Disulfide bond formation in egg white proteins through anodic oxidation

International Egg Symposium

October 6th, 2016

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Introduction of Kewpie Group

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Outline(consolidated)

Year of foundation	Nov. 30, 1919
Net sales	549,775 millions of yen (Nov. 2015)
Ordinary income	27,225 millions of yen (Nov. 2015)
Number of employees	13,941 (May 2016)
Number of subsidiaries	54

Kewport

SENGAWA キューポート

kewport

kewpie

Business Constitution

Delicatessen Products

Salads and Delicatessen Foods, Cut Vegetables

Processed foods

Jam, pasta sauces , and healthcare products including baby and nursing care products

Egg products

Egg product
Egg material

Fine Chemical Products

Hyaluronic Acid, EPA, and Egg Ingredients

Condiments products

mayonnaise and dressings

Distribution system

Food storage
Food transportation

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Network in Japan


1 Hashikami Factory


2 Goka Factory


3 Nakagawara Factory


4 Fujiyoshida Factory


5 Koromo Factory


6 Itami Factory


7 Izumisano Factory


8 Tosu Factory

Kewpie has 18 egg breaking facilities in Japan.

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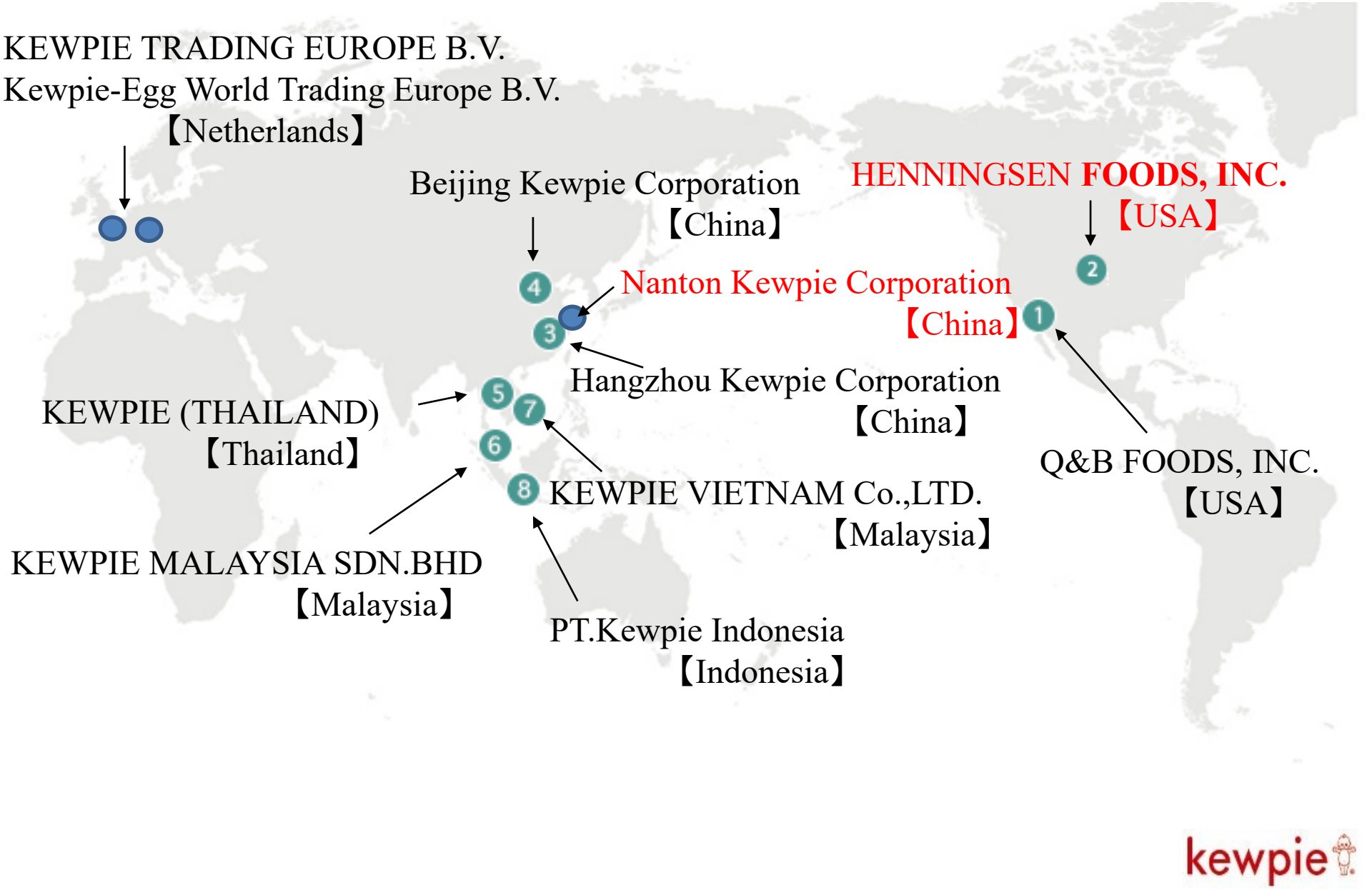
Worldwide Network



Kewpie Mayo Products in the World

North America
Mexico

Europe

China

Malaysia

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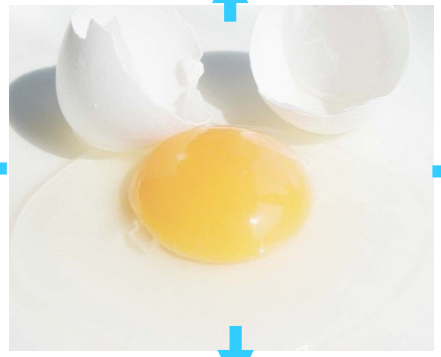
Egg Related Products

Ingredients

- Liquid egg
- Frozen egg
- Dried egg
- Egg shell powder

Shell Egg Type

- Hardboiled egg
- Pasteurized shell egg

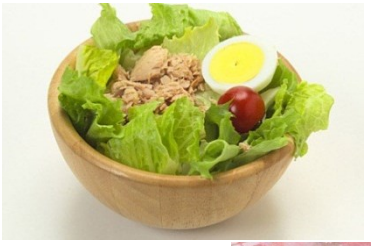


Fine Chemical

- Lecithin
- Lysozyme
- Egg shell membrane

Processed Egg

- Omelet
- Egg salad
- Sunny side up
- Baked roll egg



New Product; special whole egg

Pasteurized whole egg for egg drop

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New Product; RUMIRUN®

For your exercise and beauty

RUMIRUN®

Egg white fermented by lactic acid bacteria

Only Kewpie can provide this new protein drink

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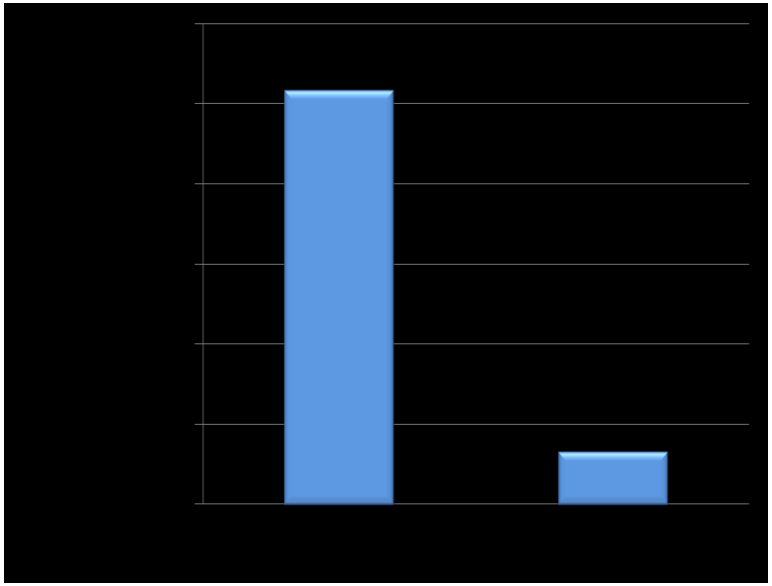
New Product; Lysopower NV

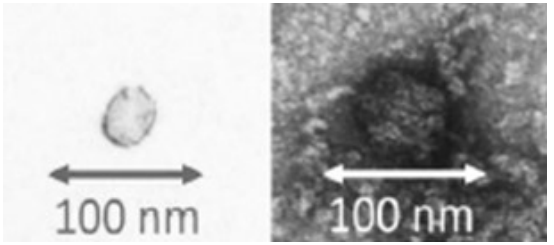
1 L type

5 L type

Sanitizer products with ethanol and lysozyme

The number of murine norovirus decreased after specially treated lysozyme was added.





Takahashi, H. *et al*. Heat-Denatured Lysozyme Inactivates Murine Norovirus as a Surrogate Human Norovirus. *Scientific Reports* 5, 11819 (2015).

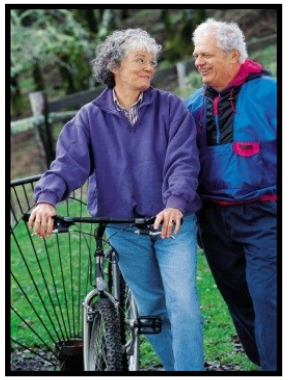
New Product; PeptiFine®

Easy to digest
(excellent bioavailability)

Higher antioxidative activity

Better flavor

Anti fatigue effect



Joint research with TUAT

Laboratory of Bioorganic Chemistry
Tokyo University of Agriculture and Technology

Institute of Technology
Kewpie Corporation

Organic chemistry
Electrochemistry

Egg science
Protein chemistry

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Full Paper

Anodic Oxidative Disulfide Bond Formation in Egg Protein

Masahito Takahashi, Akihiro Handa, Risa Kodama, Kazuhiro Chiba

First published: 18 August 2016 Full publication history

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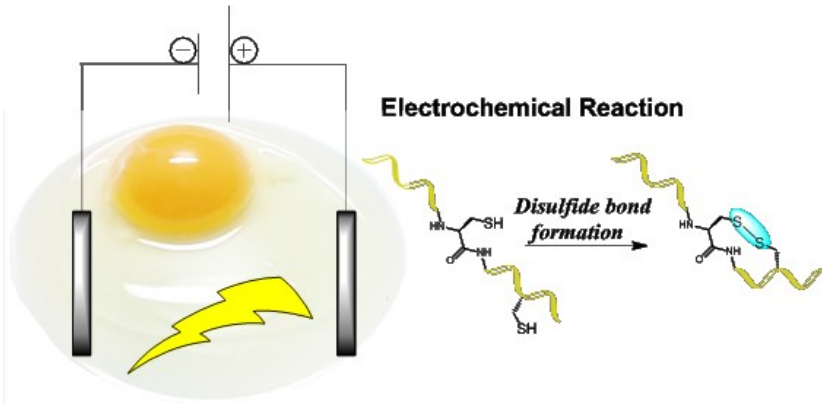

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
Letter

Anodic Oxidative Modification of Egg White for Heat Treatment

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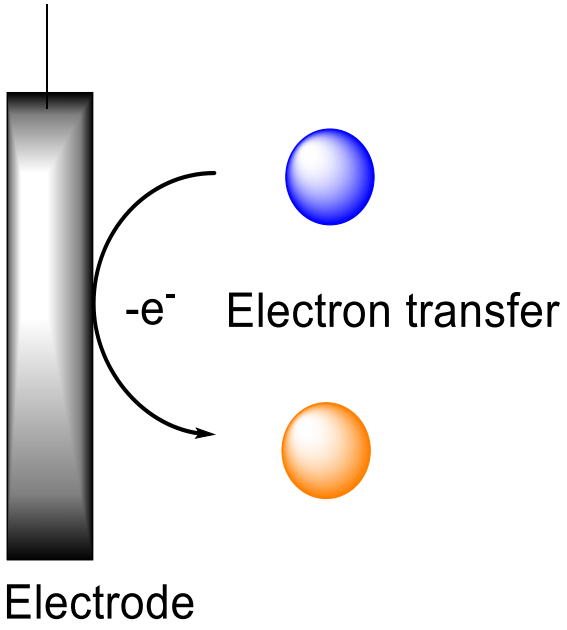
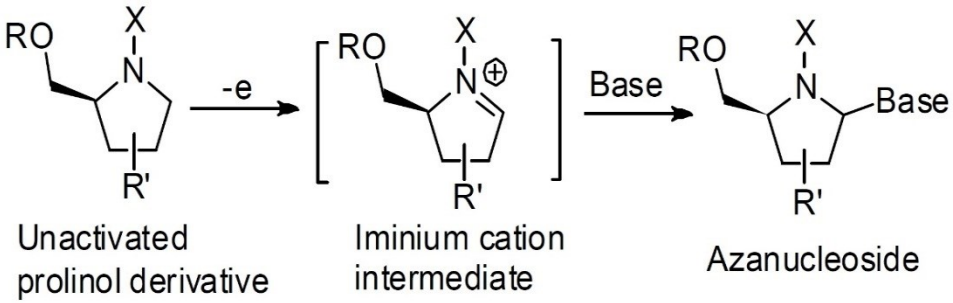
Publication Date (Web): August 12, 2016

Electrochemical reaction



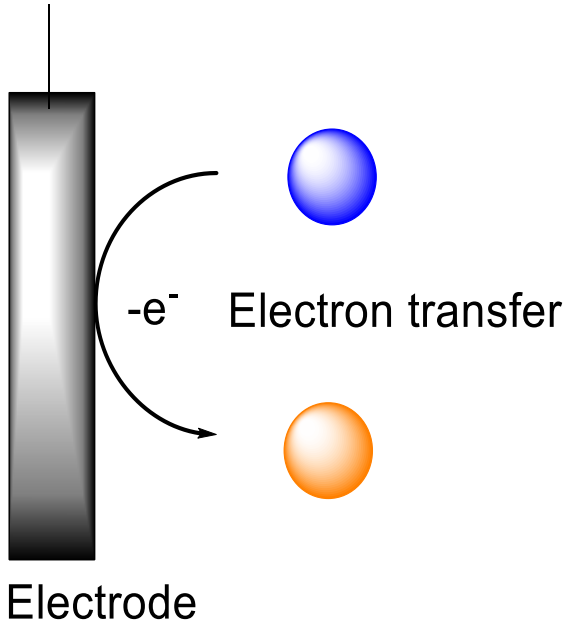
- Chemical reaction using electron transfer between electrode and substrate
- Mild condition
- Easy operation (one switch)



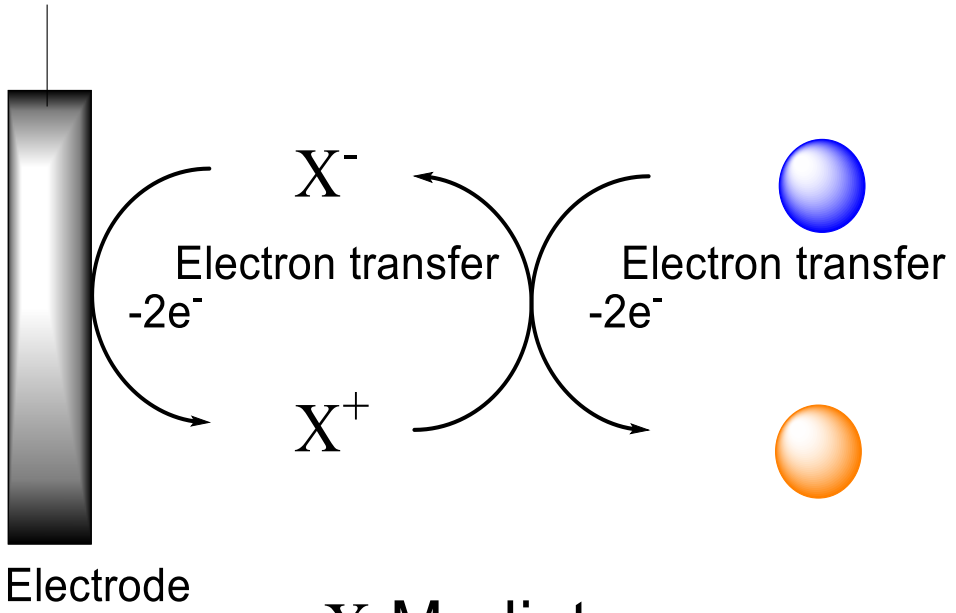
S. Kim T. Shoji, Y. Kitano, K. Chiba. Electrochemical Synthesis of Azanucleotide Derivatives using Lithium Perchlorate-Nitromethane System, *Chem. Commun*., **2013**, *49*, 6525-6527

Mediator

Direct electrochemical reaction



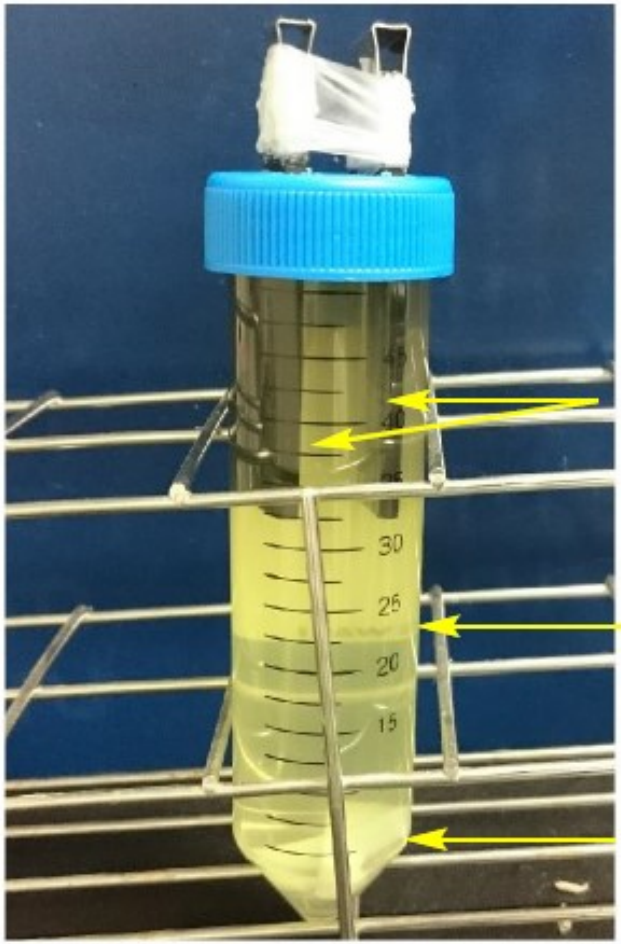
Indirect electrochemical reaction

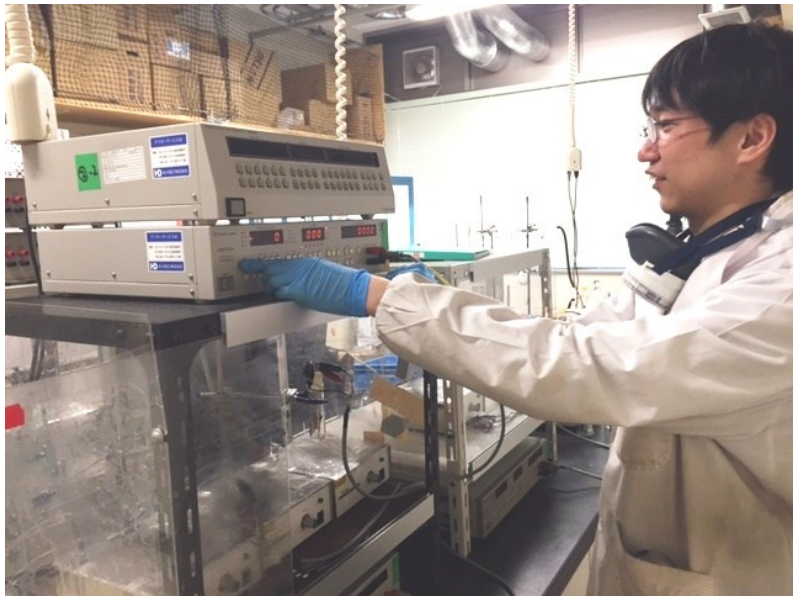


X:Mediator

1st Step
Anodic oxidation of OVA

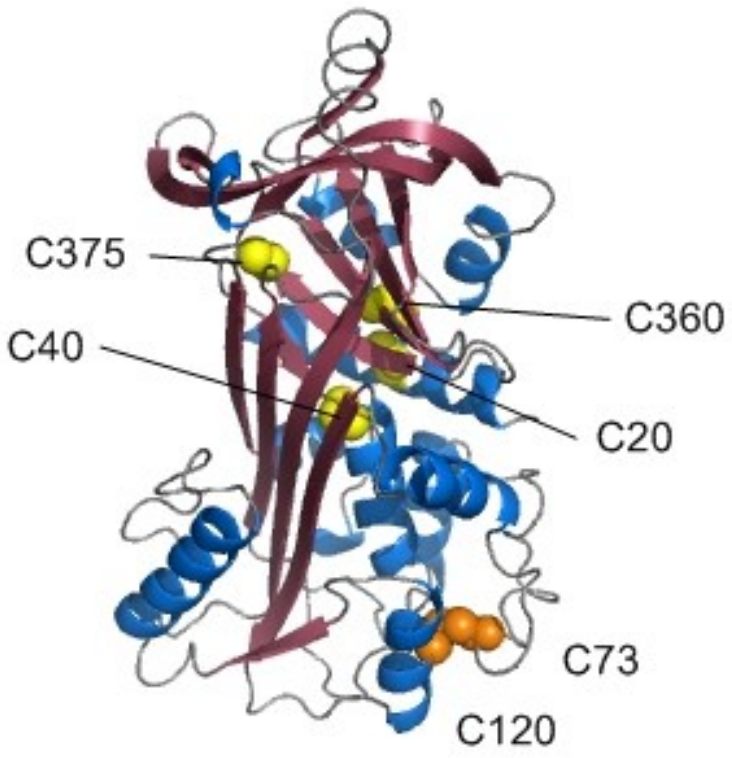
Apparatus





Power supply

Ovalbumin (OVA)



The most abundant protein in egg white

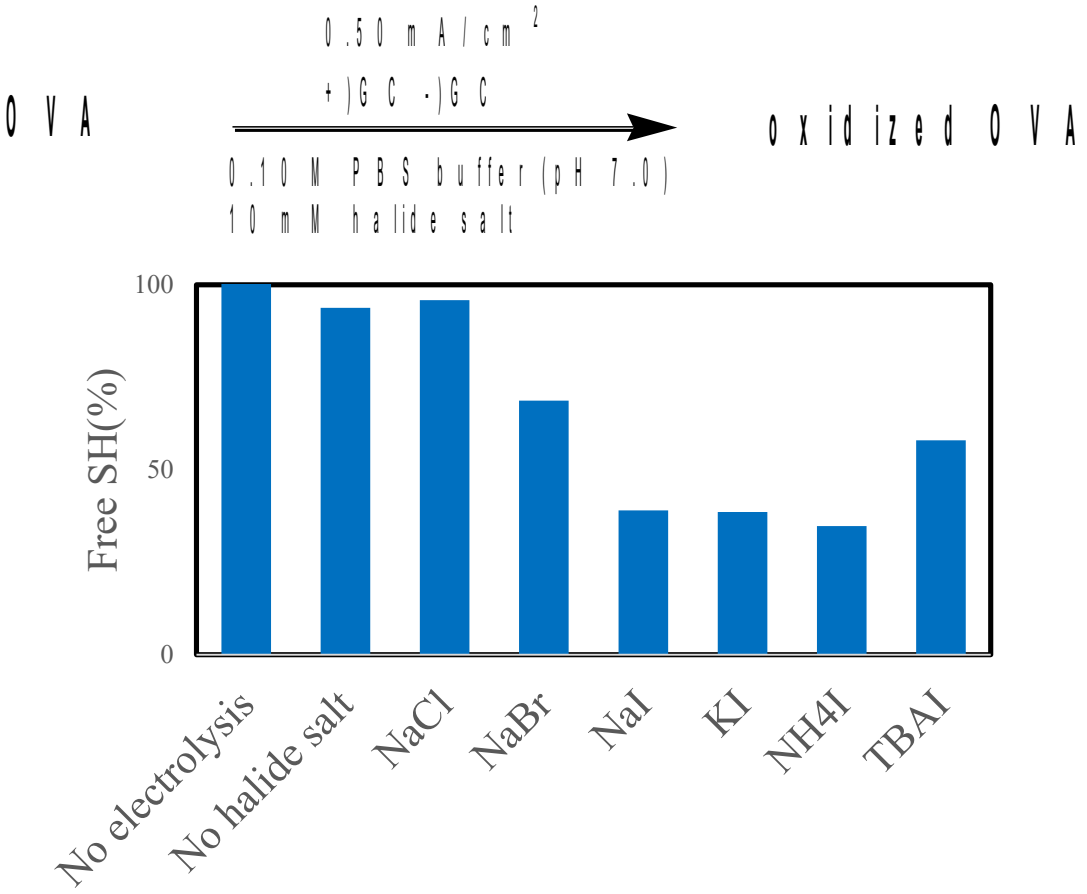
Molecular weight = 45 kDa

Four sulfidryl group (cysteine)

One disulfide bond (cystine)

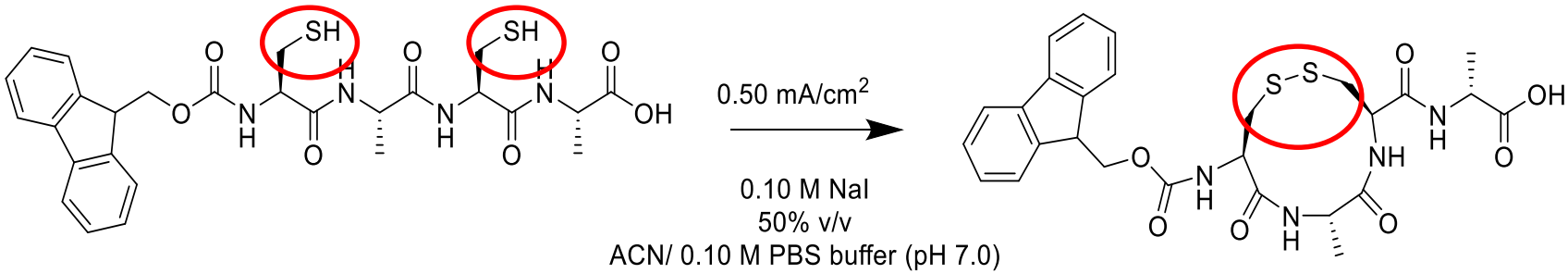
The only protein species that has sulfidryl group in egg white

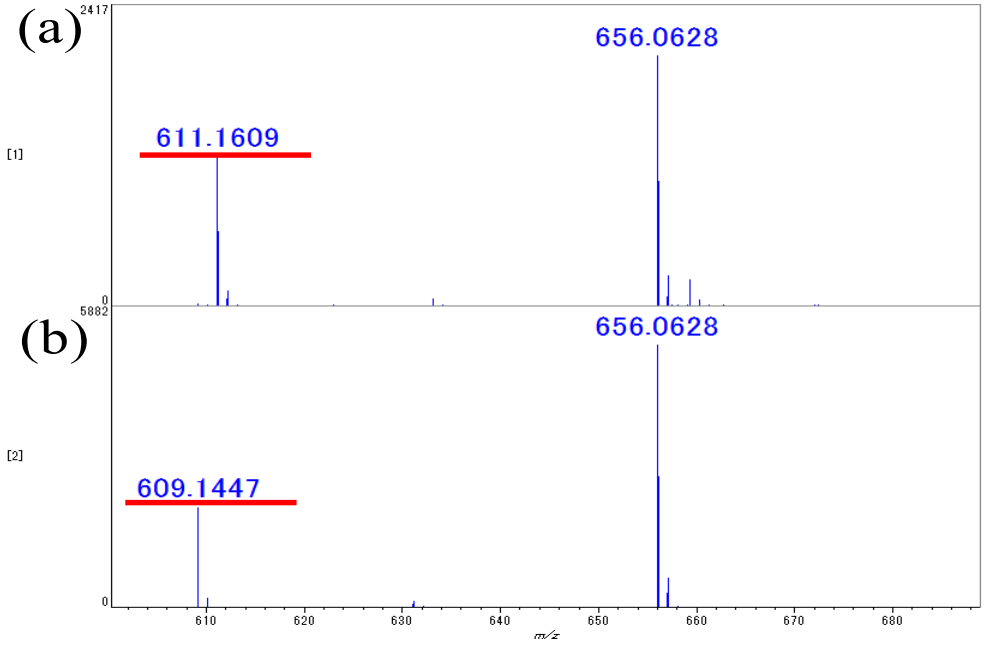
Mediator Selection



- **SH groups decreased most with iodine.**
- **What happened to SH Groups? SS bond formation, sulfonic acid, or others?**

Mass spectra of model peptide





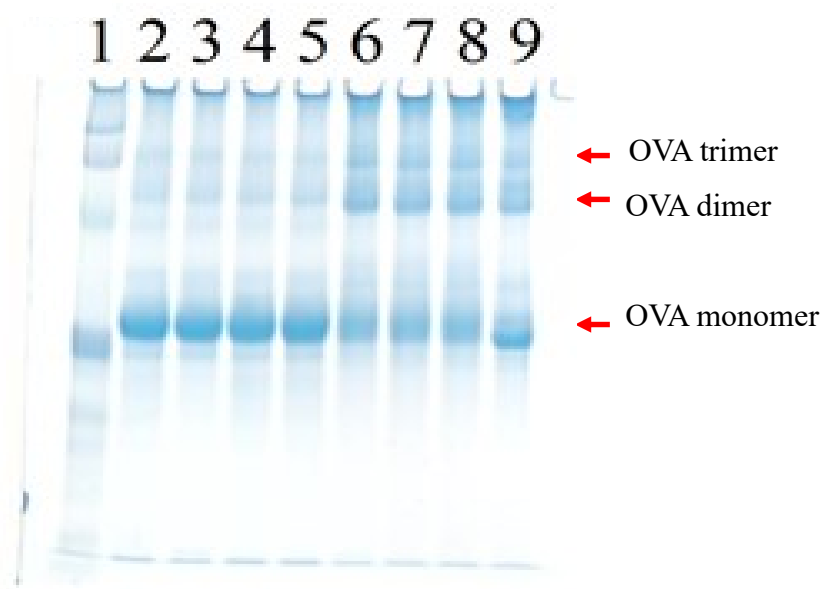
(a) before electrolysis (m/z 611.1609 represents linear peptide (calc. 611.1610))

(b) after electrolysis (m/z 609.1447 represents cyclic peptide (calc. 609.1454))

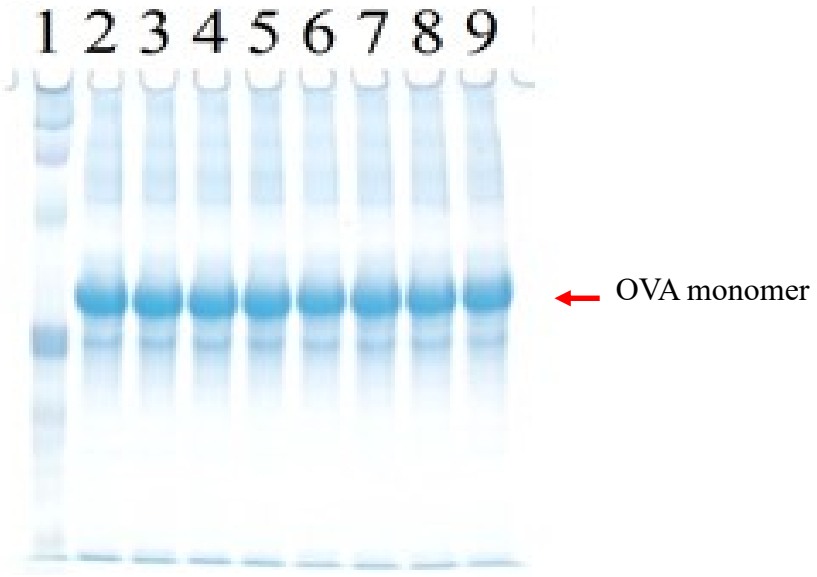
m/z 656.0628 represents CHCA (matrix for MALDI-TOF-MS).

SDS-PAGE

-) 2-mercaptoethanol



+) 2-mercaptoethanol



Lane 1: marker; Lane 2: no electrolysis; lane 3: no halide salt; Lane 4: NaCl; Lane 5: NaBr; Lane 6: NaI; Lane 7: KI; Lane 8: NH_4I; Lane 9: TBAI).
OVA; ovalbumin

Anodic oxidation of OVA occurred, and OVA intermolecular disulfide bonds were formed.

2nd Step
Anodic oxidation of egg white for heat treatment

Evaluation methods

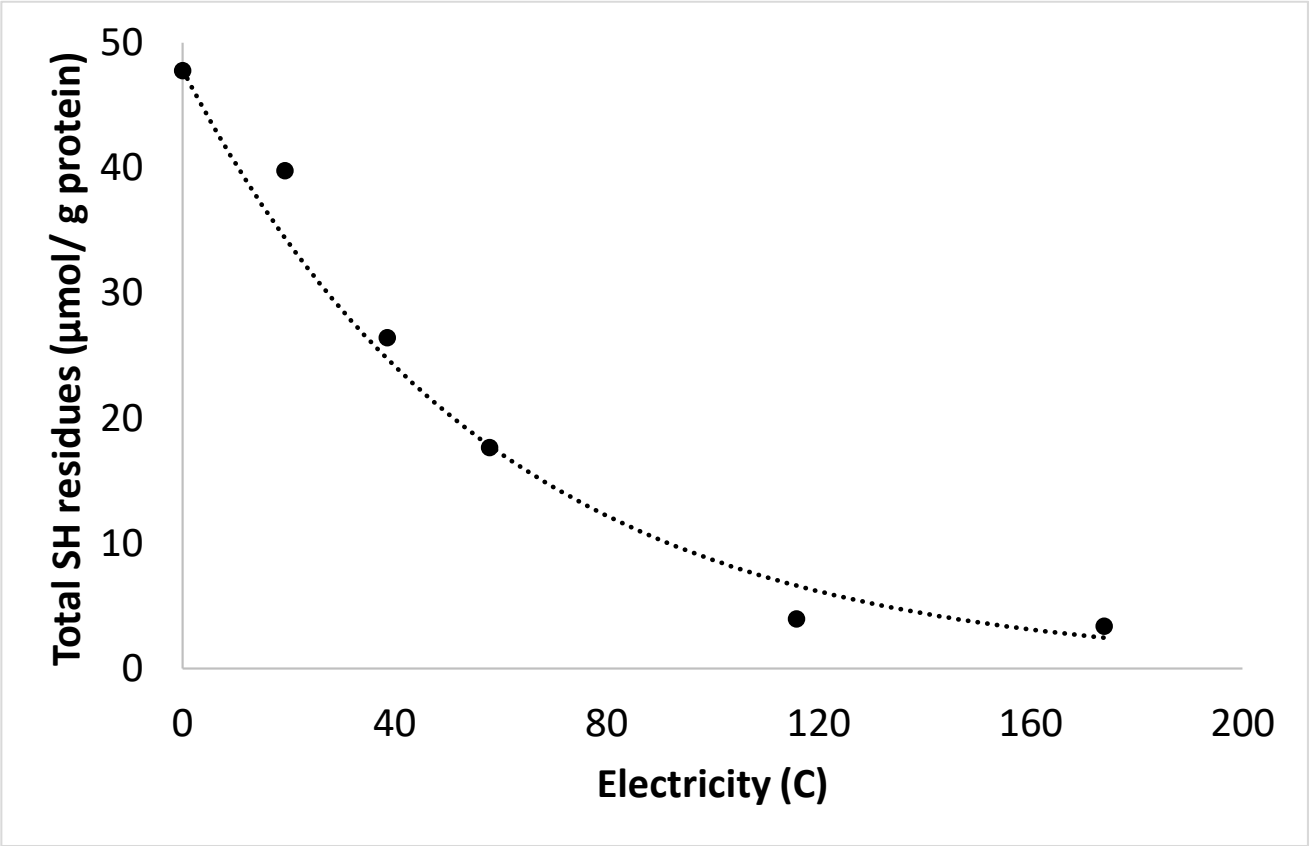
1. Structural changes in the protein

- Total SH
  ⇒Ellman's test
- Surface hydrophobicity
  ⇒ANS (Excitation wavelength 390nm, Fluorescence wavelength 470nm)
- Electrophoretic analysis
  ⇒Native PAGE, SDS-PAGE（with and without 2-mercaptoethanol）

2. Functional properties of heat-induced gels

- Hydrogen sulfide
  ⇒Kitagawa gas detector tube system (Komyo rikagaku kogyo, Kanagawa, Japan)
- Gelation temperature
  ⇒Rheometer (AR-G2, TA instruments Japan, Inc.)
- Breaking strength
  ⇒TA XT plus Texture analyser (Stable Micro Systems, Surey, UK)
- Microstructure of heat-induced gels
  ⇒scanning electron microscopy (SEM) (JEOL JSM-6320F)

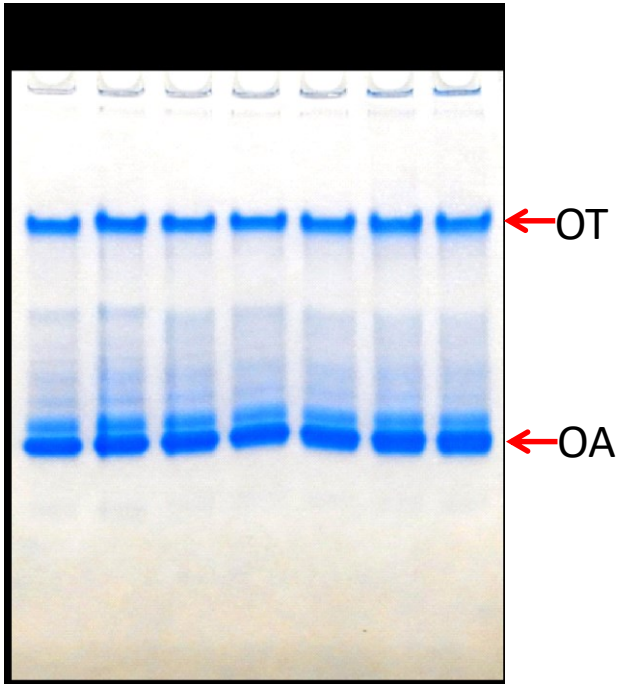
Total SH



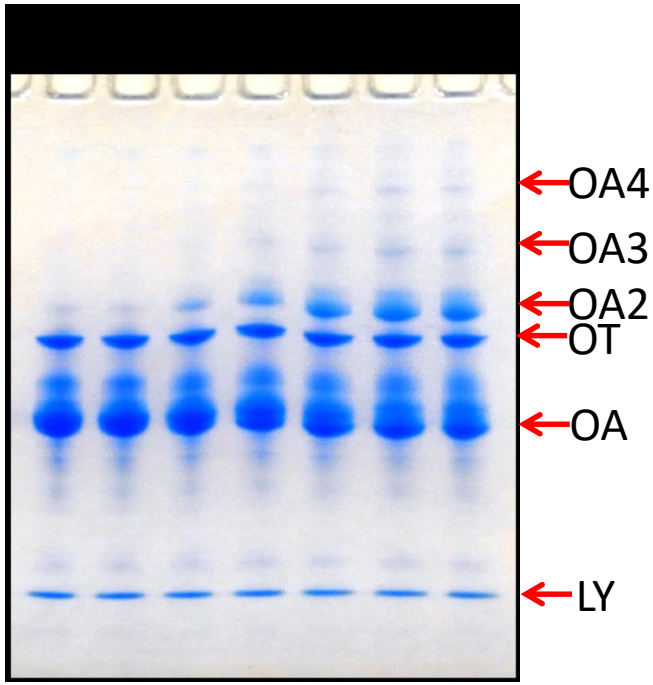
Total SH decreased with increasing electricity.

Electrophoretic analysis

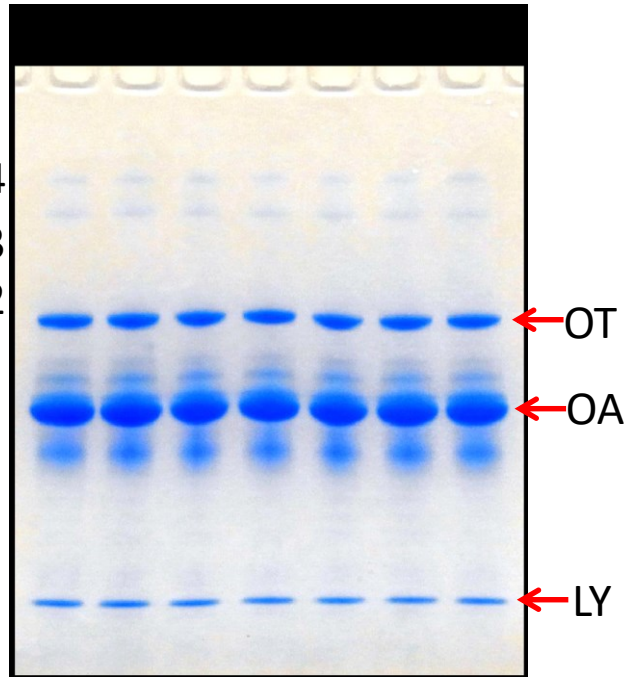
Native PAGE



SDS-PAGE without 2-ME



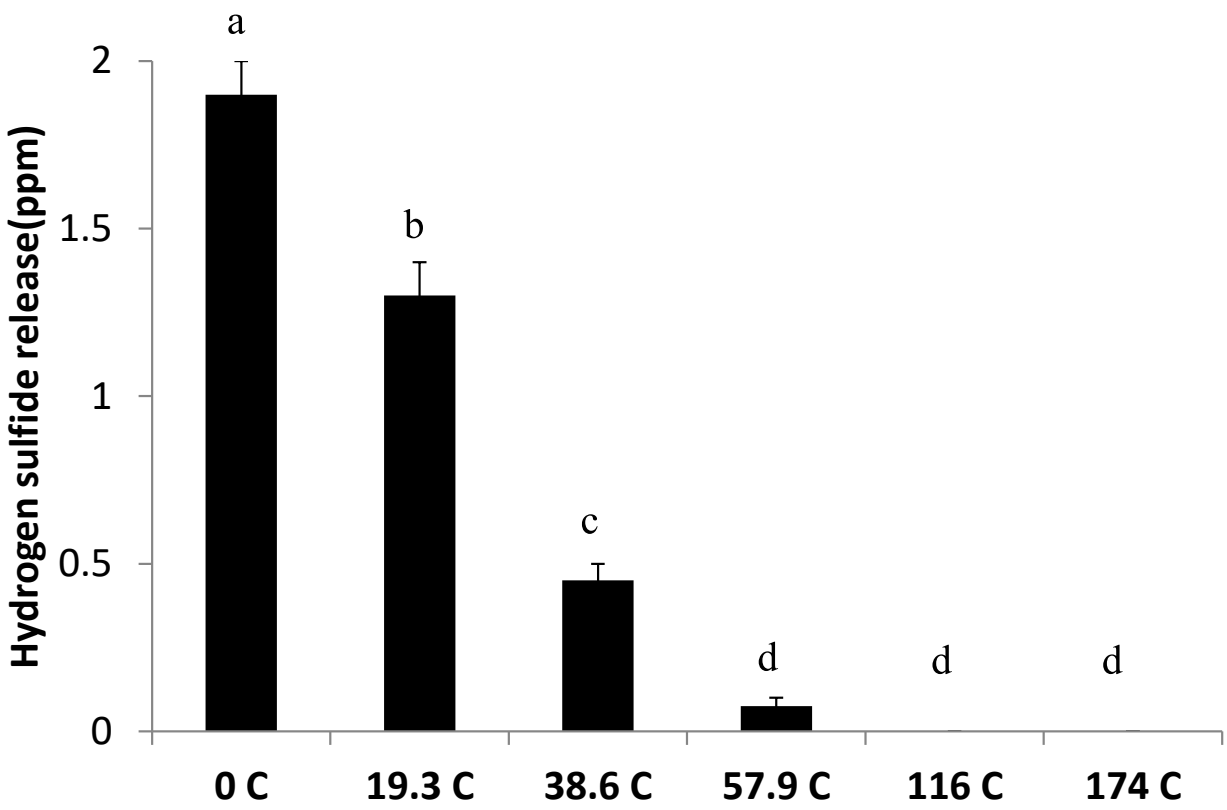
SDS-PAGE with 2-ME



Lane 1: 0 C without salts;
Lane 2: 0 C; Lane 3: 19.3 C; Lane 4: 38.6 C; Lane 5: 57.9 C; Lane 6: 116 C; Lane 7: 174 C

OVA aggregates by disulfide bond were found.

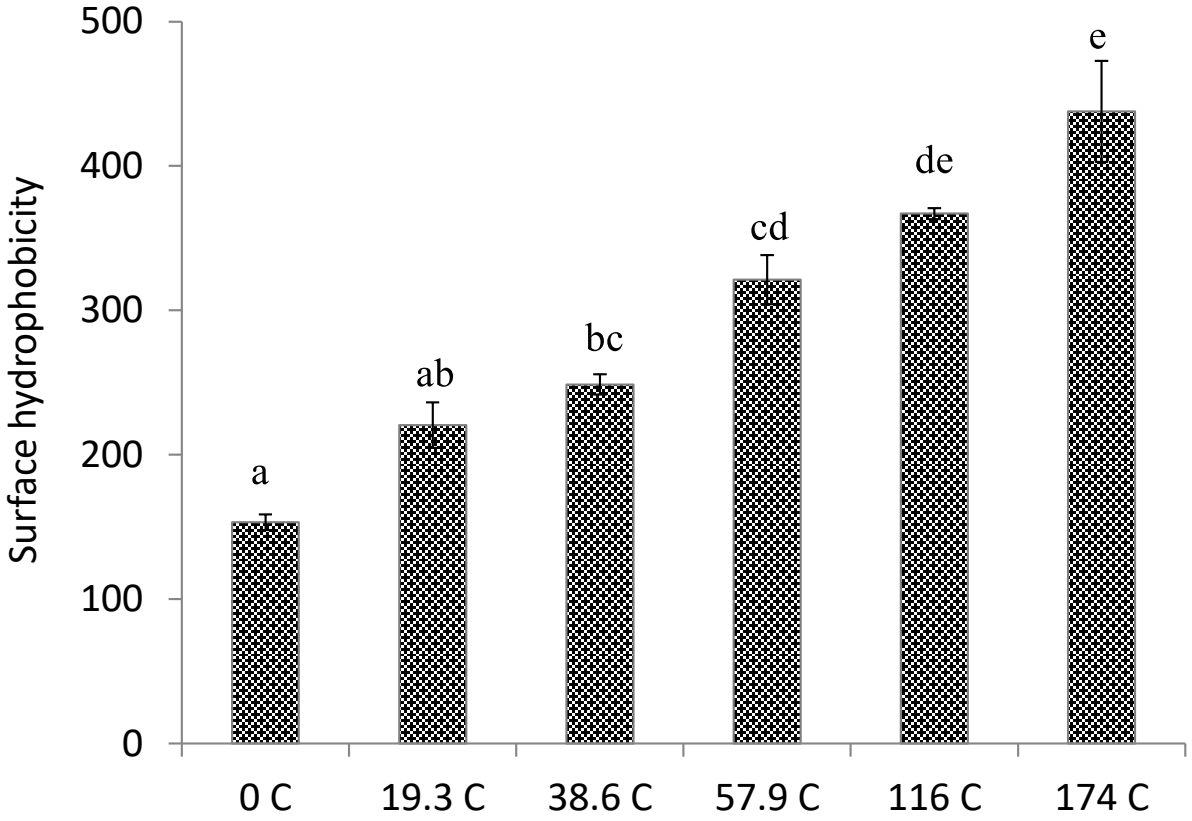
Hydrogen sulfide release



Means with different letters are significantly different from each other:$p<0.05$.
Each sample was measured in triplicate.

Hydrogen sulfide release was restrained with more electricity.

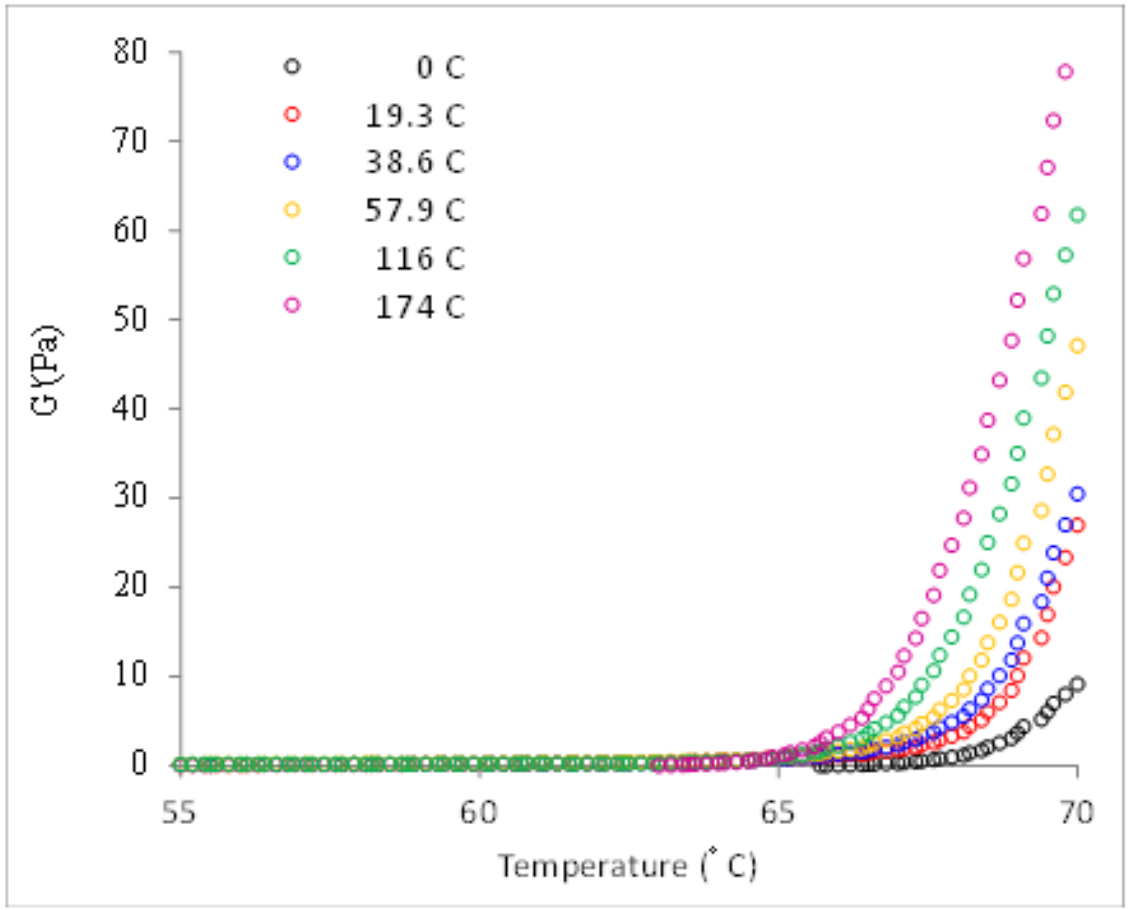
Surface hydrophobicity



Means with different letters are significantly different from each other:$p<0.05$.
Each sample was measured in triplicate.

Surface hydrophobicity increased with increasing electricity.

Changes in gelation temperature



174 C 64.8 °C

116 C 65.6 °C

57.9 C 66.0 °C

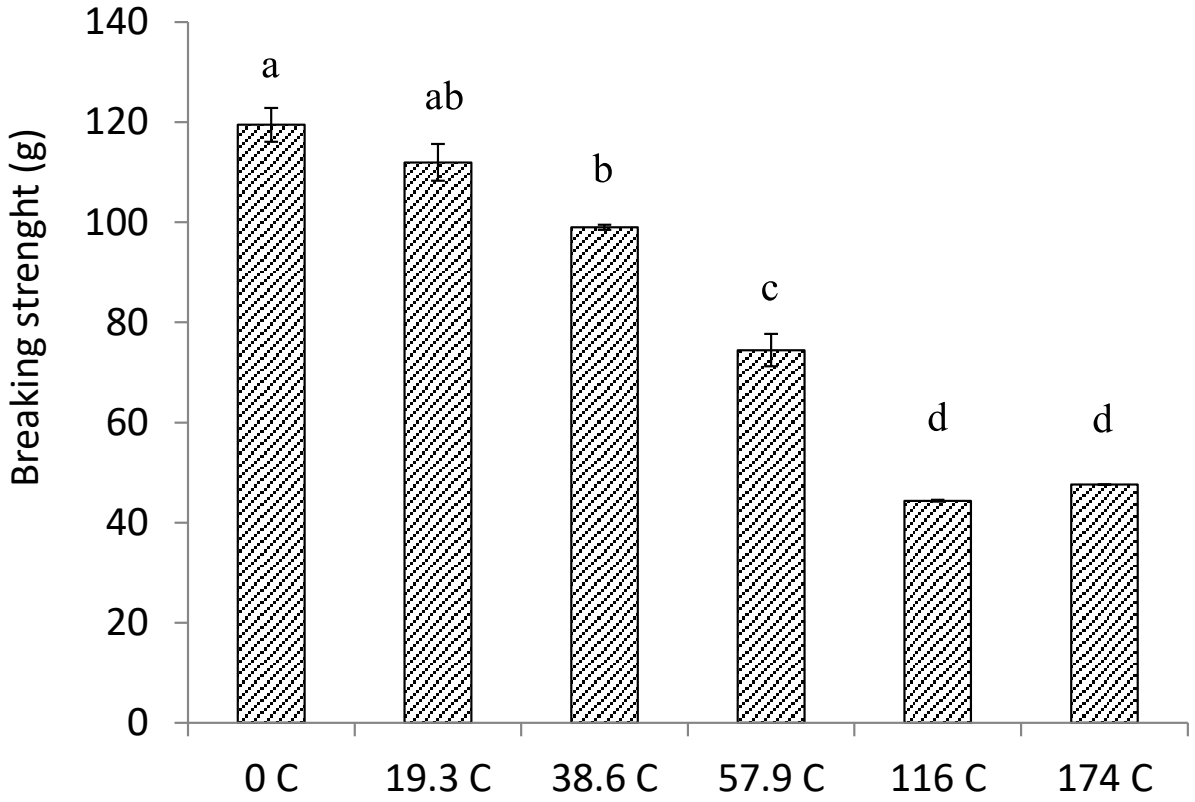
38.6 C 66.6 °C

19.3 C 67.1 °C

0 C 68.1 °C

Gelation temperature decreased with increasing electricity.

Breaking strength of heat-induced gel

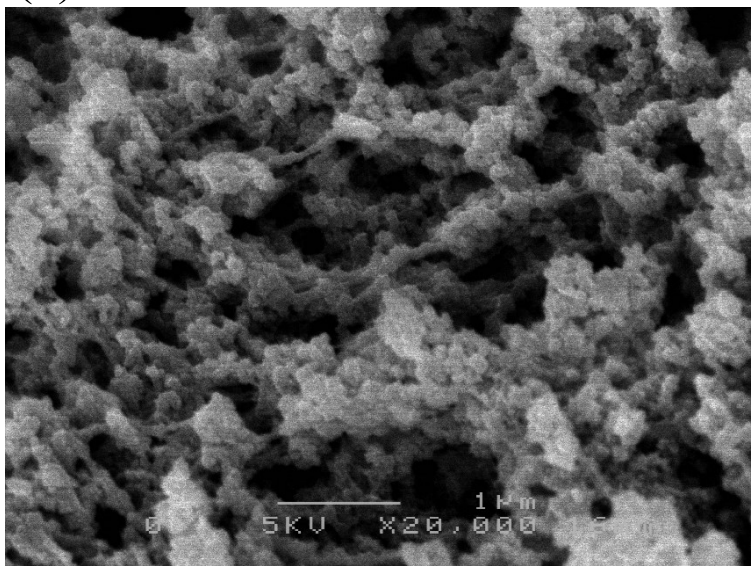


Means with different letters are significantly different from each other.
Each sample was measured in triplicate (A: 0 -57.9 C, B: 0 -174 C) or duplicate (A: 116 C, A: 174 C).

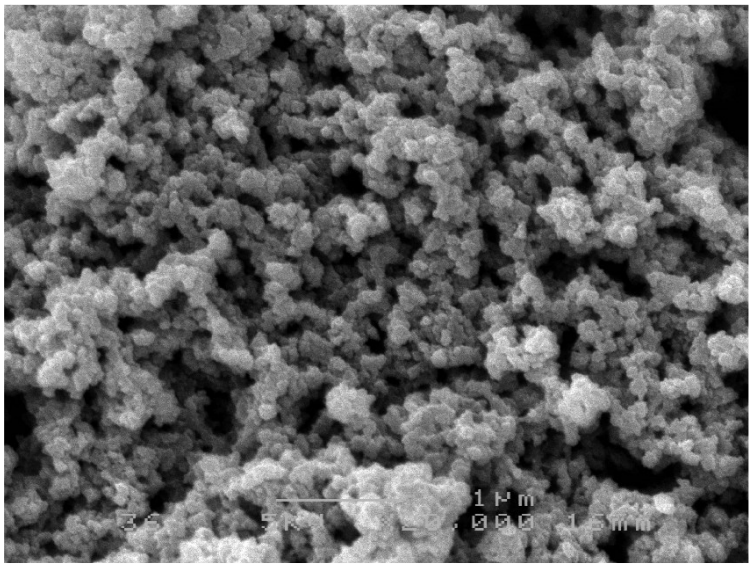
Breaking strength decreased with increasing electricity.

Investigation (SEM images)

(a)



(b)



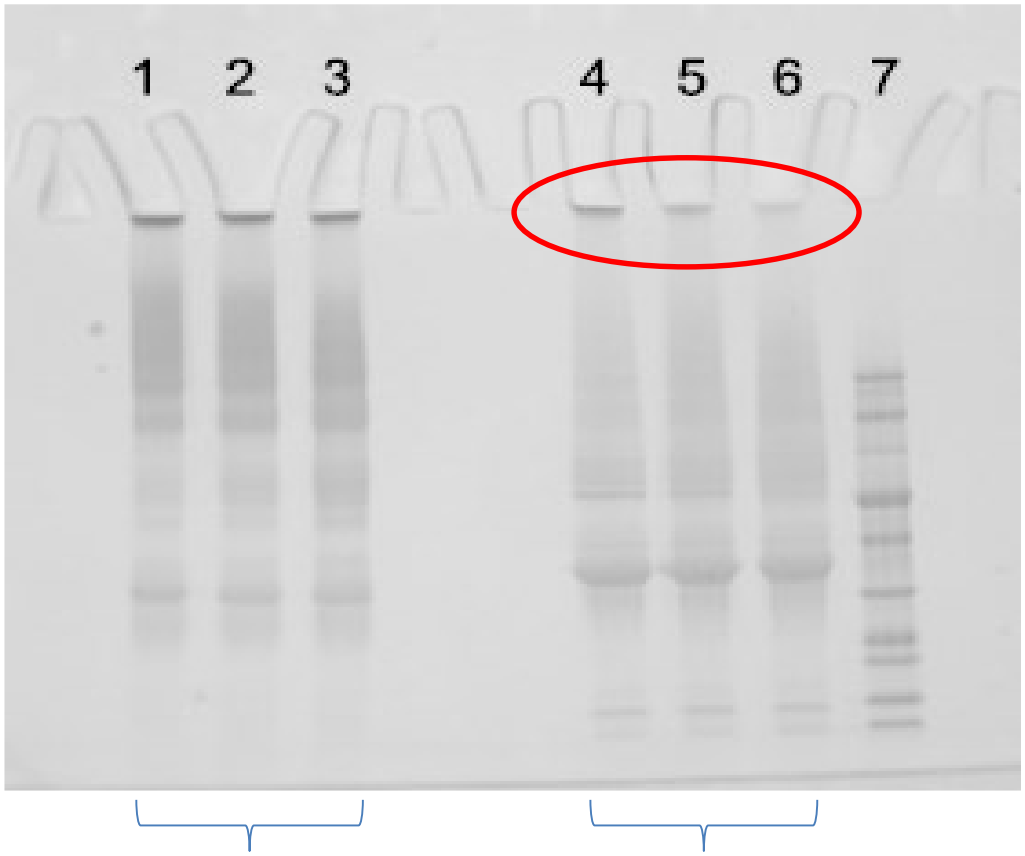
SEM images (x 20,000) of heat-induced gels
of non-oxidized egg white (a) and oxidized with 174 C (b).

Network

Random aggregates

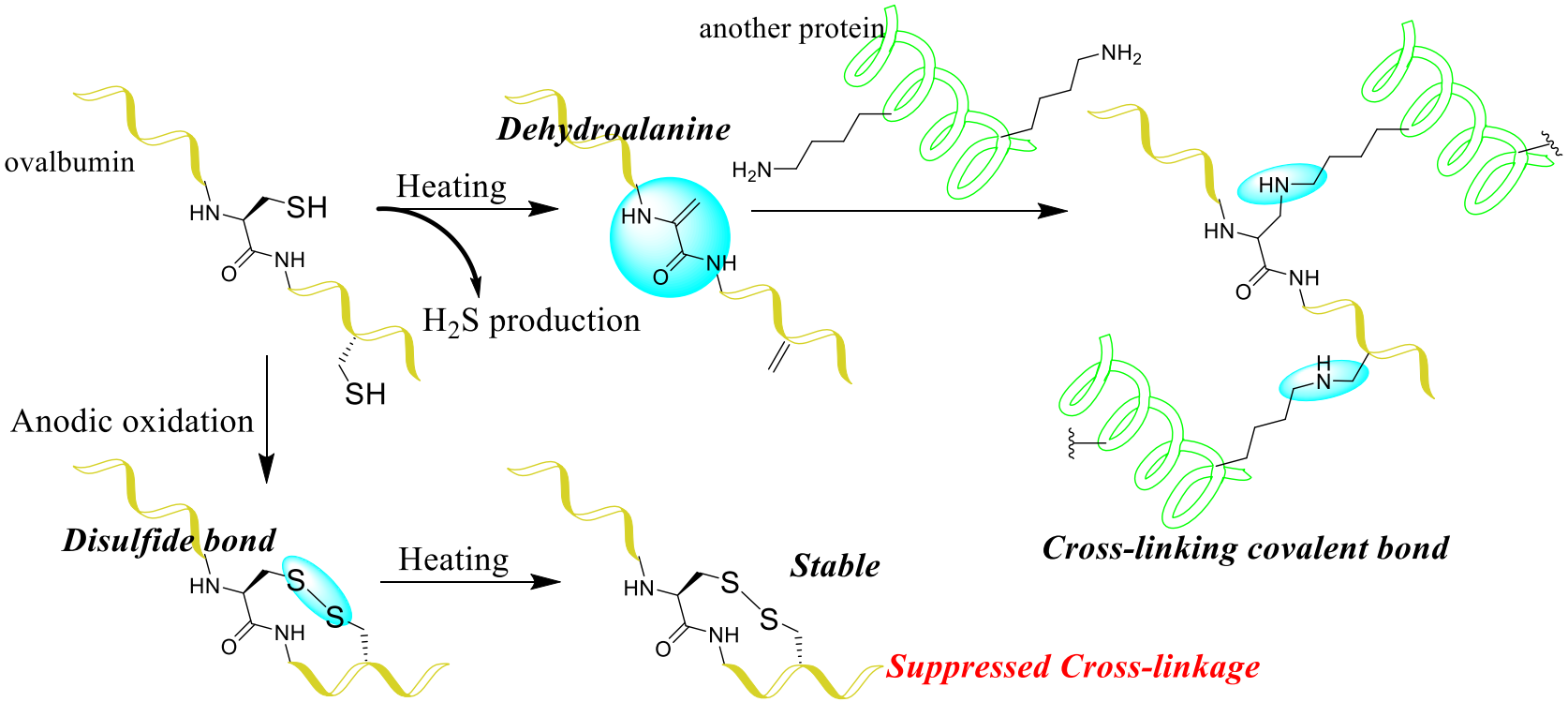
Investigation (protein interaction)

Lane 1, 4; 0C
Lane 2, 5; 38.6C
Lane 3, 6; 174C
Lane 7; markers



Covalent bonds other than SS bonds decreased with electrolysis.

Investigation (protein interaction)



Protein cross-linkage formation by covalent bonds

Conclusion

1. **Anodic oxidation of egg white caused SS bond formation in OVA and OVA polymers were formed.**

2. **The properties of anodic-oxidized egg white are as follows.**

   ①No hydrogen sulfide generated upon heating
   ②Lower gelation temperature
   ③Weaker heat-induced gel

3. **The possible reasons are as follows.**

   ①Sulfidryl groups of OVA proteins are blocked as SS bonds.
   ②Surface hydrophobicity increased.
   ③Gel structure changed into more like random aggregates and a smaller number of covalent bonds were involved.

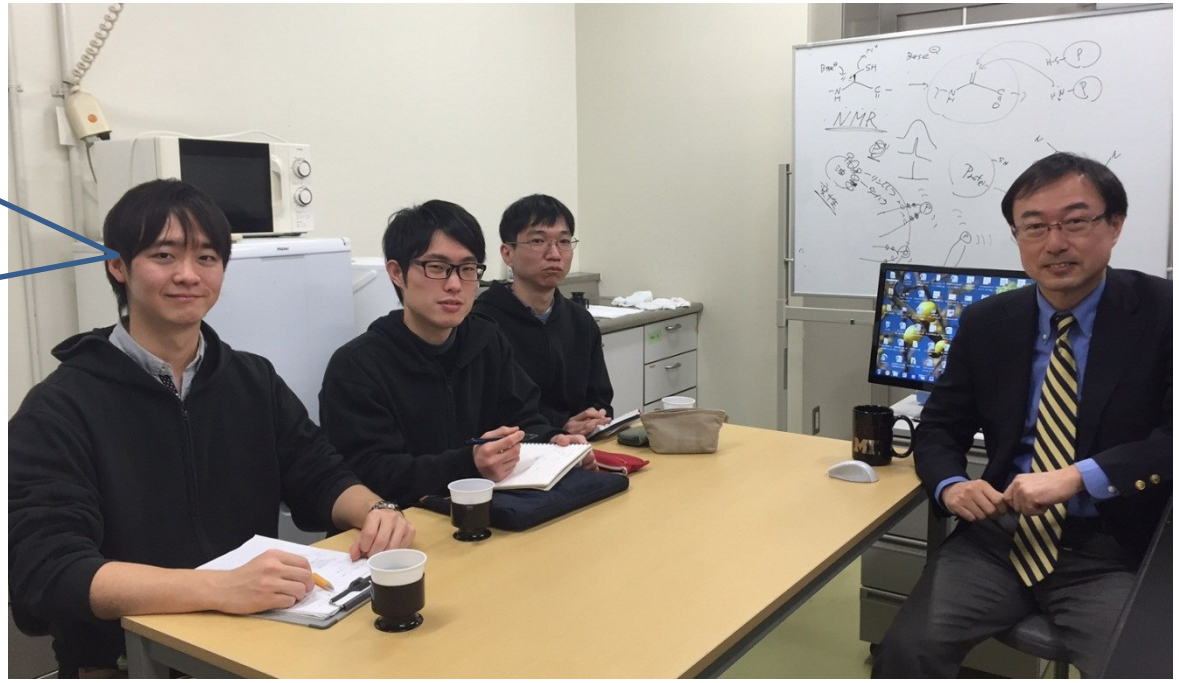
TAT

kewpie

Thank you for your attention!

Please stop by my poster!

"Dynamic Structure Analysis of Egg Yolk Denaturation with Phosphorus-31 Nuclear Magnetic Resonance"



Kewpie laboratory in TUAT