Lesson Plan: Protein Denaturation (and Reversal) Using Egg Proteins

Grade Level: High School Chemistry

Lesson Duration: 90 minutes

Focus: Real-world food and biotech applications

NGSS Connections:

Core Ideas:

- **HS-PS1-3**: Structure and properties of matter
- **HS-LS1-6**: Structure and function of biomolecules
- **HS-ETS1-2**: Design a solution to a complex real-world problem

Practices: Planning and conducting investigations, analyzing data, constructing explanations
Crosscutting Concepts: Structure-function, cause-effect, stability-change

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Lesson Objectives

By the end of the lesson, students will be able to:

1. Identify and explain what protein denaturation is.
2. Observe and compare denaturation caused by heat, pH, shear, pressure, and solvents.
3. Understand that denaturation is often irreversible. However, under special circumstances it can be reversed, as in the case of "unboiling."
4. Connect these phenomena to real-world uses in cooking, food manufacturing, and biotechnology.

Background and Introduction

Chicken eggs consist of 65% egg white (also called albumen) and 35% yolk. The egg white is mostly protein (~85% of the dry matter) while the yolk is about 1/3rd protein and 2/3rd lipids. This difference in composition alters the temperature required to cook and solidify the whites and the yolk. Thus, you can boil an egg and get a hard white with a soft, runny yolk because the whites denature at a lower temperature than the yolks (Fig. 1). That cooking process is protein denaturation – the egg proteins are unraveling from their native globular structures, creating new associations with one another, and forming a gel network that sets the structure.

Figure 1 - Soft boiled egg with gelled white and soft, runny yolk.

Denaturation is defined as the transformation of a well-defined, folded structure of a protein that is formed under physiological conditions to an unfolded state under non-physiological conditions (Fig. 2). Note that denaturation can affect secondary, tertiary, and quaternary protein structures, but it does not alter primary structures (that is, the amino acid sequence). While denaturing proteins can reduce protein solubility and reduce the activity of some proteins like enzymes, it can also improve desirable food properties like emulsifying, foaming, gelling, and digestibility. Further it increases palatability – a raw egg white doesn't sound very appetizing, while a fried egg is a tasty breakfast.

Thermal denaturation is the most common form of protein denaturation with respect to food. But, anything that alters the higher protein structures can denature, such as pressure, shear, acids, and solvents.

For more information related to protein folding and human health impacts, explore this TED-Ed resource: https://ed.ted.com/lessons/how-to-unboil-an-egg-eleanor-nelsen/digdeeper

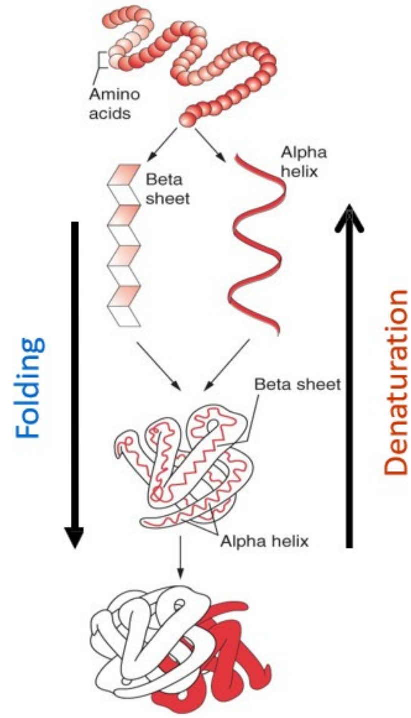

Figure 2 - Protein denaturation unfolds proteins, that is, it reduces quaternary, tertiary, and secondary protein structuring.

Lesson Structure

1. Engagement (15 minutes) – Hook & Introduction

- **Demonstration:**
  - Via either in-class examples or slide deck, give examples of foods with proteins that have been denatured in by heat, pH, shear, solvent, and pressure.
  - Show a chopped, hard-boiled egg white vs. a raw egg white. Ask: "What happened to this egg white to get from the raw to cooked version? Can we undo it? What if I told you scientists can *unboil* an egg?"
- **Mini-lecture:** (This would work well as a review lecture. If it is the students' first exposure to these concepts, you would likely want to create a larger lecture to explain further.)
  - Define proteins and their structural organization (primary, secondary, tertiary, quaternary structures).
  - Define denaturation and its impact on function.
  - Denaturation causes: heat, pH, shear, pressure, solvents.

- Introduce concept of renaturation: In most food systems, denaturation is irreversible. But in the lab, it could be reversed (i.e., renaturation – e.g., "unboiled" egg) using urea and vortex mixing. (Will likely present video demonstrating this after the exploration time, just encourage students to consider it for now.)
    - Bonus: Begin a demo by placing two samples in beakers of 10M urea: (1) ground hard-boiled egg white, and (2) ground fried egg white. The boiled egg white will dissolve in the urea while the students are running their experiment. Conversely, the fried egg will not dissolve because it has been modified (Maillard reaction).

- **Discussion Questions:**
    - Give an example of a protein that you've consumed recently. Was it denatured? If so, how?
    - Why would you want to renature a protein? How do you think protein renaturation might occur?

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2. Exploration (45-50 min) – Hands-On Experiment

Materials Needed:

- Egg white proteins
    - The most accessible option here is egg whites from fresh eggs.
    - Alternative options:
        - Fresh egg yolks. Egg whites contain ~85% protein while the yolks contain ~66% protein and 33% fat. This difference will affect the results, particularly the texture of the materials. Using yolks could enrich your lab and discussions by explaining effects of other components in the system (in this case, lipids) on protein denaturation.
        - Egg white powder. This would allow for discussion of processing of egg components. The egg white powder can also be stored for longer.
        - Vegan egg replacers (e.g., "JUST" egg). This opens discussion into egg vs plant protein structures/denaturation.
- Treatments and equipment – see Table 1 for standard and alternative treatment options.
- Test tubes
- Dropper pipettes
- Lab goggles and gloves

Table 1 – Denaturation treatments for egg proteins divided by denaturation factor.

Group	**Factor**	**Standard Treatment + Alternatives**
A	Heat	Standard: Add 5 mL of egg white to a glass test tube. Insert the test tube into rapidly boiling water for 5 min. Remove the test tube from the boiling water and allow to cool. Take note of any changes including volume, color, and smell. Then remove the egg white from the tube and assess its texture by touching it. Alternative heat treatments: * Rapidly boiling water for 1-45 min. * Boiling water for 1 min + off heat (but still in water for 3-15 min). * Oven baking. * Microwave. FYI: A good primer on heat's effects on eggs is available here: https://www.seriouseats.com/sous-vide-101-all-about-eggs
B	pH	Standard: Carefully add 3 mL of egg white into 10M KOH with a dropper pipette. After 5 min, remove the egg white from the solution and assess. Be sure to only touch with gloves as this is a VERY caustic solution! Alternative pH treatments: * Adjust time in solution. * 1M KOH. * 1-10M HCl. * Food-based acids (e.g., vinegar, lemon juice) or bases (e.g., baking soda).
C	Shear	Standard: Add 50 mL of egg white to a bowl. Using a hand mixer + whisk attachment, whisk the egg white to a stiff peak. Immediately assess the egg whites. Alternatives: * Whisking by hand. * Whisking to a soft peak. * Add tartaric acid – this adjusts the pH and can help stabilize the egg whites. * Alternative shearing equipment (blender, food processor, etc.)
D	Solvent	Standard: Carefully add 3 mL of egg white into neat ethanol with a dropper pipette. After 5 min, remove the egg white from the solution and assess. Alternatives: * Water. * 50% aqueous ethanol. * 1-10M urea. * 2.5 M Na_2CO_3.
E	Pressure	Traditional pressure cooker or Instant Pot are the primary options here. Alternatives would be changes in time, temp, or pressure levels.

Procedure:

1. Divide students into 5 groups, one for each factor in Table 1. Each group should discuss their given factor and select 3-5 different treatments therein. Students could also design their own conditions to assess.
2. Before beginning the experiment, all groups should predict how their different treatments will affect the egg white.
3. Perform the experiments and record observations and findings.

Observation Table
Each group documents:

- Treatment details (including time subjected to each treatment)
- Visual change
- Texture change
- Smell
- Volume of egg white before and after treatment

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3. Explanation (20 minutes) – Conceptual Understanding

- **Discussion of Results:**
    - This should be student-led. Have the groups report out their results and discuss their findings.
    - Connect their findings with various foods, or other denatured protein products.
    - **Video demo:** Show TED-Ed video on "How to unboil an egg".
        - If the bonus demo was performed, view the egg white proteins in urea. Discuss protein solubilization and protein modifications.
        - Outcome: Solubilized, refolded proteins—not food-safe, but biotechnologically important (e.g., enzyme recovery).

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4. Elaboration (25 minutes) – Real-World Applications & Deeper Thinking

- **Research & Discussion:**
    - Have students investigate how their group's factor denatured protein. Resource: https://www.khanacademy.org/test-prep/mcat/biomolecules/amino-acids-and-proteins1/a/protein-folding-and-denaturation

- Discuss denaturation and food safety using ceviche as an example. Resource: https://www.pbs.org/video/how-is-ceviche-cooked-s64x4m/
- Challenge students to think about other protein sources and how the denaturation factors from today's experiment would alter those proteins. (Option: have lab groups pair up, work through this, then share findings with the class.)

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5. Evaluation (10 minutes) – Assessment & Reflection

- **Exit Ticket Questions:**
    1. Define protein denaturation and provide an example (beyond eggs).
    2. How did your group's factor denature proteins? Name at least one bond that was broken.
    3. You should have observed that denaturation factors affected egg texture. Give an example of where you could use this factor to alter a protein other than egg whites.
- **Optional:** Lab report summarizing their experiment and findings.

Conclusion

This lesson provides an engaging, hands-on approach to exploring protein denaturation using egg proteins, reinforcing chemistry concepts while connecting to real-world applications.

Student Lab Worksheet: Protein Denaturation (and Reversal) Using Egg Proteins

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Background Reading

Figure 3 - Soft boiled egg with gelled white and soft, runny yolk.

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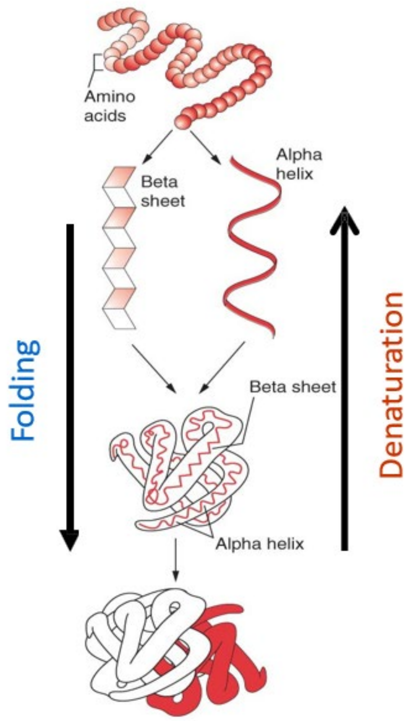

Figure 2 2 - Protein denaturation unfolds proteins, that is, it reduces quaternary, tertiary, and secondary protein structuring.

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Pre-Lab Questions

1. What is a protein, and why is its structure important in food?
2. How is denaturation different from breaking down a protein completely?
3. List three examples of how denaturation is used in cooking.
4. Why do you think an egg changes from runny to firm when cooked?
5. Predict: What might happen to an egg protein if you add vinegar?

Materials and Procedure

<u>Materials</u>

- Egg white proteins
- Treatments and equipment – see Table 1 for standard and alternative treatment options.
- Test tubes
- Dropper pipettes
- Lab goggles and gloves

<u>Procedure</u>

Procedure:

1. Your teacher will divide your class into 5 groups, one for each factor in Table 1. Each group should discuss their given factor and select 3-5 different treatments therein. Students could also design their own conditions to assess.
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<u>Results</u>

Group + Factor: ___________________

Condition	Visual + Volume Change	Texture Change	Time/Temp Notes

Optional sketches or photos:

Other observations:

Discussion Questions

1. What changes did you observe in texture, color, or thickness?
2. Which condition caused the most visible denaturation?
3. What kinds of bonds/interactions were likely disrupted?
4. Were the changes reversible or permanent?
5. How might these effects be useful in cooking or food processing?

Reflection Questions

1. What was the most surprising result in your lab?
2. How could denaturation be a problem in food processing?
3. Protein denaturation methods can be used together, rather than one at a time. Choose two methods and predict what effect(s) their combination would have on egg whites.