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# Anodic Oxidative Modification of Egg White for Heat Treatment

**ABSTRACT:** A new functionalization of egg white was achieved by an electrochemical reaction. The method involves electron transfer from thiol groups of egg white protein to form disulfide bonds. The oxidized egg white produced less hydrogen sulfide during heat treatment; with sufficient application of electricity, almost no hydrogen sulfide was produced. In addition, gels formed by heating electrochemically oxidized egg white exhibited unique properties, such as a lower gelation temperature and a softened texture, presumably due to protein aggregation and electrochemically mediated intramolecular disulfide bond formation.

**KEYWORDS:** *egg white, protein, electrochemical reaction, anodic oxidation, gelation*

## INTRODUCTION

Heat treatment is one of the most important food preparation methods. Heating induces changes in the flavor, color, and texture of food materials through processes such as the formation of volatile substances, the Maillard reaction,[1] and gel formation. Furthermore, harmful microorganisms are destroyed by heat treatment. These changes render our foods tasty, easy to eat, and safe. Although heat treatment is important for food processing, it can sometimes have undesirable effects. For example, hard-boiled eggs can release hydrogen sulfide gas,[2] which has an unpleasant odor. Heat treatment often imparts physical strength to some sol-like and gel-like food materials and modifies their textures, but excess heat treatment can make the texture too hard and causes foods to lose their flavor, especially for soft-textured foods; soft pudding is preferred to hard-textured pudding, for example.

Chicken eggs are one of the most common foods, and their characteristics change dramatically with heat treatment. A variety of foods can be prepared by heating chicken eggs (e.g., hard-boiled egg, fried eggs, omelets, and puddings). The production of hydrogen sulfide is a characteristic phenomenon associated with the heat treatment of eggs. The unique smell of hydrogen sulfide, which in small amounts can enhance the flavor of hard-boiled eggs, can be unpleasant in large amounts. New techniques for preventing the production of hydrogen sulfide are thus needed in food manufacturing, as egg white is a widely used material in food manufacturing due to its gel-forming properties. In addition to its unpleasant odor, the production of hydrogen sulfide is nutritionally disadvantageous, as hydrogen sulfide production involves the amino acid cysteine, which is an important source of sulfate for the body. After releasing hydrogen sulfide, cysteine is altered into the highly reactive amino acid dehydroalanine, which reacts with and forms covalent bonds with other amino acids, such as lysine, with which it cross-links to form lysinoalanine.[3−5] Such cross-linked amino acids, unfortunately, are less digestible and have poorer nutritional value due to their resistance to proteolytic enzymes.

Heat treatment of egg white induces the formation of gels that can be eaten (i.e., hard-boiled eggs). The thiol groups of egg white protein cysteine residues play an important role in the gelation process.[6−8] Dry heat treatment of egg white[9,10] leads to the formation of water-soluble protein aggregates that impart hardness to the heat-induced gels, and in this aggregation process, the cysteine residues of the egg white proteins lose their thiol groups.

We previously reported a technique for thiol oxidation via an electrochemical reaction using iodide salts.[11] Electrochemical techniques[12,13] are widely used in the food industry and include electrical heating[14,15] and the application of electric pulses[16,17] with a high-energy electric field. The effects of such electrochemical techniques on food materials mainly come from physical effects such as Joule heat and pulse waves. These techniques play important roles in the food industry; however, electrochemical techniques have a potential to modify food materials more effectively by inducing chemical reactions, because the nature of the chemical reaction is electron transfer. The anodic oxidation we have reported can induce chemical reactions that lead to indirect electron transfer between electrodes and substrates; the anode oxidizes iodide anions to iodine cations, and then the iodine cations oxidize thiol groups in proteins. Such indirect electrochemical reactions are useful for the reaction of large molecules such as proteins for its ease of electron transfer, but the application of food manufacturing is rarely reported. In our previous paper, we clarified the mechanism of the indirect anodic oxidation process, but the properties of oxidized protein were not investigated. We hypothesized that egg white would exhibit unique properties following anodic oxidation because the egg white oxidized by dry heat treatment enhances the breaking strength of heat-induced gels. We therefore investigated the effect of anodic oxidation on the thiol groups of egg white protein cysteine residues upon heat treatment and its potential importance in the food industry.

## MATERIALS AND METHODS

**Materials.** Egg white was prepared from laid eggs. The yolk and chalaza were carefully separated, and the egg white was homogenized in a blender (Waring Products, New Hartford, CT, USA) and then centrifuged. The ovomucin was removed to prevent adhesion of insoluble components to the electrodes during electrolysis. The protein concentration was determined according to the method of Bradford.[18] All reagents were purchased from Tokyo Chemical Industry (Tokyo, Japan), Wako Pure Chemical Industries (Osaka, Japan), Kanto Chemical (Tokyo, Japan), DRC (Tokyo, Japan), or Bio-Rad (Hercules, CA, USA) and were used without further purification. Deionized water was obtained using a Millipore purification system.

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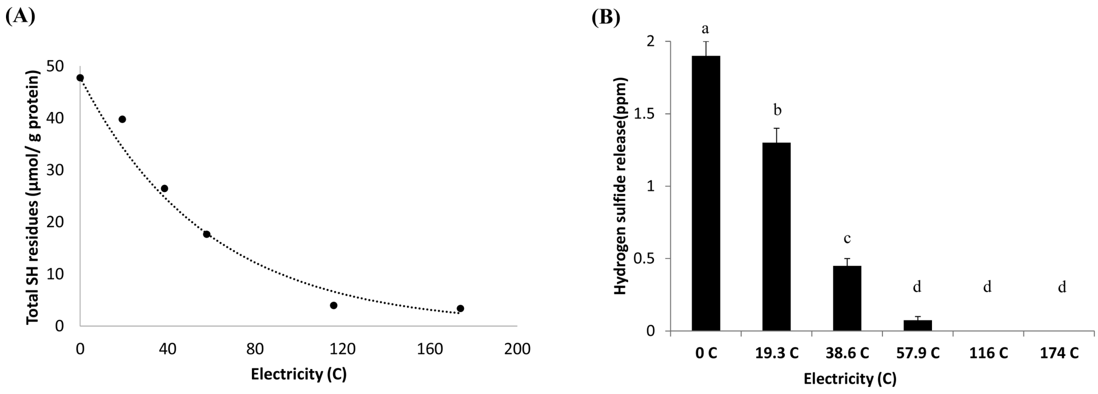


**Figure 1.** Changes in total thiol groups (A) and hydrogen sulfide release (B) of anodic oxidized egg white. Means with different letters are significantly different from each other. Each sample was measured in triplicate.

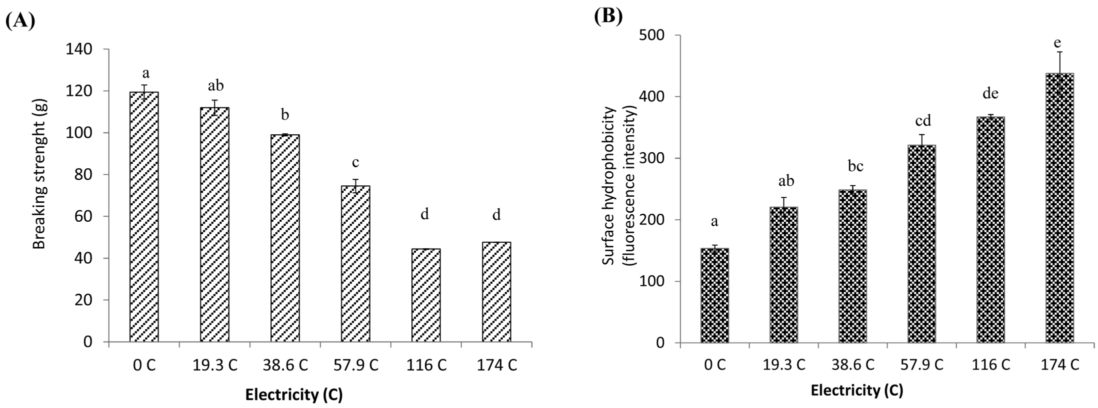


**Figure 2.** Changes in breaking strength of heat-induced gels (A) and surface hydrophobicity (B) of anodic oxidized egg white. Means with different letters are significantly different from each other. Each sample was measured in triplicate (A, 0−57.9 C; B, 0−174 C) or duplicate (A, 116 C; B, 174 C).

**Anodic Oxidation of Egg White.** Electrochemical reactions were performed using an HABF-501 potentiogalvanostat (Hokuto Denko, Tokyo, Japan). Sodium chloride (0.1 M) and sodium iodide (10 mM) were added to the egg white, and then the pH of the sample solutions was adjusted to 9.0 with 1 M aqueous HCl. Glassy carbon electrodes were inserted into the resulting reaction mixture (50 mL), and electrolysis was performed with stirring at a constant current density of 0.50 $mA/cm^2$ at room temperature (time on duration = $1.0 \times 10^3$ s for 6.0 C).

**Properties of Oxidized Egg White.** The concentration of thiol groups in egg white was measured using Ellman's test with 5,5′-dithiobis(2-nitrobenzoic acid).[19] Total thiol groups were measured in the presence of 0.25% sodium dodecyl sulfate (SDS). Surface hydrophobicity of egg white was measured using 1-anilino-8-naphthalene sulfate according to the method of Hayakawa and Nakai.[20] Fluorescence intensity was measured at excitation wavelengths of 390 nm and emission at 470 nm. Native polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE both in the presence and in the absence of 2-mercaptoethanol, a thiol-reducing reagent, were carried out according to the method of Laemmli[21] using an STC-808 electrophoresis unit (TEFCO, Tokyo, Japan) or an XV Pantera MP System (DRC, Tokyo, Japan). After electrophoresis, the gels were washed three times with deionized water for 5 min. The gels were stained with Quick-CBB (Coomassie Brilliant Blue) (Wako Pure Chemical Industries) for 3 h and stored in deionized water.

Heat treatments for aggregation formation were carried out with 4% egg white solutions at 95 °C for 30 min at pH 9 (diluted and adjusted by aqueous NaOH).

Hydrogen sulfide produced by heat treatment (80 °C for 30 min) with 10% egg white solution was measured using a gas detector tube for hydrogen sulfide ranging from 0.1 to 6.0 ppm (Kitagawa gas detector tube system, Komyo rikagaku kogyo, Kanagawa, Japan).

**Preparation and Functional Properties of Heat-Induced Gels.** Cylindrical gels (25 mm diameter, 15 mm height) were prepared by heating sample solutions at 80 °C in a water bath (NTB-221, Tokyo Rikakikai, Tokyo, Japan) for 40 min in a vinyl chloride plastic casing.

The breaking strength of heat-induced gels was measured using a TA XT plus Texture analyzer (Stable Micro Systems, Surey, UK) with a flat-bottomed plunger (8 mm diameter) at a crosshead speed of 1 mm/s.

**Microstructure of Heat-Induced Gels.** The microstructure of heat-induced gels was investigated using scanning electron microscopy (SEM). Gel samples (1 $mm^3$) were fixed in 2.5% glutaraldehyde (0.1 M cacodylate buffer, pH 7.3), postfixed in 1% osmium tetroxide (0.1 M cacodylate buffer, pH 7.3), and then dehydrated. The samples were freeze-dried (−20 °C) using a freeze-dryer, mounted, sputter-coated with gold−palladium, and examined under a JSM-6320F SEM (JEOL, Tokyo, Japan) at 5 kV.

**Statistical Analysis.** Statistical significance of differences among groups was assessed by one-way ANOVA followed by Tukey's HSD test. All statistical analyses were performed using the software JMP10 (SAS Institute Inc., Cary, NC, USA). *P* values <0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

The correlation between the amount of electricity applied and the number of thiol groups in egg white protein was investigated first (Figure 1A). As hypothesized, the number of thiol groups decreased and the oxidation reaction progressed


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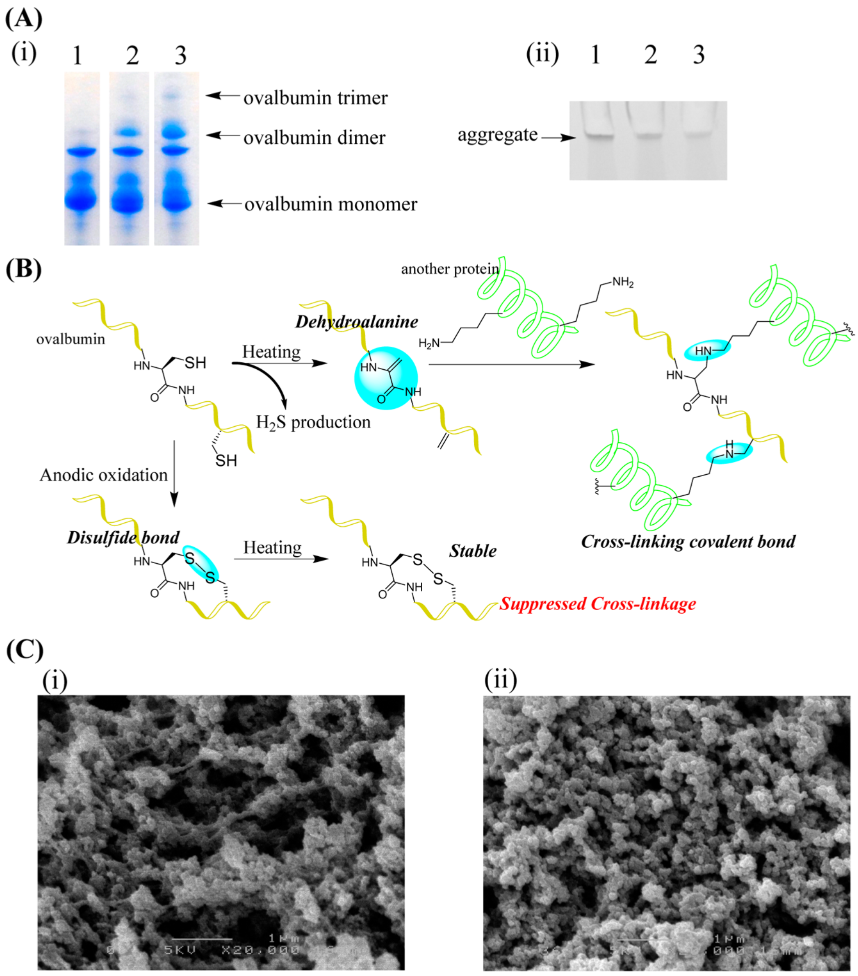


**Figure 3.** (A) SDS-PAGE patterns of anodic oxidized egg white: (i) without 2-mercaptoethanol; (ii) with 2-mercaptoethanol after heat treatment. Lanes: 1, 0 C; 2, 57.9 C; 3, 174 C. (B) Reaction mechanism of softened gels. (C) SEM images (×20,000) of heat-induced gels from nonoxidized egg white (i) and oxidized for 174 C (ii).

as more electricity was applied. Among the major proteins in egg white, only ovalbumin contains thiol groups. The thiol groups are not in the oxidized form and are not located on the surface of the protein.[22,23] From these past studies and our present experimental results, we concluded that the unexposed thiol groups must be oxidized via an indirect anodic oxidation reaction mediated by iodide salts. In general, electron transfer between electrodes and polymers such as proteins is limited, as solid−solid reactions are rare. However, in our method, electron transfer occurred between the anode and iodide ions, and proteins reacted with the activated iodine cations, indicating that electrochemical reactions involving unexposed residues in the protein occurred. Iodine cation is small enough to enter the inside of a protein and have moderate reactivity to oxidize thiol groups, and these properties might be advantageous in the oxidation of buried residues. During heat treatment, this anodic oxidized egg white produced less hydrogen sulfide than nonoxidized egg white, and almost no hydrogen sulfide release was observed if a sufficient amount of electricity was applied (Figure 1B). Suppression of hydrogen sulfide was accomplished by thiol oxidation; cysteine residues were oxidized to cystine residues via disulfide bond formation. Cystine residues exhibit lower $\beta$ elimination reactivity than thiol groups.

Next, the breaking strength of egg white gels formed by heat treatment was measured (Figure 2A). Gels produced from anodic oxidized egg white had a softer texture than those produced from nonoxidized egg white. Fewer thiol groups were observed in egg white treated with both dry heat[9] and anodic oxidation, but these treatments had opposite effects on gel strength. Dry heat treatment alone produced hard gels, whereas anodic oxidation led to the production of soft gels. To elucidate the reason for this difference, the surface hydrophobicity of anodic oxidized egg white was examined (Figure 2B), as this property is known to be positively correlated with denaturation of protein and gel strength.[23] As the amount of electricity applied increased, the surface hydrophobicity increased, and protein unfolding occurred. Increasing surface hydrophobicity enhanced hydrophobic interactions between egg white proteins, which would be expected to increase the gel strength and make the gels harder. Our results, however, suggest that the softer gels produced from anodic oxidized egg white were not due to changes in surface hydrophobicity and hydrophobic interactions and that another factor must therefore have a strong influence on the gel properties.

As mentioned, anodic oxidation oxidizes thiol groups and suppresses the release of hydrogen sulfide, which could affect the gel properties. By applying enough electricity, the number of thiol groups in egg white was reduced to nearly zero (Figure



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1A). However, PAGE analysis of anodic oxidized protein clearly showed the presence of ovalbumin monomers (Figure 3A(i)), which is suggestive of the formation of intramolecular disulfide bonds in ovalbumin. Intermolecular interactions were limited, presumably due to the relative absence of nonoxidized reactive thiol groups, thus reducing the gel strength. In addition, the suppression of hydrogen sulfide release indicates that the formation of dehydroalanine (a highly reactive amino acid generated by desulfurization) from cysteine was also suppressed. Dehydroalanine can react with other amino acids, such as lysine or cysteine, to form cross-linked amino acids, such as lysinoalanine or lanthionine, respectively. This cross-linking involves the formation of covalent bonds, which are far stronger than hydrophobic interactions. Limited cross-linking results in the formation of fewer intermolecular covalent bonds, which would be expected to result in softer gels. To test this hypothesis, the effect of electrochemical treatment on protein cross-linking was examined. In SDS-PAGE analysis, cross-linkages are broken as a result of the cleavage of disulfide bonds by the reducing reagent, but other cross-linkages, such as those involving lysinoalanyl bonds, would be stable under reducing conditions. PAGE analyses clearly showed that cross-linkage aggregation (Figure 3A(ii)), and not cross-linking associated with disulfide bond formation, is suppressed by anodic oxidation.

SEM were compared to evaluate the effects of electrochemical treatment on gel structure (Figure 3C). A less clear network structure and more randomly aggregated clusters were observed in the gel with more electrochemical treatment.

Finally, the gelation temperature of egg white was measured (Figure 4). As the amount of electricity applied increased, the

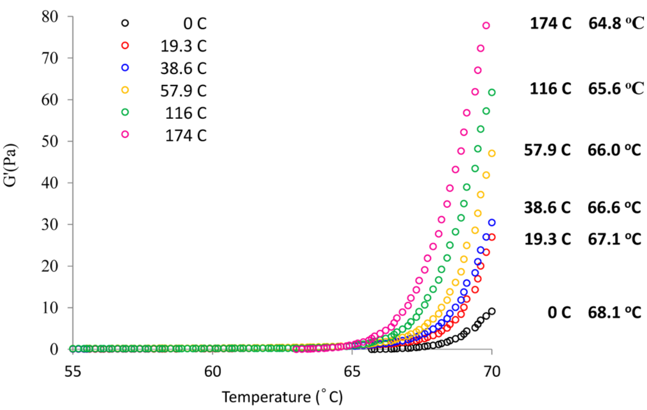


**Figure 4.** Changes in gelation temperature.

gelation temperature of anodic oxidized egg white decreased. Upon anodic oxidation, the ovalbumin in the egg white solution formed dimers or trimers prior to gelation; therefore, the number of intermolecular interactions needed to initiate gelation upon heating might be lower. In addition, surface hydrophobicity also significantly increased during anodic oxidation (Figure 2B), which had a positive effect on gelation. From these results, anodic oxidation of egg white can be regarded as a method to improve the functional properties of egg white gels. By anodic oxidation, the hardness of gels can be controlled and the production of too hard gels can be avoided because the anodic oxidized egg white forms softened gels. This might be an advantage in food manufacturing because heat treatment for pasteurization (especially after heated egg products such as hard-boiled egg and fried egg are filled in a bag) sometimes creates an undesired hard texture and imposes a limitation on food processing. In addition, a low gelation temperature could be a benefit when the egg white is mixed with meat and heated to make processed meat products. The gelation temperature of egg white is higher than that of meat; the heating process of such processed meat products sometimes creates an undesired too hard texture for meat. The anodic oxidized egg white could resolve this problem because it form gels at low temperature.

This paper indicates that anodic oxidation treatment can be used to increase the uses of egg white. By oxidizing protein thiol groups, hydrogen sulfide production is suppressed. In addition, gels formed from electrochemically oxidized egg white exhibit unique properties, such as a lower gelation temperature, and a softer texture. Thus, egg white gels produced using this method represent a new food material. Furthermore, our study demonstrates the usefulness of the electrochemical technique for inducing indirect electron transfer. Further applications would be expected by combining the technique described here with existing electrochemical processes.

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## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b02785.

Original figures of PAGE analysis and detailed description of an apparatus for an electrochemical reaction (PDF)

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### Notes

The authors declare no competing financial interest.

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