Dilution makes protein coagulation more difficult

Model 1. Heat *denatures* (or unfolds) proteins – when proteins *denature*, the exposed hydrophobic parts of the protein join together with exposed hydrophobic parts of other proteins, clumping together in a process called *coagulation.* We observe this process in the cooking of an egg – the clear, runny white becomes a white solid mass of denatured and coagulated protein[1].

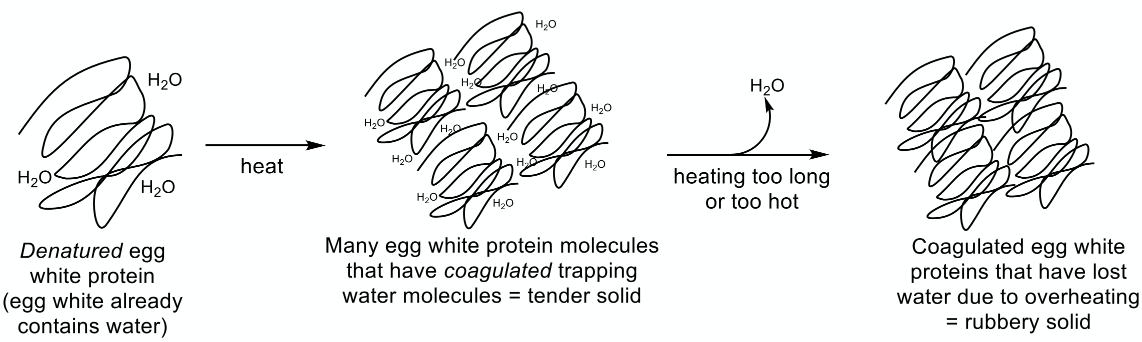


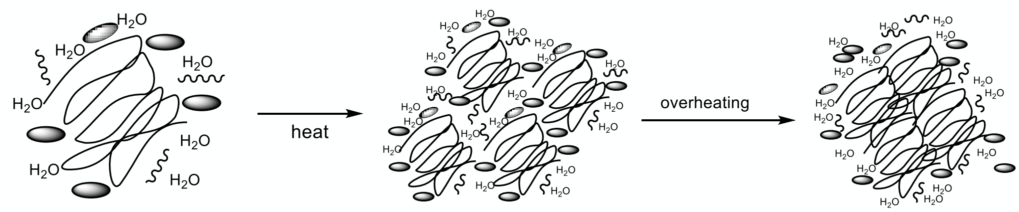
Figure 26.1. Coagulation of egg white protein – as in the frying or hardboiling of an egg.

When we add other liquids and/or molecules like starch, sugar and/or fat (from milk, butter, cheese etc) to the eggs, we *dilute* the protein mixture and *raise the temperature* at which the proteins begin to coagulate. *Dilution* of the proteins surrounds the protein molecules with many more water (and/or sugar or fat) molecules, which makes it harder for the proteins to find one another and stick their hydrophobic parts together…so we have to raise the temperature, which makes the molecules move around that much more rapidly and find each other. When the coagulated protein network does finally form in these diluted mixtures, the solid matrix of coagulated protein is tender and fragile – the large networks of coagulated proteins are filled with water, sugar and/or fat molecules – as in a custard.

If you heat a custard too much or too fast, the proteins will *curdle* instead of *thicken.* In curdling, proteins denature and form hard, tight lumps of coagulated protein that exclude the water, sugar and/or fat molecules (in custard making, the effect is called *syneresis*). Because custards are so sensitive to overheating, they are often cooked in an

[1] It is helpful to complete Activity X_Higher Order Protein Structure before attempting this activity

oven, while sitting in a *water bath*. The water bath keeps the temperature constant during cooking. Custards or creams that include starch are more stable to heat.



Egg white protein *denatured* in the presence of extra water with sugar and fat molecules (proteins have been *diluted*)

Diluted egg white proteins have *coagulated*, trapping water, sugar and fat molecules - the extra molecules interfere with the protein-protein interactions. The network of protein is weaker and more fragile.

Dilute mixtures of protein that get heated too hot or too fast will *curdle*. The protein clumps together in tight lumps, excluding the other molecules.

Figure 26.2. Egg white protein coagulation diluted with water, sugar and fat molecules

Acids (like vinegar or lemon juice) aid in protein *denaturation*, addition of acid to a protein mixture will either cause *denaturation* or make it happen more easily (e.g. at a lower temperature)[2].

Questions:

1. Custard made of 1 beaten egg, 1 cup milk and 1 tablespoon of sugar begins to thicken at 180˚F.
    a. What is causing the thickening?
    b. Why is the thickening happening at 180˚F, when a beaten egg normally sets at 165˚F?

a. The denaturing and coagulation of the protein.

b. Proteins diluted so need to increase the temperature so the molecules move around and find each other.

[2] For a lesson on how acid affect proteins see Activity 6_Higher Order Protein Structure

2. If you replace the whole eggs in a custard with just yolks (or mostly yolks), you will need more yolks (in comparison to the whole eggs) and the custard will be softer and lack the structure of a custard made with more egg white. Why is this?

Less protein in the yolk and more fat which makes coagulation more difficult.

3. The boiling point of water is 212˚F/100˚C, while a typical oven temperature is 325-350˚F. No matter how hot you make the oven, the water will only ever reach 212˚F/100˚C, upon which it will boil and eventually evaporate away. Considering that custards are best cooked at ~180˚F, why does the water bath method of cooking custards work particularly well?

Prevents overheating which would lead to curdling.

Egg white foams….cooking with the wrist

Model 2. *Denaturing* a protein uses some kind of *energy* to break the *hydrogen bonds* holding the protein together and subsequently unravel its globular structure. The unraveled protein now has exposed *hydrophobic* regions that can stick together (coagulate) with exposed *hydrophobic* regions of other proteins.

Figure 26.3. An egg white foam

Agitation can also denature proteins – specifically the kind of aggressive agitation that introduces large amounts of air into the protein mixture, creating a *foam*. When cooks use a whisk or wire beater to beat egg white, the protein will (over time) stiffen into a white foamy semisolid. This stiffening and change in color from clear to white, mirrors what we see in heat *denaturation* of egg white protein.

When egg white protein is *denatured* by whipping or beating air into the mixture, the *denatured* proteins *coagulate* and trap air bubbles in addition to water. The *denatured* proteins cluster around and stick their exposed hydrophobic portions into the air bubbles. The large numbers of trapped air bubbles give the *coagulated* protein matrix a soft, semisolid texture. The semisolid foam of *denatured* protein and air bubbles can be mixed with other ingredients and then heated.

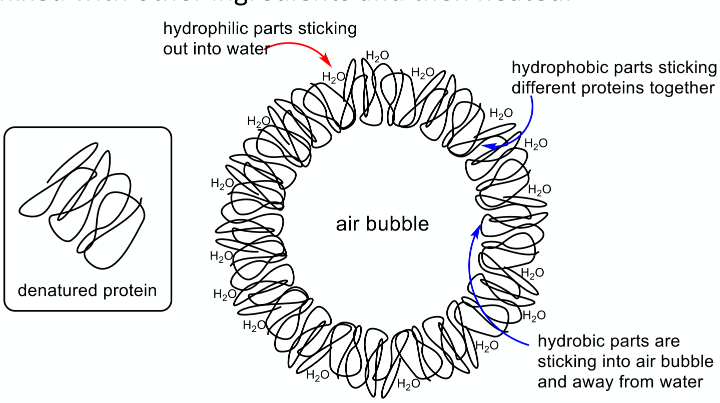


Figure 26.4. Denatured egg white protein coagulating around an air bubble. This creates a foam.

Heating accomplishes additional protein *denaturation* within the foam – especially of ovalbumin. Ovalbumin is not particularly susceptible to *denaturation* by agitation, and when the foam is heated, ovalbumin finally denatures – hardening the foam even further.

Heating expands the air bubbles in the foam which causes considerable rise, and it also dries out the foam as water molecules evaporate. When the proteins finally set enough from the heat – the gas bubbles are trapped and can't expand anymore. The result is a puffy/tall, hardened yet airy solid. Meringues like the one shown here are a classic examples of a cooked egg white foam. The structure of the meringue is created almost entirely by egg white protein.

Figure 26.5. A meringue tops this lemon tart

As we saw with custards – addition of other molecules dilutes the proteins and makes it more difficult to form the coagulated protein network that stiffens, thickens or solidifies. In particular, when making an egg white foam, *fat molecules* are particularly destructive.

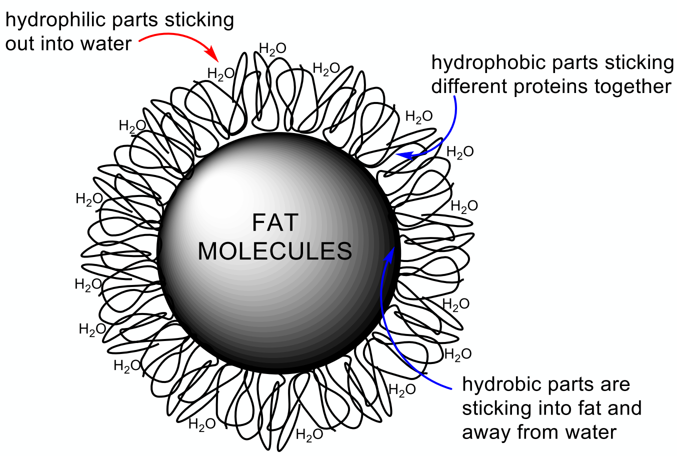
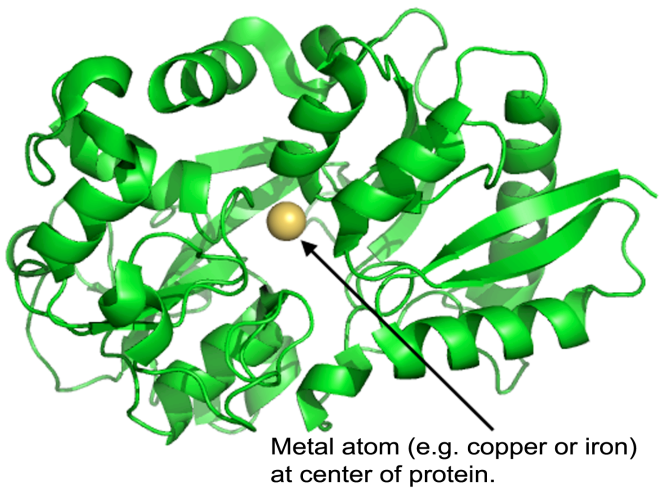


Figure 26.6. Protein denatured by agitation in the presence of fat molecules

Addition of ANY fat to a foam stabilized by denatured protein is disastrous to the foam. The denatured protein preferentially clusters around the fat molecules (since fats are very hydrophobic) and the air bubbles escape or never form in the first place.

Interestingly, it has been known for centuries that egg white proteins form better foams when beat or whipped in a copper bowl. The secret lies with the protein ovotransferrin (also known as *conalbumin*). Remember that ovotransferrin is present in egg white in order to bind iron – that's its job. Well, when a cook beats egg whites in a copper bowl – the ovotransferrin binds the copper from the bowl! Copper and Iron are both metals after all…and ovotransferrin will bind copper as easily as iron. When ovotransferrin (a.k.a. conalbumin) binds copper it becomes *more stable* and therefore *denatures at a higher temperature*. The result is that the egg white foam is more elastic and stable.

When the egg white foam is heated, the air bubbles can expand further before the copper bound ovotransferrin coagulates – this makes the cooked foam taller or puffier[3].



[4]

When ovotransferrin binds the copper metal from the bowl, the egg white foam takes on a yellowish-golden hue. The golden yellow copper atoms trapped by ovotransferrin, and the color is seen in the egg white foam itself. If you don't have a copper bowl (they are expensive and hard to clean…) then addition of acids like cream of tartar (i.e. tartaric acid) can improve the formation of an egg white foam.

Figure 26.7. Ovotransferrin binding a metal at the center. The green ribbons represent the protein, and the yellow sphere is the metal ion.

Questions:

1. Instructions for creating an egg white foam begin with separating the egg yolks from the egg whites – in fact, these instructions are very explicit that *all* egg yolk *must* be removed before beating the egg whites. Why is it so important to have every last molecule of egg yolk separated before you start whipping the egg whites?

Fat in egg yolk will make it more difficult to make the foam.

[3] *Nature* **308**, 667-668 (12 April 1984) *Why whip egg whites in copper bowls?* Harold J. McGee, Sharon R. Long & Winslow R. Briggs
[4] This image of metal bound ovotransferrin was author generated using PDB 1IEJ from J.Mol.Biol. (2001) **309:** 937-947.

2. What might explain the fact that copper bound ovotransferrin denatures at a higher temperature than regular (copper free) ovotransferrin? Use the words *non-covalent interactions* in your answer.

Skip

3. Iron has a red color. If you crush up an iron supplement tablet and add a pinch to egg whites (the whites should be beaten in a glass or stainless steel bowl), you can turn the egg white foam pink. What is the chemical explanation behind this phenomenon?

Skip

4. Why does the addition of cream of tartar improve egg white foam formation (Hint: review Activity 6 for the effects of acid on protein structure)?

Prevents disulfide bond formation

5. Most modern cooks make an egg white foam using an electric mixer, but centuries ago, cooks were making egg white foams from beating by hand with a whisk. To make hand beating an egg white foam easier, chefs would recommend beating the egg whites in a bowl that is held over another bowl of hot water. What is the chemistry behind this recommendation?

Skip

I will not eat green eggs…

Model 3. Heating proteins causes them to *denature* and *coagulate* into a solid, but heating also causes chemical reactions within the proteins. In particular, the characteristic "eggy" odor of hard boiled eggs can be attributed to the degradation of cysteine amino acid residues within the albumen protein to produce H_2S (*hydrogen sulfide*). The sulfurous odor of H_2S is what we associate with cooked eggs. In high concentrations, H_2S can be objectionable.

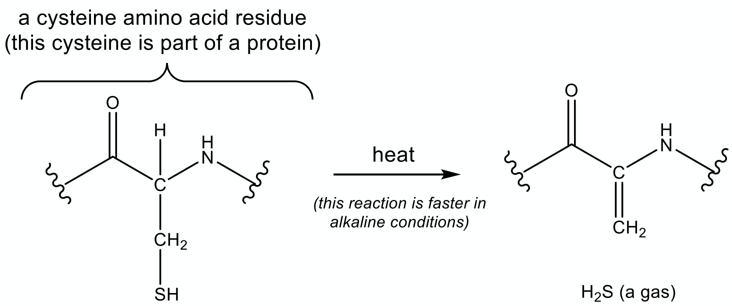
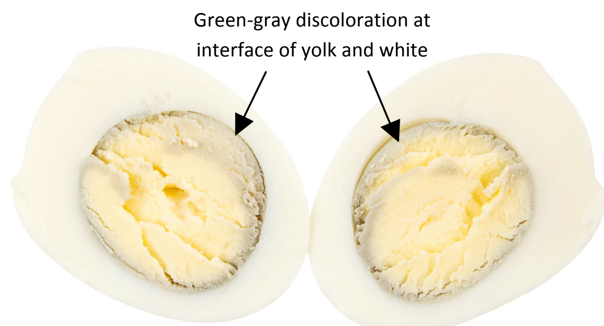


Figure 26.8. The reaction of cysteine and heat to produce H_2S.

The cysteines within the egg white albumen protein react more readily to produce H_2S when the protein has unfolded (*denatured*). This exposes cysteines that were previously buried inside the protein and allows them to react.

The reaction to produce H_2S also happens more readily in *alkaline* conditions (pH > 7). Sometimes when hardboiling eggs, a green gray discoloration can occur at the interface of the yolk and white, and can even (in some cases) discolor the entire yolk. This greenish color is caused by the compound *iron sulfide* (FeS). The source of the sulfur (S) in FeS comes from the H_2S released by albumen cysteines, while egg yolk is particularly iron (Fe) rich (remember which type of yolk was rich in iron?)

The reaction to produce FeS happens when the eggs are heated for extended periods of time.



$$\underset{\textit{a gas}}{H_2S} + \underbrace{Fe(OH)_2}_{\textit{iron in egg yolks}} \longrightarrow FeS + 2\,H_2O$$

Figure 26.9. A) The green gray discoloration appears the longer the egg is cooked. It starts at the interface of yolk and white and then moves inward. B) The reaction of hydrogen sulfide and iron to make the green iron sulfide.

Boiling eggs in water for 15 minutes is a sure way to make iron sulfide.

Heating eggs at 140˚F for 5 minutes or at 160˚F for 1 min is essential in order to kill Salmonella. Salmonella is a type of bacteria that can be found inside raw eggs. The bacteria come from the chicken that laid the egg – not necessarily from poor handling of eggs in the grocery store or farm. Since the bacteria are living inside the egg, washing the surface of the egg has no effect on removing them. Since Salmonella bacteria are living organisms, they prefer warm temperatures to grow; bacterial growth is slowed at cold (refrigerator) temperatures and the bacteria are killed by hot temperatures. There is no way to tell if an egg is harboring Salmonella, you simply have to prepare egg dishes in order to kill Salmonella were it present.

Question:

6. Some cookbooks recommend that you add lemon juice or vinegar to the water when hardboiling eggs in order to reduce the "eggy" smell. What is the chemistry behind this reasoning? (it would help to remember that vinegar and lemon juice both contain acids).

   Prevents H_2S (hydrogen sulfide) formation

7. Why (upon extended heating) does the egg yolk turn green and not the egg white?

   H_2S reacts with iron in the yolks.

8. Why is extended heating of the egg a necessary step in generating the green iron sulfide?

   Have to form H_2S which requires heat so that it reacts with the iron

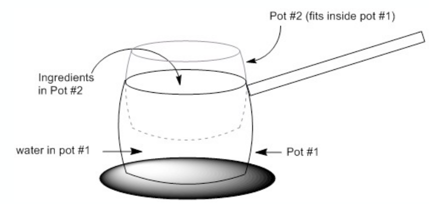
9. In a particularly bad case of salmonella poisoning, the cook had left contaminated 1 week old eggs on the countertop at room temperature overnight (she obviously did not know the eggs harbored Salmonella). She then used these eggs to make soft-boiled eggs the next morning. The eggs were heated in a bowl of hot (not boiling) tap water for 30 minutes. Why did these conditions create such a terrible case of Salmonella poisoning?

   Temperature not hot enough to kill the bacteria.

Putting it all together:

10. Pastry cream and crème anglaise are egg thickened pourable creams.
    a. Making crème anglaise requires the use of a *double boiler*. The bowl with the egg yolks, vanilla, milk, cream, salt and sugar sits over another bowl full of boiling water. The bowl of ingredients is only ever heated with the steam from the boiling water. Why is the use of this *double boiler* essential to successful crème anglaise? Use the words *diluted* and *protein coagulation* in your answer.
    b. To the right, the ingredients for pastry cream are compared to the ingredients for crème anglaise.
        i. How is pastry cream different from crème anglaise? cornstarch
        ii. How might that difference explain the fact that pastry cream is more heat stable (it can be brought to higher temperatures without curdling)? The protein is more dilute = harder to coagulate

A double boiler



Pastry Cream	Crème Anglaise
Egg yolks	Egg yolks
Vanilla	Vanilla
Milk	Milk
Heavy cream	Heavy cream
Sugar	Sugar
salt	Salt
cornstarch	

11. As some cooks have observed, older eggs that have been sitting in the refrigerator for a while smell more "eggy" when they are hardboiled.
    a. What molecule is creating the eggy smell? H_2S
    b. Why do you create *more* of this eggy smelling molecule when the eggs are older? (you may want to revist Activity 25 and review what happens to an egg as it ages) Skip globulin breakdown

12. These vanilla soufflés were made first by beating egg white into a stiff, glossy foam, then *folding* in a "base" to add flavor to the whites, then baked. The base contained egg yolks, milk, butter, flour, sugar and vanilla bean
    a. Based on what we know about egg white foams, why is it so important to carefully *fold* in the "base", and why is it important to fold it in *after* the egg white foam has been created? Not disrupt the foam and lose air; easier to make foam when base not present
    b. A soufflé base is supposed to have enough starch and protein to reinforce the walls of the air bubbles that exist in the egg white foam – this gives the soufflé a stronger structure that can stand up straight out of the dish. The

base doesn't actually create any bubbles. Does the "base" described here meet those requirements? How?

Yes, the proteins + starch will give structure

c. When the soufflé hits the oven, it rises dramatically. In (C) we can see the soufflé rose higher than the ramekin! What causes this dramatic rise?

expansion of the trapped air

d. In part (D) we can see that the soufflé in (C) shrunk a little after cooling. Considering your answer to part (C) – why did this soufflé (and *every* soufflé made before or after) shrink a little after cooling?

contraction of cooled air


(A) Base folded into egg white foam


(B) Soufflés ready for the oven


(C) Soufflé immediately after baking


(D) Same soufflé a little while later....it lost height.

Soufflé photos © http://parsleysagesweet.com/ Used with Permission

13. Raw egg yolks contain an enzyme called *alpha amylase* - an enzyme that breaks starch molecules into individual sugar molecules. When making a custard or cream with starch, the primary ingredient is egg yolks, but the *alpha amylase* can totally ruin the product by chewing up the starch molecules. The solution is to heat the raw egg yolks above 180˚F before adding them to the starch. What is the reasoning behind this solution? What would happen to the alpha amylase above 180˚F?

Denatures the amylase (it is a protein)
⇓
can no longer function as enzyme.