AN INVESTIGATION INTO THE DEPENDENCE OF EGG PROTEIN DENATURATION ON TEMPERATURE.

Aim

The aim of this investigation was to investigate how the rate of denaturation of egg white proteins is dependent on temperature and to experimentally determine the Activation Energy of the denaturation process.

Introduction.

The original idea for this project came from a lesson on boiling temperature and vapour pressure when we learned why it takes longer for an egg to hard boil at high altitude (due to the lower boiling temperature of water). This topic stimulated many thoughts. How is the time it takes to boil an egg dependant on temperature? Can the time taken to exactly hard boil an egg be predicted over all temperatures? Below what temperature do eggs cease to hard boil?

I decided that the investigation would concentrate on determining one important parameter which is the Activation Energy barrier to egg protein denaturation. If this can be determined then predictions of the egg's behaviour during boiling at a range of temperatures can be made and then tested.

Background

This project has two main theoretical bases, the principles of kinetics and process of the nature of protein denaturation, which I will describe below.

Part A: Kinetics and the Arrhenius Equation

The way temperature affects the rate of a reaction is explained by the Figure 1 below[1].

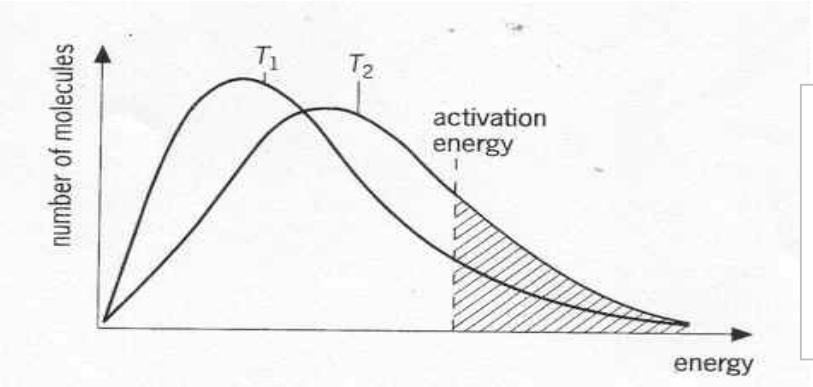


Figure 1 [1]: Maxwell-Boltzman graph where $T_2 > T_1$.

Only collisions with more energy than that of Activation Energy (the minimum energy that must be surpassed in order for a chemical reaction to take place) will cause a reaction. Therefore, in the graph above, the shaded area represents those collisions.

According to theory, as temperature increases, the molecule velocities increase, and therefore, both the frequency of collision between molecules is greater and a greater proportion of collisions cause a reaction. In figure 1, this is apparent. At the lower temperature, T_1, the fraction of molecules reacting is less than of T_2 (shaded area on graph). The rate of reaction is proportional to the number of molecules with more energy than Ea and increases exponentially with temperature.

The relationship between reaction rate and temperature is expressed by the **Arrhenius equation** which relates the rate constant of a reaction k to the absolute temperature T:

$$k = Ae^{-(Ea/R.T)}$$

where k= rate constant, Ea= Activation energy, T= Reaction Temperature, R= Gas constant and A = Arrhenius constant which is a factor that relates to the orientation of collision; only molecules colliding in the correct orientation with sufficient energy react.

Note that the Arrhenius equation is an exponential function and only applies when the activation energy lies within the exponential decay part of the curve to the right hand side of the Boltzman distribution graph in Figure 1.

Part B: Proteins & Denaturation

Proteins are formed by a combination of amino acids containing often 50 to 1000 amino acid residues). All proteins, independent of their nature (shape, complexity etc…) have structures, which are divided into four categories: primary, secondary, tertiary and quaternary.

The primary structure is mainly concerned with protein polypeptide chains (subunits) and with its amino acid sequence. In the secondary structure, there are different types of energetically stable three-dimensional structures of the polypeptide chain (also referred to as conformations). For some proteins, their polypeptide chain might form a β-pleated sheet and for others it might follow the spiral a-helix conformation. The tertiary structure is the overall three-dimensional appearance of the protein which is held together by strong intermolecular forces (e.g. Hydrogen bonding). For example, a globular protein such as in egg white, is approximately spherical and folding is extensive to obtain a compact tertiary structure. Lastly, the interaction of various polypeptide chains in a non-covalent way to pattern the protein molecule is said to present the quaternary structure.

Denaturation is when the biological activity of a protein is lost and disruption in the secondary, tertiary and quaternary structure of a protein occurs due to changes in temperature, pH, ionic strength, or due to an addition of organic solvents. For instance, when egg white is exposed to heat, it thickens and changes color. At that point, denaturation has occurred and all its structure has been disrupted, except for its primary structure, and an alternative energetically stable three dimensional structure is formed. It is the energy barrier to this process of permanently disrupting the three dimensional structure of the egg protein that is the focus of this investigation.

Methodology

A common procedure (Hill, G & Holman, J (2001))[2] to determining the Ea, is by measuring the time of reaction (in this case of final time of denaturation of egg proteins determined as the time when the film of egg white between the two microscope slides became opaque) at various different reaction temperatures using the Arrhenius equation:

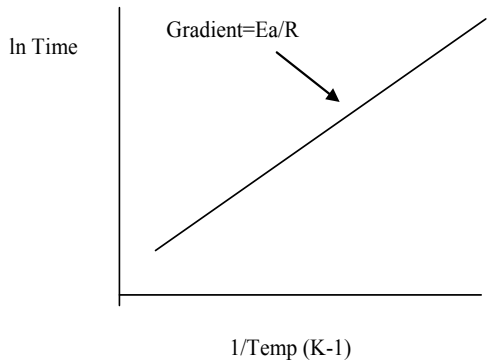
$$k = Ae^{-(Ea/R.T)} \Rightarrow \ln k = \ln A^{-(Ea/R.T)}$$

and since k is proportional to 1/Time:

$$\ln k = -\ln Time + a\ constant \rightarrow \ln Time = (Ea/R.T) - \ln A + a\ constant$$

Now, we can plot a graph, lnTime versus 1/Temperature (in Kelvin) and calculate the gradient. Since we recognize the gas constant(R=8.3145 JK-1mol-1), we can determine the Activation energy:

Gradient = Ea/R → Ea = R x Gradient



EXPERIMENTAL PROCEDURE

The focus of the experimental work was to measure how long it took egg white and egg yolk to denature over a range of temperatures. The development of a suitable procedure was far more time consuming than originally anticipated since it proved difficult to experimentally determine exactly when the egg sample had 'boiled' (denatured). In the end some procedures yielded results and these experiments are described below. The final successful experiments only focused on the egg whites.

The procedure was as below:

1. The egg white was separated from the egg yolk in a small beaker and a 500ml beaker was filled with tap water to heat over a flame.

2. With a syringe, a drop of egg white was put on the center of a preweighed microscope glass slide and then using another clean preweighed microscope glass slide, I pressed them together (with egg white in between) and wiped up the sides of the slides. They were weighed again.

3. Afterwards, the diameter of the circular shaped liquid egg white pressed between the two slides was measured.

4. Then, at different temperatures of the heated water slides were added to the water and were closely observed, as the stopwatch was running.

5. When I noticed denature of the egg white, I stopped the stopwatch and simultaneously placed the two slides in room temperature water to cool down.

6. In each experiment, recorded was the time the egg white took to denature and temperature it was at.

Results

Egg white results

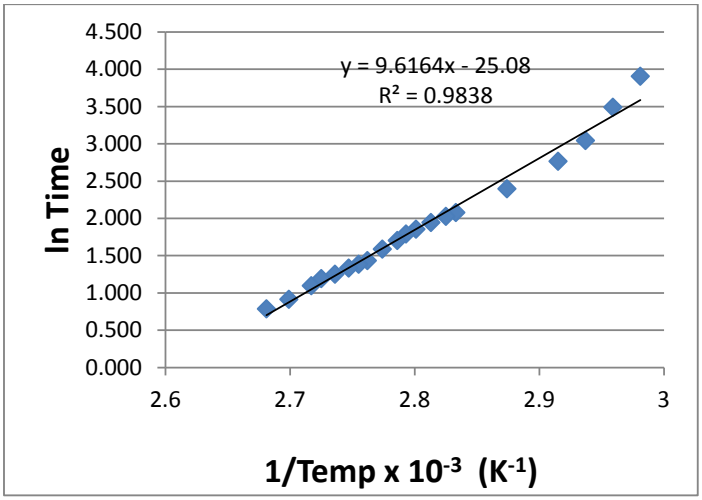
Diameter (+/- 0.1 cm)	Mass of egg white (+/- 0.005 g)	Temperature of water (+/- 0.5 °C)	Time of denaturation (+/- 0.5 sec)
2.5 by 5.0	0.01	25.0	Never denatured
2.5 by 5.0	0.01	30.0	Never denatured
2.5 by 4.5	0.01	35.0	Never denatured. Not even after 15 min.
2.5 by 5.0	0.01	40.0	Never denatured. Not even after 10 min.
2.5 by 5.0	0.02	45.0	Never denatured. Not even after 5 min.
2.5 by 5.0	0.01	50.0	Never denatured. Not even after 5 min.
2.5 by 5.5	0.01	55.0	Never denatured. Not even after 5 min.
2.5 by 5.0	0.01	60.0	Never denatured. Not even after 5 min.
2.5 by 5.0	0.01	62.5	49.9 sec.
2.5 by 5.0	0.01	62.5	49.7 sec.
2.5 by 5.0	0.01	65.0	32.8 sec.
2.5 by 4.5	0.01	67.5	21.0 sec.
2.5 by 5.5	0.01	70.0	15.9 sec.
2.5 by 5.5	0.01	75.0	11.0 sec.
2.5 by 5.0	0.01	80.0	8.0 sec.
2.5 by 5.0	0.01	81.0	7.6 sec.
2.5 by 5.0	0.01	82.5	7.0 sec.
2.5 by 5.0	0.01	84.0	6.4 sec.
2.5 by 5.0	0.01	85.0	6.0 sec.
2.5 by 5.0	0.01	86.0	5.5 sec.
2.5 by 5.5	0.01	87.5	4.9 sec.
2.5 by 5.0	0.01	89.0	4.2 sec.
2.5 by 5.0	0.02	90.0	4.0 sec.
2.5 by 5.0	0.01	91.0	3.8 sec.
2.5 by 5.5	0.02	92.5	3.5 sec.
2.5 by 5.0	0.01	94.0	3.3 sec.
2.5 by 5.5	0.01	95.0	3.0 sec.
2.5 by 5.0	0.01	97.5	2.4 sec.
2.5 by 5.0	0.01	97.5	2.5 sec.
2.5 by 5.0	0.01	100.0	2.1 sec.
2.5 by 5.0	0.01	100.0	2.2 sec.

ANALYSIS

In order to find the activation energy I need to calculate ln Time and 1/Temperature values for the reaction temperatures where denaturation occurred

Temperature(k)	Time(sec.)	ln Time	1/ Temp.(k^{-1})
298.0	-----	-----	
303.0	-----	-----	
308.0	-----	-----	
313.0	-----	-----	
318.0	-----	-----	
323.0	-----	-----	
328.0	-----	-----	
333.0	-----	-----	
335.5	49.7	3.906	2.981×10^{-3}
338.0	32.8	3.490	2.959×10^{-3}
340.5	21.0	3.045	2.937×10^{-3}
343.0	15.9	2.766	2.915×10^{-3}
348.0	11.0	2.398	2.874×10^{-3}
353.0	8.0	2.079	2.833×10^{-3}
354.0	7.6	2.028	2.825×10^{-3}
355.5	7.0	1.946	2.813×10^{-3}
357.0	6.4	1.856	2.801×10^{-3}
358.0	6.0	1.792	2.793×10^{-3}
359.0	5.5	1.705	2.786×10^{-3}
360.5	4.9	1.580	2.774×10^{-3}
362.0	4.2	1.435	2.762×10^{-3}
363.0	4.0	1.386	2.755×10^{-3}
364.0	3.8	1.335	2.747×10^{-3}
365.5	3.5	1.253	2.736×10^{-3}
367.0	3.3	1.194	2.725×10^{-3}
368.0	3.0	1.099	2.717×10^{-3}
370.5	2.5	0.916	2.699×10^{-3}
373.0	2.2	0.788	2.681×10^{-3}

Graph 1. Plot of ln Time against 1/Temperature



Calculation to determine Activation energy, Ea.

Gradient from Excel derived linear equation

$= 9.6164 \times 10^3 = 9616$

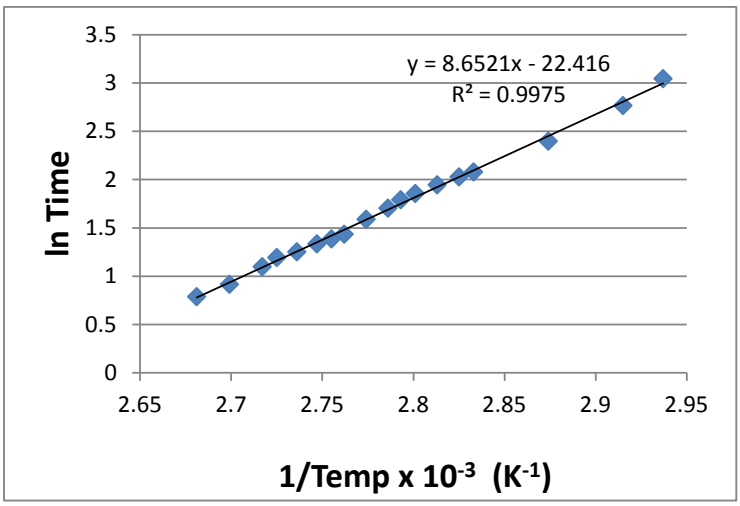
Gradient= Ea/R so Ea= 9616x 8.314

Ea= 79974 J mol^{-1}

Ea = 80kJ mol^{-1}

The two data points corresponding to the lowest reaction temperatures at 335.5 and 338.0 K do not appear to conform to the linear plot. I have removed these two points as anomalous in the graph below and recalculated Ea.

Graph 2. Plot of ln Time against 1/Temperature with discarded data points



Gradient from Excel derived linear equation

$= 8.6521 \times 10^3 = 8652$

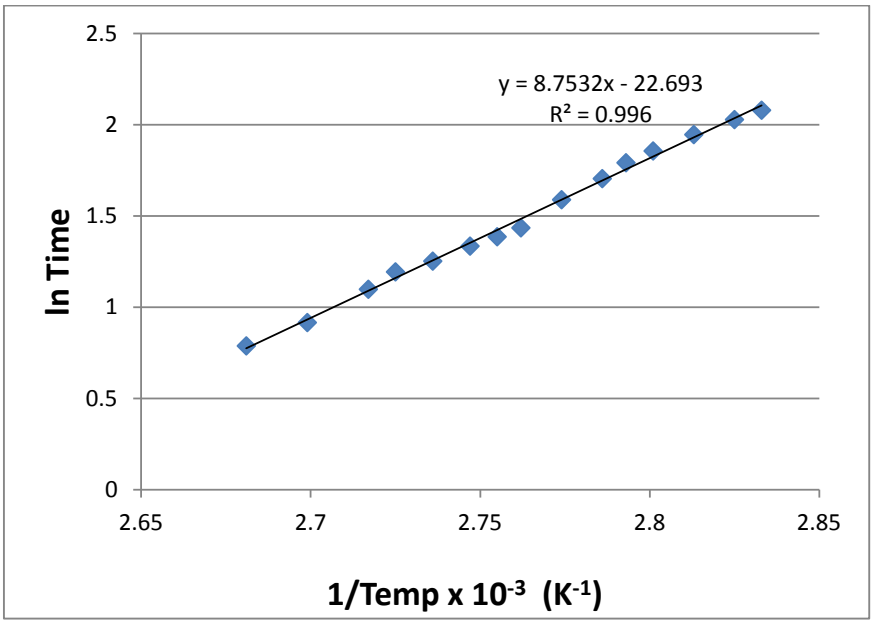
Gradient= Ea/R so Ea= 8652x 8.314

Ea= 71932.73 J mol^{-1}

Ea = 72kJ mol^{-1}

By cutting the data back further to a maximum 1/Temp value of 2.766×10^{-3} which represents the closely spaced data points the graph becomes

Graph 3. Plot of ln Time against 1/Temperature with further discarded data points



and Ea= 8753x 8.314

Ea= 72772.44 J mol^{-1}

Ea = 73kJ mol^{-1}

The calculated Es results are tabulated below along with the R^2 correlation value that relates to how good the linear fit was in the graphs (with 1 being perfect fit)

	Graph 1	Graph 2	Graph 3
Ea (kJ mol^{-1})	80	72	73
R^2	0.9838	0.9975	0.996

The best value is from graph 2 and the value from Graph 3 gives some idea as to the amount of uncertainty arising from the plots.

My final experimental value for Ea of egg protein denaturation = 72 ± 1 kJ mol^{-1}

Conclusion and Evaluation

The initial aims of the investigation have been met. It has be seen that denaturation did not take place at 60°C and below. Above this temperature the rate of protein denaturation increases rapidly with temperature.

I was able to calculate an Activation Energy for the activation energy of egg protein denaturation and it was

$Ea = 72 \pm 1$ kJ mol^{-1}

I could not find an exact **literature value** for the Ea of egg protein (albumin). One article[3] studied the effect at acidic pH's (which will change the Ea because acidic pH also denatures proteins) and gave the values as 36.7 and 50.0 kcal./mole which correspond to **150-200 kJ mol^{-1}**

My value is about a half or a third of this literature value. When I reflect on the simplicity of the method I am impressed that this investigation has arrived at a value that is so sensible in size.

It is also significant that the Arrhenius equation seems appropriate for the determination of egg protein denaturation as long as the temperature range for the measurements is kept within specifically defined limits. This is because the Arrhenius equation strictly applies to ideal gas reactions only although it has been widely used in the study of liquids and solution reactions where collision theory still holds and only the Arrhenius constant A is affected by the change of state.

However the denaturation reaction of proteins is not a collision reaction (it depends on the protein chains rotating and intermolecular forces breaking and reforming) and the theoretical basis of the equation no longer so obviously holds. There is no obvious reason why the plot of ln(Time) v 1/Temperature should have been so clearly linear. It is maybe the most interesting finding of this investigation that the relationship in the Arrhenius Equation still seems appropriate.

References

1. http://www.webchem.net/notes/how_far/kinetics/rate_factors.htm, last accessed 3rd March 2012

2. Hill, G & Holman, J (2001). *Chemistry in Context: Laboratory Manual and Study Guide*, 5th Edition, pp 54-55, Surrey, Nelson

3. Investigations on proteins and polymers. VII. The denaturation of egg albumin, Robert J. Gibbs, M. Bier, F.F. Nord, Archives of Biochemistry and Biophysics, Volume 35, Issue 1, January 1952, Pages 216–228, Last accessed at http://www.sciencedirect.com/science/article/pii/S0003986152800670 on 4th March 2012

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4. http://chemistry.about.com/od/biochemistry/a/proteinstructur.htm, last accessed 26th February 2012