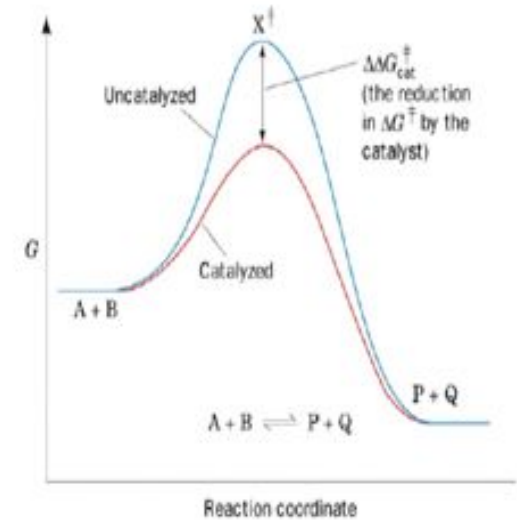

You Are What Your Pepsin Eats

— The Effect of Temperature on
the Rate of Pepsin Protein
Digestion in Egg Whites —

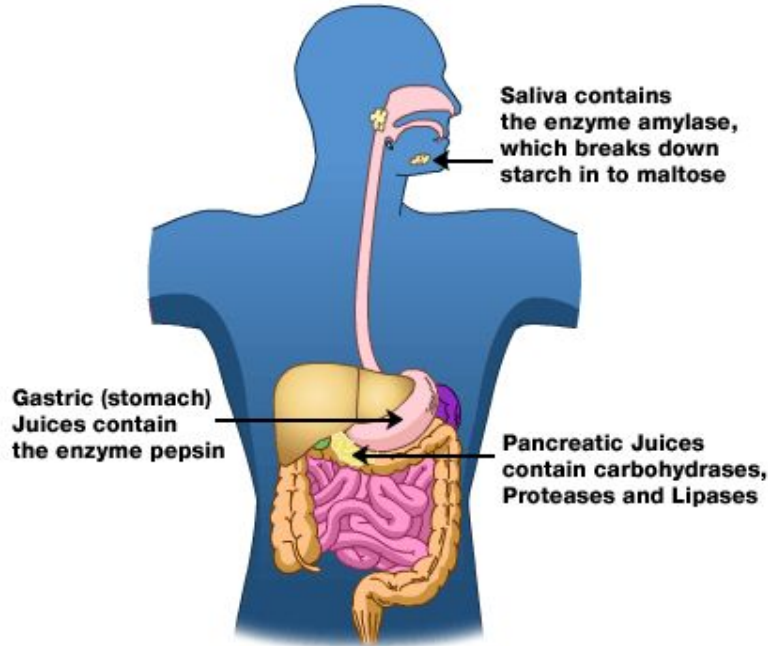
Quinn Johnshoy & Lizzie Plender

Introduction - A Molecular View

- Enzymes as reaction helpers (biocatalysts) (Trivedi, Rathore, Kamble, Goyal, & Singh 2013)
- Pepsin & acidic environments
 - Protein digestion
 - Mucous lining of stomach (Kristo & Pintye-Hodi 2013)
 - 6 carboxyl groups, H-bonds
 - Valence structures, optimal activity, & denaturation (Edelhoch 2002)



Introduction - Pepsin & Human Digestion

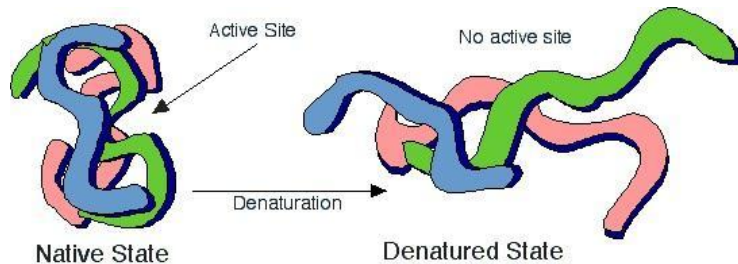


- Hydrolyzing Proteins & amino acids
- Longevity of digestion
 - 3-4 hrs in stomach (Kondyjoyan, Daudin, & Champanelle 2014)
- Pepsin supplements (Do, Kong, Penet, Winetzky, & Gregory 2015)

Introduction - Conditions Affecting Denaturation

pH

- Acidic environment
 - pH of 1.0-4.0
- Quaternary structure = enzymatic function (Edelhoch 2002)



Temperature

- High kinetic energy in environment = denaturation
 - Irreversible denaturation
- Little kinetic energy = little enzymatic activity
(Cadena, Ramos, Peimbert, Hernandez, & Zubillaga 2011)

Hypothesis - Pepsin & Temperature

- Protein substrate = egg whites



Our hypothesis is that the pepsin will break down the protein in the egg whites more efficiently at human body temperature (37°C) than at any other temperature because that is the temperature in which pepsin functions in the human body.



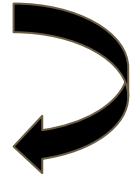
Materials & Methods



30x pieces
egg whites,
about 0.5 g
each, record
initial mass



2mL 5% pepsin
solution per tube
(30 tubes total)



-Incubate 48 hrs
-Measure
change from
initial mass



-6 tubes/temp.
-temps. @ 1°C, 22°C,
30°C, 37°C, 50°C



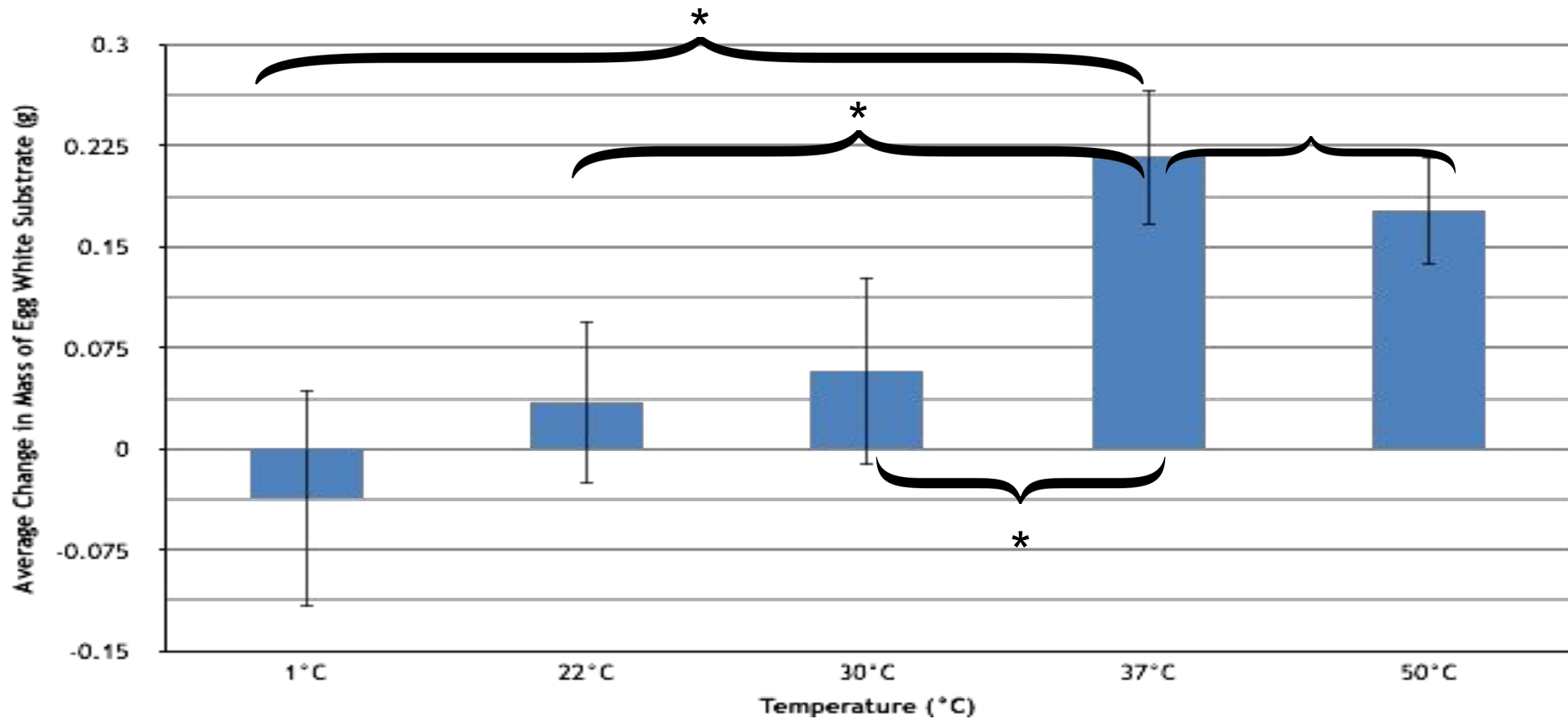


Figure 1. Average change in mass (in grams) of egg white substrate over 48 hours in incubators of varying temperatures, as an indicator of pepsin enzyme efficiency and temperature(s) of plausible enzymatic denaturation. Error bars represent +/- 1 standard deviation, (*) indicates p-value $< \alpha = 0.05$

Results - T-Tests & P-Values

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

	1 °C	22 °C	30 °C	37 °C	50 °C
Mean change in mass (g)	-0.0370	0.0345	0.05783	0.21667	0.17667
P-value compared to 37°C	2.1486x10 ⁻⁴	2.2950x10 ⁻⁴	0.0016424	N/A	0.14364

$$\bar{x} = \frac{\sum x_i}{n}$$

Discussion - Hypothesis

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

- Alternative Hypothesis rejected (overall)
 - Egg whites @ 37°C had the highest average change in mass, based on means
 - P-value for 37°C & 50°C = rejection of alternative
- 1°C egg whites = only ones w/ average increase in mass
- 50°C & 37°C = very similar trend, indication that pepsin can maintain quaternary structure @ high temperatures?

Discussion - Limitations & Interpretations

- Sources of error/limitations
 - Water weight lowers change in mass
 - Temperatures may not have been constant over the 48 hours
- Alternate interpretations
 - Experiment conducted in incubators and not in the human body, could vary results



Discussion - Big Picture

- Importance of study
 - Human digestion (Kondyjoyan, Daudin, & Champanelle 2014)
 - Gastric Health
 - Diseases linked to decreased levels of pepsin (Samuels et al. 2015)
 - Optimal body temp. & sickness (Cadena, Ramos, Peimbert, Hernandez, & Zubillaga 2011)
 - Enzymes = vital biological molecules (Trivedi, Rathore, Kamble, Goyal, & Singh 2013)
- Further research- arctic fish and pepsin (evolution) (Carginale, Trinchella, Capasso, Scudiero, & Parisi, 2004)



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Literature Cited

- Camarillo-Cadena, M., Garza-Ramos, G., Peimbert, M., Pérez-Hernández, G., & Zubillaga, R. (2011). Thermal denaturation of β -Glucosidase B from *Paenibacillus polymyxa* proceeds through a lumry-eyring mechanism. *The Protein Journal*, 30(5), 318-323. <http://link.springer.com.dml.regis.edu/article/10.1007/s10930-011-9334-0/fulltext.html>
- Carginale, V., Trinchella, F., Capasso, C., Scudiero, R., & Parisi, E. (2004). Gene amplification and cold adaptation of pepsin in Antarctic fish. A possible strategy for food digestion at low temperature. *Gene*, 336(2), 195-205. doi:<http://dx.doi.org.dml.regis.edu/10.1016/j.gene.2004.04.030>
- Do, D.H., Kong, F., Penet, C., Winetzky, D., & K. Gregory. (2016). Using a Dynamic Stomach Model to Study Efficacy of Supplemental Enzymes during Simulated Digestion. *LWT – Food Science and Technology*, 65, 580-588. <http://www.sciencedirect.com.dml.regis.edu/science/article/pii/S0023643815301481>
- Edelhoch, H. et. al. (2002). The denaturation of pepsin IV. The effects of temperature. *Biochimica et Biophysica Acta*, 38, 113-122. <http://www.sciencedirect.com.dml.regis.edu/science/article/pii/0006300260912002>.
- Kondjoyan, A., Daudin, I-D., & Santé-Lhoutellier, V. (2015). Modelling of pepsin digestibility of myofibrillar proteins and of variations due to heating. *Food Chemistry*, 172, 265-271. <http://www.sciencedirect.com.dml.regis.edu/science/article/pii/S0308814614013351>
- Kristo, K. & Pintye-Hodi, K. (2013). Effects of pharmaceutical processing on pepsin activity during the formulation of solid dosage forms. *Pharmaceutical Development and Technology*, 18(1), 17-21. doi:10.3109/10837450.2012.717946.
- Samuels, T., Hoekzema, C., Gould, J., Goldblatt, M., Frelich, M., Bosler, M., & ... Johnston, N. (2015). Local Synthesis of Pepsin in Barrett's Esophagus and the Role of Pepsin in Esophageal Adenocarcinoma. *Annals Of Otology, Rhinology & Laryngology*, 124(11), 893-902. doi:10.1177/0003489415590657
- Trivedi, V., Rathore, R., Kamble, P., Goyal, M., & Singh N. (2013). Pepsin, Papain, and Hyaluronidase Enzyme Analysis: A Review. *International Journal of Research in Pharmacy and Science*, 3(1), 01-18. <http://www.ijrpsonline.com/pdf/0235.pdf>.

Pictures Cited

<http://www.rpi.edu/dept/chem-eng/Biotech-Environ/Projects00/temph/enzyme.html>
http://www.abpischools.org.uk/page/modules/enzymes/enzymes3.cfm?coSiteNavigation_allTopic=1
<https://www.studyblue.com/notes/note/n/enzymes-flashcards/deck/826713>
<https://www.caloriecount.com/calories-egg-white-i1124>
<http://www.biosciencetechnology.com/product-releases/2014/05/analytical-lab-balance>
<http://www.medicaexpo.com/prod/sartorius-group/product-69922-508714.html>
https://www.numberanalytics.com/anova_upload.php
<http://www.discover6sigma.org/post/2007/02/sampling/>
http://sphweb.bumc.bu.edu/otlt/MPH-Modules/BS/BS704_Probability/BS704_Probability11.html
<http://www.panasonic-biomedical.co.uk/ProdMIR.htm>
<http://www.plentyofguides.com/heartburn-and-chest-pain>
<http://regisrangers.com/information/logos/index>
https://www.google.com/search?q=hard+boiled+eggs&biw=1280&bih=630&source=lnms&tbm=isch&sa=X&sqi=2&ved=0ahUKEwjI_T5g5nMAhVklYMKHfM9BxEQ_AUIBygC#imgrc=5IF7KeR9lIXuUM%3A
<http://www.carolina.com/specialty-chemicals-p-r/pepsin-laboratory-grade-25-g/879377.pr>
<http://www.faqs.org/docs/docbook/html/iso-grk4.html>
<http://www.psych.utoronto.ca/courses/c1/chap7/chap7b.html>