Food Bioprocess Technol (2010) 3:150–154
DOI 10.1007/s11947-009-0257-0

COMMUNICATION

# Mechanical Properties of Ovalbumin Gels Formed at Different Conditions of Concentration, Ionic Strength, pH, and Aging Time

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Received: 7 November 2008 / Accepted: 28 August 2009 / Published online: 25 September 2009


**Abstract** Ovalbumin gels were prepared by heat treatment at constant pH and ionic forces. Ovalbumins are widely utilized as emulsifying or binding agents. However, due to their protein origin, mechanical properties of ovalbumins are enclosed in a wide range of rheological responses depending on concentration, ionic strength, pH, and aging time. The objective of this work was to study the effect of processing conditions (pH, ionic strength, and protein content) on the textural attributes of an ovalbumin protein system by means of uniaxial compression. Gels were prepared by dispersing proteins (purity 98%) (8.3–12.5% *w*/*w*) until complete dissolution in deionized water at 90°C by 45 min, pH (6.3–9.1) was adjusted using citric acid, and the ionic strength (0–100 mM of NaCl) was adjusted using NaCl. The storage of gels was done at 63°C (24–168 h). The rheological tests of gels were done by uniaxial compression. A rupture force peak was observed at high protein content together with an increase in the Young's modulus. At fixed conditions of ion content (NaCl 50 mM) and pH of 7, the gels presented a maximum in fracture force and Young's modulus after 7 days of storage. The addition of minimum amount of citric acid increases the stability of ovalbumin gels. This information is useful to ensure that the final product will remain stable during storage time at longer shelf lives.

**Keywords** Aging time · Gels · Ovalbumin · Proteins · Rheology · Uniaxial compression

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## Introduction

The ability to form a gel is an important function of proteins in food systems (Totosaus et al. 2002). Most food protein gels are formed by denaturation, aggregation, and gelation during heating process (Geveke 2008). This is because heating protein dispersions causes molecular unfolding, which leads to the partial aggregation of proteins and hence gelation (Choi et al. 2008; Icier and Bozkurt 2009). Most proteins can form gels. Ovalbumin, a protein that can form gels, is the most abundant protein in the egg white. It is classified as a phosphoprotein containing both carbohydrates and phosphorus. Additionally, ovalbumin exhibits interesting functional attributes, such as emulsification capacity, which allows its use for the modification of food texture (Pietrasik 2003).

The emulsifying and stabilizing capacity is caused by the lipoproteins which are mainly hydrophilic colloids that

migrate to the water–oil interface favoring therefore the formation of emulsions. Egg proteins facilitate oil dispersion contributing to the product consistency; this is the case of products such as mayonnaise and sauces. In the industry (i.e., baking, cosmetics, and drugs), these proteins are used because of their gel-forming capacity.

There have been many studies on the effects of salts on denaturation and gelation of globular proteins (Shimada and Matsushita 1980; Weijers et al. 2002). Sodium chloride affects thermal stability of globular protein near neutral pH and, at lower concentrations, it stabilizes the globular protein molecule (Matsudomi et al. 1991). The strength of globular protein gels increases with increased ionic strength by adding salts (Kohnhorst and Mangino 1985). However, to our knowledge, there are no published works about the combined effect of pH, ionic strength by adding salts, and stored time on the mechanical behavior of gels of ovalbumin. In addition, the ions in solution increase electrostatic repulsions among protein aggregates, thus modifying the structure, solubility, and textural attributes of the resulting gel (Borbas et al. 2003).

Some common rheological properties in mechanical testing are: fracture or rupture, elongation at fracture, and fracture stress (Foegeding et al. 1995). It is possible to obtain information from the ovalbumin structure and its gel behavior by means of fundamental tests and to relate the mechanical response to the textural attributes (Totosaus et al. 2005).

One of the most important material functions in the rheological characterization of solid materials is the Young's modulus, a property associated with the rigidity of the material. Larger values of Young's modulus correspond to more rigid materials. Most food products are viscoelastic materials with non-linear stress–strain curves. In this way, the capacity of a gel to exhibit rigidity or elasticity is based on the composition, concentration, ionic strength, temperature, and the protein thermal history (Weijers et al. 2002).

The objective of this work was to study the effect of processing conditions on the textural attributes of an ovalbumin protein system by means of uniaxial compression tests. These preparation conditions were studied by varying extreme settings of protein and citric acid content, ionic strength, and storage time. This research attempted to quantify and make clear the effect of preparation and process parameters on the mechanical behavior of ovalbumin gels.

## Materials and Methods

### Materials and Reagents

Dried ovalbumin powder was obtained as a commercial product (98% purity, particle size mesh of 100, lot # AN23 VII-1025; La Granja, México). The physical and chemical properties were determined in situ (see Table 1). Chemicals used were citric acid (Baker ACS grade, Mexico City, Mexico) and sodium chloride (Baker ACS grade, Mexico City, Mexico).

### Gel Preparation and Experimental Setup

The ovalbumin gel was prepared by dispersing the protein powder in deionized water at 90°C until complete dissolution (45 min) under constant mechanical agitation. Then, the samples were collected in a syringe system (2 cm diameter, 5 cm length) and placed for in a storage system at least 4 h at 25°C prior to each test. The pH and ionic strength were adjusted in the solutions using citric acid and NaCl, respectively (see Table 2). The pH of the ovalbumin solution was measured using an electrode standardized against buffer solutions of known pH. Aging time experiments were tested by using citric acid at constant pH. The ionic strength was evaluated in function of the amount of NaCl added (0, 10, 50, and 100 mM of NaCl). Samples (for aging time experiments) were put into the oven lab at 63°C for 7 days. Samples for texture analyses were taken at 24, 127, 144, and 168 h (see Table 3). The formed gels were cut with a fixed height of 2.5 cm and finally the specimens were tested by uniaxial compression to obtain fundamental rheological parameters.

### Texture Analysis

The samples were placed between two rigid parallel plates. The upper plate moved downwards and the axial force was recorded as a function of time. In this work, a texturometer MTS Sintech model 1/S Universal Machine (MTS, USA) with a load cell of 10 N was used. The geometry was cylindrical with a diameter of 2.8 cm and a speed of 10 mm/min in compression. The samples were lubricated with low viscosity oil to reduce friction during compression. Axial force and displacement data were transformed to stress and Hencky strain (Tang et al. 1996; Medina-Torres et al. 2003) to obtain Young's modulus and fracture stress. The curves of uniaxial compression were carried out up to a deformation of 70% and the contact area (transverse section) was 3.14 $cm^2$. In agreement with Hencky, the true

**Table 1** Ovalbumin physicochemical specifications

| Composition | Method | Content/100g |
|---|---|---|
| Proteins N×6.68 | NMX-F608-NORMEX 2002 | 80 |
| Humidity | NOM-116-SSA1-1995 | 6 |
| Fat | NOM-086-SSA1-1994 | 1 |
| Ashes | NMX-F-607-NORMEX 2002 | 6 |

**Table 2** Experimental conditions of samples

| Test | pH | NaCl (mM) | Ovalbumin % (*w*/*w*) |
|---|---|---|---|
| Effect of concentration | 7.8±0.1 | 0 | 12.5 |
| | 7.8±0.1 | 0 | 10 |
| | 7.4±0.1 | 0 | 8.3 |
| Effect of ionic strength | 7.6±0.1 | 10 | 8.3 |
| | 7.8±0.1 | 50 | 8.3 |
| | 7.6±0.1 | 100 | 8.3 |
| Effect of pH | 6.3±0.1 | 0 | 8.3 |
| | 7.4±0.1 | 0 | 8.3 |
| | 9.1±0.2 | 0 | 8.3 |
| Effect of citric acid at pH7.4 | 0.1% (*w*/*w*) | 0 | 8.3 |
| | 0.15% (*w*/*w*) | 0 | 8.3 |
| | 0.2% (*w*/*w*) | 0 | 8.3 |

strain ($\varepsilon_H$) for the uniaxial compression test is given by the dimensional logarithm (Eq. 1) (Peleg 1987) that relates the height change at a given time $t$ ($\Delta H$) to the current specimen height at the same time ($H$).

$$\varepsilon_H = \int_{H_0}^{H} \frac{dH}{H} = \ln H - \ln H_0 = \ln \frac{H}{H_0} \quad (1)$$

where $\varepsilon_H$=Hencky strain (m m$^{-1}$), $H$=height at time $t$ (m), and $H_0$=initial altitude (m). In order to use the Hencky strain it is important to consider that the volume of the sample is constant.

When the normal stress is calculated, distinctions based on the cross-sectional area have to be considered. Since cross-sectional area of the specimen changes constantly during the test time, the stress calculated at the initial area is called "*engineering stress*". The engineering stress does not represent the material's true stress state, therefore to calculate it, instantaneous cross-sectional area is used (Medina-Torres et al. 2003). The true stress relates the force applied on the actual cross-sectional area at time $t$, given by Eq. (2):

$$\sigma_T \equiv \frac{F_T}{A_T(t)} \quad (2)$$

where $\sigma_T$=true stress (Pa), $F_T$=force applied at time $t$ [N], and $A_T$=cross-sectional at test time—this value was obtained from experimental flow curves and Hencky correction, $t$ [m$^2$]. Thus, true strain and stress are direct measures to evaluate and classify the mechanical response in this type of proteins.

The Young's modulus was determined as the slope line drawn at the initial zone (linear zone) in the stress–strain curve. For materials with predominantly non-linear behavior (non-linear zone), the Young's modulus is determined from the interception of the stress–strain curve with a vertical line drawn at small values of strain ($\varepsilon \leq 5\%$; Medina-Torres et al. 2003).

A factorial randomized design was used for this experimental work. A statistical analysis of data was performed using one-way analysis of variance ($p<0.05$) by the use of Statistica v. 5.2 (StatSoft, Tulsa, OK, USA; see Table 4). All experiments were done in triplicate.

## Results and Discussion

The effect of ovalbumin concentration on the mechanical properties of the gels is presented in Table 4. At constant pH (7.8), we can observe a higher fracture stress for a lower

**Table 3** Experimental conditions for aging experiments

| Aging time at 63°C | Condition | NaCl (mM) | Ovalbumin % (*w*/*w*) |
|---|---|---|---|
| 24 h | Citric acid 0.15% (*w*/*w*) | 0 | 8.3 |
| | Without citric acid | 0 | 8.3 |
| 127 h | Citric acid 0.15% (*w*/*w*) | 0 | 8.3 |
| | Without citric acid | 0 | 8.3 |
| 144 h | Citric acid 0.15% (*w*/*w*) | 0 | 8.3 |
| | Without citric acid | 0 | 8.3 |
| 168 h | Citric acid 0.15% (*w*/*w*) | 0 | 8.3 |
| | Without citric acid | 0 | 8.3 |

**Table 4** Results obtained from mechanical tests

| | $\varepsilon_f$ (–) | $\sigma_f$ (kPa) | Young's modulus (kPa) |
|---|---|---|---|
| Ovalbumin concentration % (*w*/*w*) | | | |
| 8.3 | 0.59±0.02a | 25.44±1.88a | 13.57±1.44a |
| 10.0 | 0.59±0.00a | 29.86±1.24b | 20.08±2.13b |
| 12.5 | 0.60±0.02a | 55.40±2.19c | 47.72±0.79c |
| Ionic force (NaCl) mM | | | |
| 10 | 0.58±0.01a | 21.25±1.15a | 16.46±1.81a |
| 50 | 0.61±0.01b | 25.78±0.45b | 15.24±0.54a |
| 100 | 0.60±0.01b | 22.86±0.15a | 13.69±1.26a |
| pH | | | |
| 6.3 | 0.59±0.01a | 17.64±0.74a | 11.47±2.08a |
| 7.4 | 0.56±0.01b | 17.59±0.53a | 10.64±0.27a |
| 9.1 | 0.60±0.02a | 24.31±0.51b | 9.08±1.15a |
| Citric acid % (*w*/*w*) | | | |
| 0.10 | 0.67±0.01a | 38.82±0.35a | 8.72±0.18a |
| 0.15 | 0.64±0.04a | 39.75±0.39b | 12.57±3.87b |
| 0.20 | 0.62±0.05a | 34.66±0.48c | 8.52±0.52a |
| Aging time | | | |
| 24 h, citric acid 0.15% | 0.66±0.01a | 34.77±0.10a | 11.12±0.32a |
| 24 h, no citric acid | 0.57±0.00b | 13.08±1.06b | 3.46±0.32b |
| 127 h, citric acid 0.15% | 0.62±0.01c | 28.02±0.40c | 7.99±0.02c |
| 127 h, no citric acid | 0.56±0.01b | 8.77±1.24d | 2.77±0.01d |
| 144 h, citric acid 0.15% | 0.62±0.00c | 39.11±0.16e | 10.58±0.32e |
| 144 h, no citric acid | 0.48±0.02d | 01.79±0.15f | 1.26±0.14f |
| 168 h, citric acid 0.15% | 0.62±0.00c | 37.80±0.01g | 10.01±0.30e |
| 168 h, no citric acid | 0.55±0.01e | 13.74±0.82b | 3.39±0.22b |

Different letters indicate statistical differences ($p<0.05$)

fracture strain, as the albumin concentration was increased. Thus, an increase in albumin concentration produces gels with lower malleability. A 50% increment in the fracture stress was observed for the gels containing 12.5% ovalbumin versus those with 8.3% ovalbumin content.

The results in compression tests for ovalbumin gels with different ion content are shown in Table 4. At ionic force of 50 mM, fracture stress increases up to a maximum of approximately 27 kPa. At higher ion content (100 mM of Na), the fracture force shows a decrease that may be caused by the $Na^+$ ions' enhancement of electrostatic repulsions among macromolecules, thus weakening the gel structure (Creighton 1993).

Below 50 mM of NaCl, a stabilization of the molecule was observed, evidenced by its mechanical response (Burguess and Sahin 1997). Both ions in the macromolecule tend to reduce the electrostatic free energy of the protein and to increase its solubility in the aqueous media, thus affecting the mechanical properties by increasing the gel stability (Creighton 1993). Although this effect is reversed when the ion content reaches a critical value, the ions' occurrence causes the macromolecules to repel themselves, weakening the gel structure (i.e., lower fracture stress).

On the other hand, no appreciable variation was observed in the fracture stress when increasing pH from 6.3 to 7.4 (Table 4), but an important enhancement was observed when pH increased to 9.1. A similar behavior has been already reported in the literature (Burguess and Sahin 1997). The latter results indicate that there is a critical pH value as well as a critical ion content below which the mechanical properties do not show an appreciable improvement. This seems to indicate that electrostatic forces play an important role in the mechanical properties of the gel as they become significant above certain pH and ion content values. This behavior is comparable to that reported by Burguess and Sahin (1997), where they observed similar effect for ovalbumin at different ionic strength conditions; they reported an increase in the interface tension when diminishing the ionic strength and attributed this effect to changes in the protein conformation rather than to the existing electrostatic repulsions.

The effect of citric acid content (at constant pH of 7.4) on the mechanical properties of the gel fracture stress was also analyzed and displayed in Table 4. The aging time (time evolution) of textural properties is presented in Table 4 for gels with and without citric acid. The addition of a minimum amount (0.1%) of citric acid provoked a

significant improvement (about 50%) in the fracture stress but a decrease at higher citric acid concentrations. This result is reinforced by comparing the fracture stress values for the materials with 0.15% of citric acid versus aging time (Table 4), which presented fracture stress values of about 40 kPa, whereas samples with no citric acid showed lower values of about 14 kPa (Table 4). Then, the addition of citric acid (Table 4) seems to impart stability to the gel as compared to the aging time fracture stress for the material with no acid added, where the behavior is erratic and presents a drop at 144 h but the fracture stress is recovered at 168 h.

## Conclusions

Mechanical properties of ovalbumin gels, such as the fracture stress and Young's modulus, were presented on varying ovalbumin concentration, ionic strength (as NaCl concentration), pH, and aging time. A rupture force peak and an increase in Young's modulus (i.e., high firmness) were observed at higher protein contents.

NaCl concentration influences the rupture force, as ions are added up to a maximum concentration of 50 mM of NaCl after which the force diminishes. The pH affects rupture force, which increases with pH and reaches a higher value at pH of 9.1.

Finally, the storage time presented the highest fracture force and Young's modulus at fixed conditions of ion content (NaCl 50 mM) and pH (i.e., 7) at 7 days of storage.

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