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Turbidity measurement of heated egg proteins using a microplate system

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Turbidity of egg albumin and egg white heated at various pH values and NaCl concentrations were measured using a microplate system. Small samples could be treated in a short time. The effects of pH and NaCl concentration on the turbidity of the samples after heating were clearly shown by this method and quantitatively by the contour graph. Turbid samples were formed by heating at pH values around pI values of constituent proteins and at high NaCl concentration. On the other hand, transparent gels and sols were formed on heating at pH values distant from pI values and at low NaCl concentration.

INTRODUCTION

When a protein solution is heated, it becomes a turbid suspension or gel. This turbidity is due to the formation of large coagulums of heat-denatured protein molecules and scattering of the light by the coagulums. Turbidity is a good indicator for denaturation of molecules, and shows a molecular aggregation pattern which gives specific properties of the products, such as viscosity and gelling characteristics (Kitabatake *et al.*, 1989). Egg white is one of the best known food proteins to form a turbid gel on heating. However, it is possible to prepare transparent gels or sols on heating by the control of pH and ionic strength of the heating medium (Kitabatake *et al.*, 1988). This can be interpreted as the interaction between denatured molecules being controlled by the electrostatic repulsion and hydrophobic affinity among these molecules (Kitabatake & Doi, 1993). A transparent sample is made by heating at pH values distant from the isoelectric points of proteins and low salt concentration.

Since this interpretation is based on data of typical conditions, systematic experiments with a large number of samples under various conditions should be performed in order to generalize and verify it. However, it is actually difficult to do such works because of the cost and time involved.

A microplate system using a microwell plate and microreader was introduced to show the turbidity of a small amount of heated milk whey protein samples (Kitabatake *et al.*, 1994). This is a simple and rapid technique to screen many small samples of proteins or different preparations at a time, and applicable to many kinds of protein samples.

Here we show the turbidity of a large number of samples of egg white and egg albumin, the major protein of egg white, before and after heating at various pH values and salt concentrations with this method.

MATERIALS AND METHODS

Materials

Egg albumin was purified from fresh egg white by the ammonium sulphate crystallization method (Kitabatake & Doi, 1987). Crystallization was repeated five times. The egg albumin was dialyzed against distilled water and the supernatant that was obtained by centrifugation to remove the insoluble materials, was used for the experiments. Egg white was also dialyzed against distilled water and the dialysate was centrifuged to remove the insoluble materials. The clear supernatant thus obtained was adjusted to a given pH value and NaCl concentration by addition of 2 M NaOH and 5 M NaCl. Sodium azide (0·02%) was added to the supernatant as a preservative. The final protein concentration was adjusted to 70 mg/ml.

Measurement of protein concentration

Protein concentrations of egg albumin and egg white, dissolved in 20 mM sodium phosphate buffer, pH 7·5, were measured from their absorbance at 280 nm based on a value of $E^{1\%}_{1cm}$ = 7·12 (Glazer *et al.*, 1963) and 10·0.

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Microplate system for turbidity measuring

Test samples (250 μl) were put in the wells of a microwell 1 × 8 module (Nunc-Immune Module MaxiSorp F8, Nunc, Inc., Roskilde, Denmark). Turbidity was expressed in terms of the absorbance at 590 nm. The absorbance of each sample was measured with a microplate reader (Model VH 450, UBE Handy Reader, UBE Industries Ltd, Tokyo) using a 590 nm filter. For heating the sample, the 12 microwell modules which were held by a frame (Nunc, Inc.) were covered with a seal (Linbro/Titertek, Flow Laboratories Inc., McLean, VA, USA) and put in an air-tight box containing some water. This closed box was placed in an oven at 80°C for 1 h. The samples were then cooled at room temperature for 2 h. The absorbance of each well was measured as described above. Each experiment was done in five samples, and the mean was calculated. Contour graphs were prepared by a computer (Macintosh VX, Apple Japan Inc., Tokyo) equipped with Pixcel Jet printer (Canon, Tokyo) using software (DeltaGraph professional, DeltaPoint Inc., CA, USA) designed for drawing contour lines.

RESULTS AND DISCUSSION

Heating of egg albumin

Egg albumin samples before and after heating using the 96-well plate are shown in Figs 1(A) and (B). Slightly turbid samples were obtained before heating at pH 2 and at high NaCl concentrations. After heating, the range of turbidity increased and turbid samples gelled. Absorbance at 590 nm measured with a microplate reader was shown by a contour graph (Fig. 1C). Transparent samples were formed at pH values distant from the isoelectric point (pI = 4·7) and at low NaCl concentrations. These samples also gelled.

Heating of egg white

With egg white, all of the samples were transparent before heating (Fig. 2A). However, after heating, most of the samples were turbid, except those at acidic pH and low NaCl concentrations (Fig. 2B), which are shown by contour graph (Fig. 2C). Transparent samples could

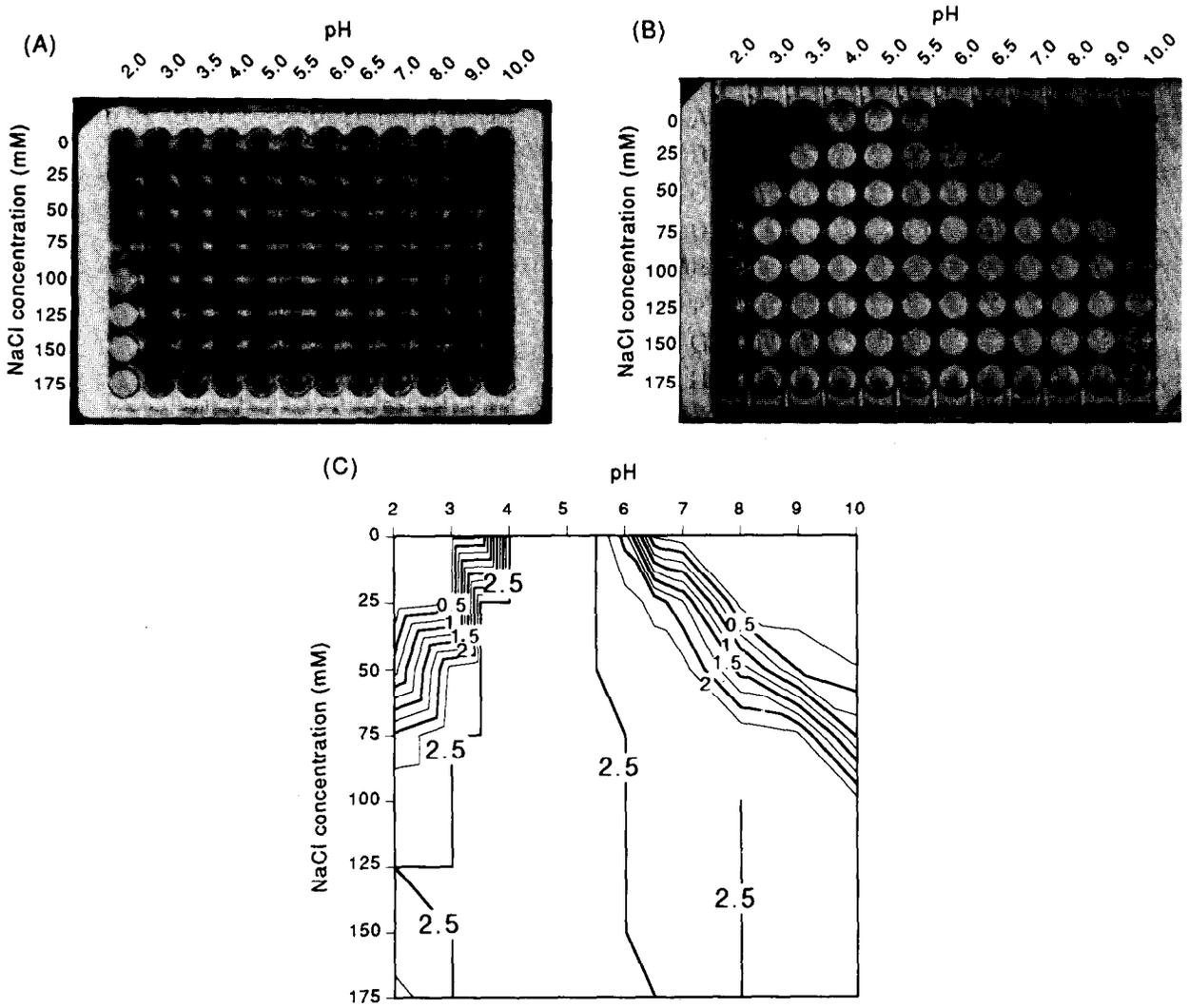

Fig. 1. Egg albumin (A) before, and (B) after heating at 80°C for 1 h. Absorbance at 590 nm was used as an indicator of turbidity and is shown by the contour graph (C). Each well contained 250 μl of ovalbumin solution (70 mg/ml). Ovalbumin was dialyzed against distilled water, and then pH, NaCl concentration and protein concentration were adjusted.

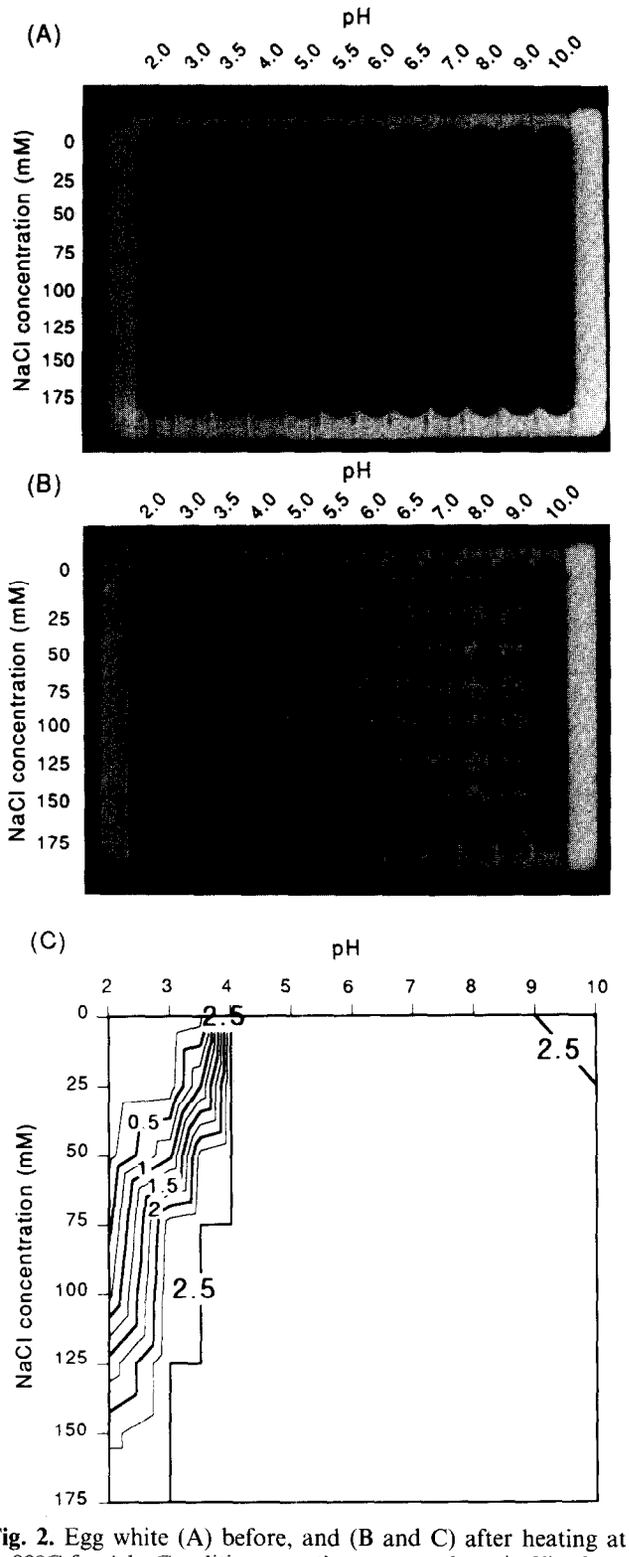

Fig. 2. Egg white (A) before, and (B and C) after heating at 80°C for 1 h. Conditions are the same as those in Fig. 1.

not be obtained at neutral and alkaline pH values, perhaps because egg white contains various protein components, some of which have pI values in a neutral and/or basic pH range.

In a broad range of pH and salt concentration the transparency of the sample of egg protein before and after heating can be observed at a glance and measured in a short time. The transparent samples after heating, observed at acidic and basic pH values for egg albumin in the 96-well plate, clearly demonstrated that transparent gels or sols were formed by heating when electrostatic repulsion between heat-denatured egg albumin molecules was relatively high. That is, when salt concentration is low and/or the pH is distant from the isoelectric point (pI = 4·7) of the egg albumin, electrostatic repulsive and hydrophobic attractive forces between heat-denatured egg albumin molecules increase or are suppressed, respectively, and then transparent gels or sols are formed even after heat treatment. This is not inconsistent with the results and interpretation reported elsewhere (Kitabatake & Doi, 1993).

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