1998).

In this study we tried to characterize in a comprehensive manner the modifications of a number of properties of albumen when it was treated in a pressure range high enough to be relevant to food sanitation (Miglioli et al., 1997). Pressure-induced variations in the foaming and gelling capacities of albumen treated in conditions that left it in soluble form were related to structural changes of albumen proteins, such as the formation of aggregates and their susceptibility to tryptic digestion. Structural modifications of ovalbumin in the complex matrix of treated albumen were studied in terms of protein recognition by suitable antibodies.

* Author to whom correspondence should be addressed (telephone +39-02-2663662; fax +39-02-70633062; e-mail francesco.bonomi@unimi.it).

[†] Dipartimento di Scienze Molecolari Agroalimentari.

[‡] Centro Interuniversitario per lo Studio delle Macromolecole Informazionali.

[§] Dipartimento di Scienze degli Alimenti.

^{||} SSICA.

MATERIALS AND METHODS

Reagents and Chemicals. Chicken eggs were collected from a local market. Chemicals were of analytical or HPLC grade. Ovalbumin (grade III), trypsin (type XIII), and rabbit anti-ovalbumin antibodies were from Sigma (St. Louis, MO). Peroxidase-labeled anti-rabbit immunoglobulins were from Bio-Rad (Richmond, CA). Chemicals were of analytical or HPLC grade.

Methods. High-pressure treatments were performed on bulk albumen solutions separated from eggs, in the presence of 10% (w/w) of sucrose or NaCl. For each treatment, 10 mL of egg solution was sealed in a plastic bag before pressure treatment. An aliquot of the sample was kept as an untreated control. Pressure treatment was carried out in water at a nominal temperature of 25 °C in an isostatic high-pressure generator ABB Model QFP-6 (ABB), with pressure settings of 450, 600, and 800 MPa. Under our conditions, the temperature increase was 2 °C/100 MPa, so that the highest temperature (~35–36 °C) was reached for samples treated at 800 MPa. The pressurization step of the samples took between 60 and 90 s depending on the final pressure achieved. During the holding time (which is referred to as the actual treatment time), and the following instantaneous decompression, the temperature of the samples decreased to 25 ± 2 °C. Immediately after the pressure treatment, the treated solutions were refrigerated and kept at 0–4 °C until further analysis.

The sanitizing effects of high-pressure treatments were evaluated on whole beaten eggs by means of a microbial challenge. The test was carried out by inoculating the mixture with a pool of spoiling microorganisms, either isolated at the Stazione Sperimentale per l'Industria delle Conserve Alimentari or from other collections. The strains used were *Streptococcus faecalis* QM-2, *Staphylococcus aureus* A110, and *Escherichia coli* ATCC 25922. Yolks and whites were gently mixed, and after addition of 10% NaCl (w/w), they were inoculated with the microbial pool before treatment for 5 min at 400, 500, or 600 MPa. Microbiological analyses on inoculated samples were carried out just after the high-pressure treatments by using the following media and techniques: *Str. faecalis*, Enterococcus agar (Oxoid), sowing on surface and incubation at 37 °C for 24 h; *Sta. aureus*, Baird Parker agar (Oxoid) containing 5% yolk and 1% potassium tellurite, sowing on surface and incubation at 37 °C for 24 h; *E. coli*, Violet red bile glucose agar (Oxoid), sowing in inclusion at 30 °C for 24 h.

For assessing foaming properties, egg albumen was beaten at for 3.5 min at 25 °C with a Philips Masterchef beater set at maximum speed. Foam density was measured by weight. Foam consistency was measured in triplicate assays run with a Stable Micro System texture analyzer equipped with a 2.5 kg detection cell. The probe had a diameter of 35 mm and was moving at 60 mm/min. Tests were performed in a cylindrical cell (50 × 70 mm) on a sample volume of 78 mL.

Heat-set gels were prepared by heating untreated or high-pressure-treated albumen as described below. The albumen samples were put in a cylindrical polyethylene box (40 × 40 mm), sealed under vacuum, and heated at 90 °C for different times (10–60 min) in a water bath. After heat treatment, the boxes were cooled at 4 °C in a water-ice bath and stored at 4 °C for 18 h until analysis. Analysis of the strength of the gel was performed using an Instron Universal Testing Machine. After removal of the upper part of the polyethylene boxes, the gels formed were carefully taken out and cut into slices of 17–18 mm height. Penetration experiments were performed by means of a circular probe (11 mm diameter), penetrating for 8 mm into the gel slice at a speed of 20 mm/min. The maximum load during the force deformation curve was taken as the gel strength. Data reported are the average of at least six repeated determinations.

Nonreducing SDS-PAGE was performed in a 12% monomer gel by using a Bio-Rad MiniGel Protein II apparatus and by omitting 2-mercaptoethanol from the sample preparation buffer (Cairolì et al., 1994).

Separation of different albumen proteins, and in particular

of native and *S*-ovalbumin, was performed by using HPLC ion-exchange chromatography (Perini, 1995). Proper amounts of albumen samples were diluted to 13 mg/mL final concentration in ovalbumin with 30 mM Tris-HCl, pH 7.2. A 0.2 mL aliquot of this solution was applied on a TSK-DEAE column (Waters, Bedford, MA; 0.8 × 7.5 cm) previously equilibrated with 30 mM Tris-HCl, pH 7.2, containing 0.06 M NaCl (buffer A). The different protein fractions were separated in 25 min by a linear gradient obtained by mixing buffer A with a 30 mM Tris-HCl, pH 7.2, containing 0.3 M NaCl (buffer B). The flow rate was 1 mL/min, in a Waters 625 HPLC, connected to a Waters 490 UV-vis detector set at 280 nm. The presence of *S*-ovalbumin was identified by comparison with samples of aged albumen in which conversion of native to *S*-ovalbumin was detected by microcalorimetry (Donovan and Mapes, 1976).

For the direct, noncompetitive ELISA test, polystyrene microtiter plates (Nunc-Immuno Plate Maxi Sorp) were coated overnight at 4 °C with 0.1 mL of albumen sample diluted with 0.1 M sodium bicarbonate, pH 9.6, to a protein concentration ranging from 0.1–1 to 0.0001–0.001 mg/mL. Coated plates were washed with buffer (phosphate buffer saline/Tween, PBST: 0.015 M potassium phosphate, 0.15 M NaCl, pH 7.4, containing 0.5 g/L Tween 20). The residual protein binding sites were blocked by incubation with 30 g/L gelatin (EIA grade reagent gelatin, Bio-Rad) in PBS (0.015 M potassium phosphate, 0.15 M NaCl, pH 7.4) for 90 min at 37 °C. After extensive washing with PBST, plates were incubated for 2 h at 37 °C with 0.1 mL of rabbit anti-ovalbumin serum (1/20000, v/v) in PBST buffer containing 1% (w/v) gelatin. Wells were then washed again with PBST, rinsed with 0.1 mL of peroxidase-labeled goat anti-rabbit immunoglobulins (1/2500), and incubated for 1 h at 37 °C in PBST containing 1% gelatin. After removal of unbound reagents by repeated washes with PBST and PBS, the antigen-antibody complexes were revealed by addition of a solution containing 0.3 mg/mL *o*-phenylenediamine and 0.03% (v/v) H₂O₂ in 0.5 M sodium citrate buffer, pH 5.0. The reaction was stopped by addition of 0.05 mL of 4 N sulfuric acid. The optical density of each well was measured at 490 nm in a Bio-Rad 3550 microplate reader. Absorbance figures were corrected for average blank readings, and Bio-Rad software was used to correlate absorbance values with antigen concentration.

The direct competitive ELISA test was carried out as described above except for the following modifications. The plates were coated with 0.1 mL of untreated ovalbumin standard solution with a protein concentration of 0.001 mg/mL. The interaction between rabbit anti-ovalbumin serum (1/20000, v/v) and coated ovalbumin was performed in the presence of different concentrations of treated albumen samples (competitor). Development with the secondary conjugate antibodies and the chromogenic substrates and quantitative plate analysis were carried out as above. Data from all of the ELISA tests are reported as residual antibody recognition, in which the antibody recognition of untreated albumen was taken to be equal to 100%.

Susceptibility of albumen samples to tryptic hydrolysis was detected by incubation of 0.4 mL of treated samples (containing 1 mg/mL protein in 50 sodium phosphate buffer, pH 6.8) with 0.5 mg of trypsin at 37 °C. The proteolytic reaction was stopped by adding an equal volume of 20% (w/v) trichloroacetic acid, and the protein precipitate was removed by centrifugation at 13000g for 15 min. The absorbance at 280 nm of the clear supernatant phase was measured, and the hydrolyzed substrate was expressed as the amount of tyrosine released in the supernatant, determined by using $A_{280} = 1.25$ for tyrosine.

RESULTS AND DISCUSSION

Preparation and Functional Properties of Pressure-Treated Albumen in Soluble Form. High-pressure treatment of egg albumen or whole egg as such reportedly results in the formation of gels (Messens et al., 1997). We found that by adding 7–10% of common food ingredients such as sucrose or NaCl to fresh

Table 1. Reduction of Microbial Count upon High-Pressure Treatment of Whole Eggs in the Presence of 10% NaCl^a

treatment pressure, MPa	micro-organism	initial inoculum, cfu/mL	final count after pressure treatment, cfu/mL	decimal reduction
400	<i>Sta. aureus</i>	3.0×10^5	2.0×10^5	0.2
	<i>Str. faecalis</i>	1.8×10^4	1.8×10^3	1.0
	<i>E. coli</i>	5.0×10^5	0	=
600	<i>Sta. aureus</i>	3.0×10^5	1.2×10^4	1.4
	<i>Str. faecalis</i>	1.8×10^4	1.8×10^2	2.0
	<i>E. coli</i>	5.0×10^5	0	=
800	<i>Sta. aureus</i>	3.0×10^5	6.0×10^2	2.7
	<i>Str. faecalis</i>	1.8×10^4	6.0×10^1	2.5
	<i>E. coli</i>	5.0×10^5	0	=

^a Whole eggs were gently mixed before dissolving NaCl and inoculating with the given amount of each bacterial species. Pressure treatment was carried out at nominal 25 °C for 5 min at the given pressure.

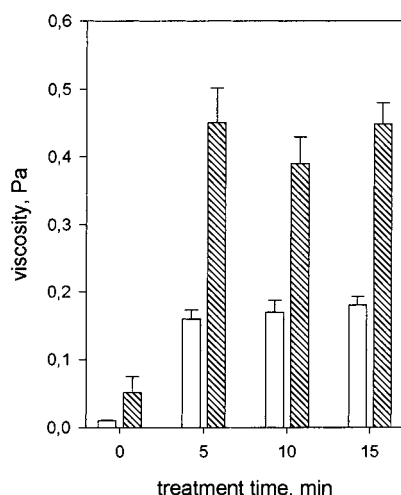


Figure 1. Changes in viscosity of pressure-treated egg albumen. Sucrose (shaded bars) or NaCl (open bars) was separately added to fresh egg albumen to a final concentration of 10% (w/v). Viscosity was measured before and after treatment at 600 MPa for the times given.

albumen, no major precipitation or formation of gels occurred for exposure to pressures up to 800 MPa for 10 min at room temperature. Only a small turbidity was evident in albumen treated for 10 min at 800 MPa in the presence of 10% NaCl.

The reduction in water activity caused by the addition of the two "protectants" did not reduce the sanitizing effects of high-pressure treatment (Maggi et al., 1995). The results of experiments in which mixed whole egg samples with added 10% NaCl were inoculated with a large number of common egg-contaminant microorganisms and treated for 5 min at increasing pressure are reported in Table 1. A substantial, pressure-dependent sanitation effect was evident, with the sample treated for 5 min at 600 MPa being of equal or better quality than pasteurized albumen. For this reason, we chose 5 min at 600 MPa as our standard treatment. The results reported here confirm previous observations on the barosensitivity of different microorganisms in food systems (Gola et al., 1997).

Although the albumen treated in the presence of 10% of either NaCl or sucrose remained soluble, the viscosity of the treated material increased remarkably. As shown in Figure 1, the increase in viscosity was independent of the treatment time but was much larger in the

Table 2. Strength of Gels Formed upon Heat Treatment of Pressure-Treated Albumen^a

treatment pressure, MPa	gel strength, N		
	no addition	10% NaCl	10% sucrose
untreated	5.63 ± 0.20	3.35 ± 0.14	4.62 ± 0.07
600	3.31 ± 0.29	3.89 ± 0.15	3.11 ± 0.21

^a Sucrose or NaCl was separately added to fresh egg albumen. The strength of gels formed upon heat treatment at 90 °C for 30 min was measured on the untreated sample and on the sample treated for 5 min at the given pressure.

sample treated in the presence of sucrose. In this context, it has to be remembered that 10% NaCl (≈ 1.7 M) is by far more osmotically active than 10% sucrose (≈ 0.55 M) and that the two compounds differ with regard to their water-binding properties. This, in turn, could affect their activity as "protectants" of macromolecular structures. Indeed, at the same concentrations used here, sucrose was demonstrated to be by far less effective than NaCl in protecting purified ovalbumin against loss of structure upon high-pressure treatment (Iametti et al., 1998). Also, Donovan et al. (1975) reported that 10% sucrose increased the heat stability of egg white proteins due to reduced water activity. In this and other studies (Woodward and Cotteril, 1983) the higher heat stability observed in the presence of 10% NaCl was attributed to changes of ionic strength in the liquid phase immediately surrounding the proteins.

The soluble nature of the product obtained upon high-pressure treatment of albumen in the presence of either protectant could make it practical for direct use in food, provided that the treated material retains the functional properties of albumen. The heat-gelling properties of treated and untreated albumen/protectant mixtures were studied by exposing samples at 90 °C for different times, and the strengths of gels obtained after 30 min of heating are summarized in Table 2. Addition of either protectant to untreated albumen decreases the strength of heat-induced gels, with 10% NaCl causing a greater decrease than 10% sucrose. The strength of the heat-induced gels obtained from pressure-treated albumen increased slightly for the albumen treated in the presence of NaCl but decreased markedly for the other two samples. The strength of the treated sample containing sucrose was much lower than that of the one containing NaCl. For all the pressure-treated samples, prolonged heating (up to 60 min) produced gels of increasing strength. As suggested by the weakness of the gel formed in the absence of a pressure treatment, the addition of salt inhibits the protein-protein interactions within the conformers present at high temperature, which are responsible for heat-induced gel formation. The effect of pressure on the albumen proteins in the presence of salt results in the appearance of protein conformers that are more likely to interact and thus give a firmer gel upon short heating times.

The properties of foams formed by treated and untreated albumen samples were assessed by using a texture analyzer. With this approach, consistency of the foam is expressed by the ratio between the positive signal stemming from probe penetration and the negative signal produced by the adhesion of the foam to the probe on its return trip. Consistency values are listed in Table 3 along with foam density. Foams obtained from reference samples had a greater consistency and a lower density than those obtained from pressure-treated albumen. The density of the foam obtained from the albumen pressure-treated in the presence of NaCl

Table 3. Properties of Foams Formed by Egg Albumen Containing NaCl or Sucrose before and after Treatment at 600 MPa for 5 min^a

	no addition	10% sucrose		10% NaCl	
	untreated	untreated	treated	untreated	treated
A1, ^b g·s	1275 ± 123	2267 ± 183	1228 ± 111	2615 ± 188	1322 ± 123
A2, ^c g·s	881 ± 75	1326 ± 87	872 ± 121	1655 ± 145	901 ± 99
consistency, A1/A2	1.45	1.71	1.41	1.58	1.47
foam density, g/mL	na ^d	0.26 ± 0.01	0.31 ± 0.02	0.25 ± 0.01	0.18 ± 0.02

^a Treated or untreated egg albumen was beaten for 3.5 min at 25 °C with a Philips Masterchef beater set at maximum speed. Results are the average of triplicate experiments. ^b Data refer to the measured area during probe penetration (A1). ^c Data refer to the measured area during probe release (A2). ^d Not available.

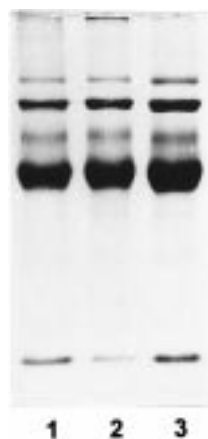


Figure 2. SDS-PAGE analysis of high-pressure-treated albumen. Albumen samples containing 10% NaCl were treated for 5 min at the given pressure. Aliquots (0.005 mg of protein) of the treated material were analyzed by nonreducing SDS-PAGE. Lane 1, albumen treated at 600 MPa; lane 2, albumen treated at 400 MPa; lane 3, fresh, untreated albumen.

was much lower than that of any other sample, whereas the foam obtained from the albumen pressure-treated in the presence of sucrose was the highest. This could be related to the different denaturation upon pressure treatment of the major protein component of egg albumen, that is, ovalbumin (Iametti et al., 1998).

Changes in the Protein Composition and Properties in High-Pressure-Treated Albumen. Treated albumen samples were analyzed by SDS-PAGE and by HPLC ion-exchange chromatography to study the possible correlation between the functional properties described above and changes in the protein pattern in high-pressure-treated albumen.

SDS-PAGE in the absence of reductants was used to detect the formation of disulfide-stabilized protein aggregates. The SDS-PAGE pattern of albumen treated at 400 or 600 MPa for 5 min is shown in Figure 2. Polypeptide distribution in high-pressure-treated albumen was similar to that in fresh albumen, indicating that pressure processing did not cause formation of protein aggregates stabilized by disulfide interactions.

To detect possible chemical modification of major protein components, high-pressure-treated albumen samples were analyzed by HPLC ion exchange chromatography by using conditions developed to identify the S-form of ovalbumin. The relative amount of the S-form of ovalbumin represents a useful index of aging in eggs (Donovan and Mapes, 1976). The HPLC profile of an albumen sample treated at 600 MPa for 5 min is compared in Figure 3 with that of fresh albumen. The virtual identity of the protein patterns in Figure 3 indicates that the S-form of ovalbumin was not present in high-pressure-treated albumen (Perini, 1995). This,

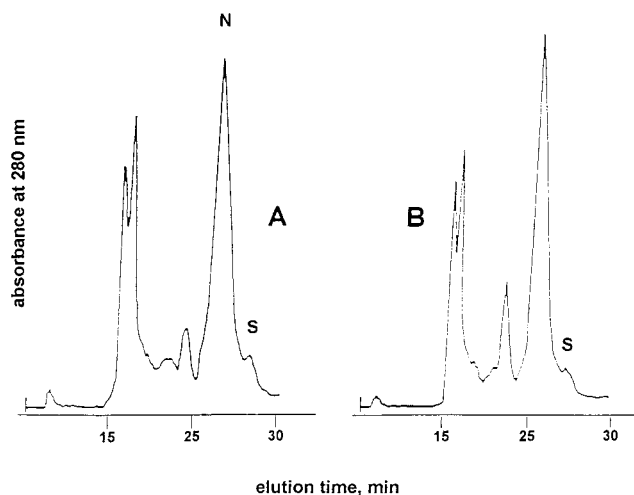


Figure 3. Ion-exchange HPLC of fresh and pressure-treated albumen. Aliquots (2.6 mg of total protein) of untreated (A) or pressure-treated albumen (600 MPa for 5 min, 10% NaCl, B) were analyzed by ion-exchange HPLC. N and S indicate the native and S forms of ovalbumin, respectively.

along with the evidence provided by SDS-PAGE, indicates that the proteins in pressure-treated albumen are similar to those of fresh albumen.

Susceptibility of the Treated Albumen to Proteolysis. The susceptibility of high-pressure-treated albumen samples to the action of trypsin has practical relevance from technological and nutritional points of view. In fresh albumen, the major protein component (i.e., ovalbumin) is completely resistant to digestion by trypsin in the absence of a prior denaturation step. Indeed, susceptibility to tryptic hydrolysis has been taken as an index of structural integrity for many proteins, including ovalbumin and egg lysozyme. As reported in previous studies, purified ovalbumin that was pressure treated at neutral pH in the presence of either sucrose or NaCl showed an increase in its susceptibility to proteolysis (Iametti et al., 1998).

High-pressure treatment of albumen resulted in an increase in the susceptibility to trypsin with increasing pressure from 400 to 600 MPa, as shown in Figure 4. The increase in the susceptibility to proteolysis observed after treatment in the presence of sucrose was larger than that observed in the presence of NaCl. This confirms that NaCl and sucrose have different effects on the albumen proteins as for their unfolding, as hypothesized above in the discussion of the strength of gels formed by albumen that was pressure-treated in the presence of different protectants. The different influences of sucrose and NaCl on tryptic proteolysis of high-pressure-treated albumen also parallel what was observed for pressure-treated ovalbumin (Iametti et al., 1998), thus circumstantially confirming the role of

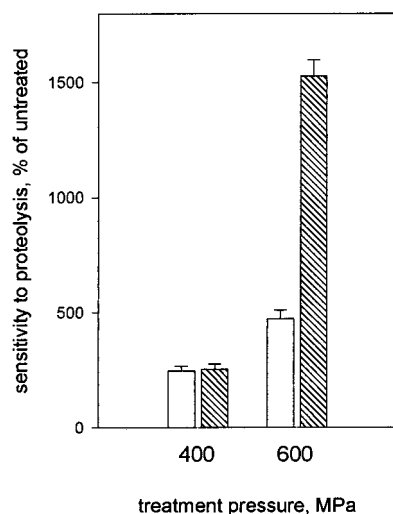


Figure 4. Susceptibility to trypsin of albumen treated at different pressures in the presence of NaCl or sucrose. NaCl (open bars) or sucrose (shaded bars) was added to separate aliquots of fresh egg albumen to a final concentration of 10% (w/v). Albumen was then treated at the given pressure for 5 min. Susceptibility to trypsin is expressed as percent of that of untreated albumen.

Table 4. Residual Recognizability of Ovalbumin by Anti-Ovalbumin Antibodies in Pressure-Treated Albumen^a

treatment pressure, MPa	recognizability by antiovalbumin antibodies, % of untreated albumen	
	sucrose	NaCl
400	42 ± 3	45 ± 7
600	39 ± 7	42 ± 2

^a Sucrose or NaCl was separately added to fresh egg albumen to a final concentration of 10% (w/v). Samples were treated for 5 min at the given pressures and analyzed through direct, noncompetitive ELISA. Results are the average of triplicate experiments.

ovalbumin in assessing the modifications of albumen induced by high-pressure.

Modification of Epitopic Regions of Ovalbumin in Pressure-Treated Albumen Samples. Treatments aimed at reducing the human immune response to egg proteins in food are of potentially great relevance in human nutrition, given the high number of individuals who are sensitive to this class of products. In this regard ovalbumin, which represents 51% of albumen proteins, is identified as the main component responsible for human intolerance to albumen proteins. It was also observed that high-pressure treatment of isolated ovalbumin resulted in a modification of epitopic regions of the protein (Iametti et al., 1998), with a marked reduction of its recognizability by anti-ovalbumin antisera in a direct, noncompetitive ELISA format test using antibodies raised against purified ovalbumin. For practical purposes, it must be assessed whether this kind of modification was retained when the whole albumen was pressure-treated.

As shown in Table 4, the residual immunochemical reactivity of ovalbumin in albumen samples treated at 400 or 600 MPa for 5 min was 60% of that of the ovalbumin in untreated albumen. Little pressure dependence was observed. Under the same treatment conditions, loss of antibody recognition in treated albumen samples was identical to that measured on treated isolated ovalbumin (Iametti et al., 1998). The absence of differences between the two systems is of practical

interest and is somewhat surprising, considering the many differences between the model and the real system, which include the high pH of albumen, the remarkably different protein concentrations, and the presence of other albumen proteins that could make ovalbumin in whole albumen less sensitive to pressure denaturation.

To prove that the observed modifications in antibody recognition could be ascribed to structural modifications of ovalbumin and not to a different adhesion of the albumen components to the microtiter plate wells, immunochemical tests were repeated by using a competitive ELISA format. These confirmed that the residual immunochemical reactivity of ovalbumin in albumen samples treated at 400 or 600 MPa for 5 min was at least 75% of that of the ovalbumin in untreated albumen.

Sucrose and NaCl had the same effect on the loss of recognizability by antibodies, suggesting that the two protectants had no effect in preventing those particular conformational modifications of treated ovalbumin that resulted in surface epitopic changes.

CONCLUSIONS

This work demonstrates that it is possible to use high-pressure treatments to stabilize egg albumen from a microbiological standpoint while retaining most of the technologically relevant properties of the material. By adding NaCl or sucrose prior to the treatment, foams and heat-set gels could be obtained from pressure-treated albumen, although their properties were somewhat different from those obtained from the untreated material. Sucrose and NaCl have different effects with regard to the technologically relevant properties of the pressure-treated product, a fact that could be exploited to customize the product of pressure treatment for subsequent use in different food technologies.

Pressure treatment of albumen, in conditions that do not impair its potential as a food component, also results in the acquisition of highly desirable properties. In the conditions depicted here for the treatment of egg white, protein digestibility is highly increased, and *in vitro* recognition of ovalbumin epitopes by anti-ovalbumin antibodies is appreciably lowered. The latter result is comparable with previous observations on dilute solutions of isolated ovalbumin at neutral pH, suggesting the absence of significant matrix and pH effects.

If our current work will show that these two properties will be retained upon further processing of foods in which the pressure-treated albumen is used as an ingredient, the findings presented here could represent a first step toward a rather useful application of high pressure to Western-culture food.

ACKNOWLEDGMENT

We thank Mr. Aristodemo Carpen and Dr. Secondo Gola for helpful assistance with immunochemical and microbiological determinations, respectively. We greatly appreciated the continuous support of Prof. Gianfranco Dall'Aglio.

LITERATURE CITED

Cairolì, S.; Iametti, S.; Bonomi, F. Reversible and irreversible modifications of β -lactoglobulin upon exposure to heat. *J. Protein Chem.* **1994**, *13*, 347–354.

- Doi, E.; Shimizu, A.; Oe, H.; Kitabatake, N. Melting of heat-induced ovalbumin gel by pressure. *Food Hydrocolloids* **1991**, *5*, 409–425.
- Donovan, J. W.; Mapes, C. J. A differential scanning calorimetric study of conversion of ovalbumin to S-ovalbumin in eggs. *J. Sci. Food Agric.* **1976**, *27*, 197–204.
- Donovan, J. W.; Mapes, C. J.; Davis, J. G.; Garibaldi, J. A. A differential scanning calorimetric study of the stability of egg white to heat denaturation. *J. Sci. Food Agric.* **1975**, *26*, 73–78.
- Gola, S.; Maggi, A.; Rovere, P.; Scaramuzza, N.; Dall'Aglio, G. F. Inactivation of microorganisms treated with high pressures in model systems and in foods. In *High-Pressure Biology and Medicine*; Bennet, P. B., Demchenko, I., Marquis, R. E., Eds.; Rochester University Press: Rochester, NY, 1998; pp 83–89.
- Hayashi, R.; Kawamura, Y.; Nahasa, T.; Okinaka, O. Application of high pressure to food processing: pressurization of egg white and yolk, and properties of gels formed. *Agric. Biol. Chem.* **1989**, *53*, 2935–2939.
- Iametti, S.; Donnizzelli, E.; Vecchio, G.; Rovere, P. P.; Gola, S.; Bonomi, F. Macroscopic and structural consequences of high-pressure treatment of ovalbumin solutions. *J. Agric. Food Chem.* **1998**, *46*, 3521–3527.
- Johnsen, G.; Elssayed, S. Antigenic and allergenic determinants of ovalbumin—III. MHC Ia-binding peptide (OA 323–339) interacts with human and rabbit specific antibodies. *Mol. Immunol.* **1990**, *27*, 821–827.
- Kahlert, H.; Petersen, A.; Becker, W.-M.; Schlaak, M. Epitope analysis of the allergen ovalbumin (Gal dII) with monoclonal antibodies and patients' IgE. *Mol. Immunol.* **1992**, *29*, 1191–1201.
- Kato, A.; Takagi, T. Estimation of the molecular weight distribution of heat-induced ovalbumin aggregates by the low-angle laser light scattering technique combined with high performance gel chromatography. *J. Agric. Food Chem.* **1987**, *35*, 633–637.
- Maggi, A.; Gola, S.; Rovere, P.; Dall'Aglio, G. Effetti delle alte pressioni sui microorganismi. In *L'impiego delle alte pressioni nell'industria alimentare*; CNR–RAISA, Subproject 4: Parma, Italy, 1995; pp 11–16.
- Matsuda, T.; Nakamura, R. Molecular structure and immunological properties of food allergens. *Trends Food Sci. Technol.* **1993**, *4*, 289–293.
- Messens, W.; Van Camp, J.; Huyghebaert, A. The use of high pressure to modify the functionality of food proteins. *Trends Food Sci. Technol.* **1997**, *8*, 107–112.
- Miglioli, L.; Gola, S.; Maggi, A.; Rovere, P.; Carpi, G.; Scaramuzza, N.; Dall'Aglio, G. F. Microbiological stabilization of low-acid food using a combined high-pressure temperature process. In *High-Pressure Research in Bioscience and Biotechnology*; Heremans, K., Ed.; Leuven University Press: Leuven, Belgium, 1997; pp 277–280.
- Mine, Y. Recent advances in understanding of egg white protein functionality. *Trends Food Sci. Technol.* **1995**, *6*, 225–230.
- Mine, Y. Laser light scattering study on the heat-induced ovalbumin aggregates related to its gelling property. *J. Agric. Food Chem.* **1996**, *44*, 2086–2090.
- Okamoto, M.; Kawamura, Y.; Hayashi, R. Application of high pressure to food processing: textural comparison of pressure and heat-induced gels of food proteins. *Agric. Biol. Chem.* **1990**, *54*, 183–189.
- Ottesen, M.; Wallevik, K. Use of pH-stat for measuring the denaturation of ovalbumin in acid solution. *Biochim. Biophys. Acta* **1968**, *160*, 262–264.
- Perini, F. Modificazioni del punto isoelettrico dell'ovoalbumina nel corso dell'invecchiamento dell'uovo. M.S. Thesis, University of Milan, Italy, 1995.
- Takahashi, N.; Hirose, M. Reversible denaturation of disulfide-reduced ovalbumin and its reoxidation generating the native cysteine cross-link. *J. Biol. Chem.* **1992**, *267*, 11565–11572.
- Woodward, S. A.; Cotterill, O. J. Electrophoresis and chromatography of heat-treated plain, sugared and salted whole egg. *J. Food Sci.* **1983**, *48*, 501–506.

Received for review August 3, 1998. Revised manuscript received May 3, 1999. Accepted May 18, 1999. Work supported in part by grants of the Italian Ministry for the University and Scientific and Technological Research (MURST, Rome, Italy, National Project "Biocatalisi e Bioconversioni").

JF9808630