G4 lab report

Topic

-

Protein digestion rate in human stomach.

Choosing the topic

-

Most members in our G4 group want to become doctor so our topic focuses mainly on medicine and healthcare.

I spent my summer vacation in China this year. My grandfather suffered from diabetes and his doctor told him not to eat too much because that would increase his blood sugar. He had poor diet for years which caused him anemia (condition in which the body does not have enough healthy red blood cells or hemoglobin). This disease can be caused by poor diet and anorexia nervosa which is an illness of eating disorder characterized by low body weight and body image distortion with an obsessive fear of gaining weight. I suggested him to take protein powder I brought form U.S. As a bodybuilder, I knew that protein can make people healthier by putting on more muscle. But it's not all what a responsible physician needs to know! Instead, a doctor should be able to suggest patient to use different types of proteins at different time because of the different digestion rate of protein. Another reason is a good diet cannot be replaced by protein powder. That's the reason we chose our G4 topic and we would like to apply our result to healthcare fields in the future.

Research question

Is the protein digestion rate of different solid proteins different in human stomach and what factors can contribute to the difference between them? Based on the theory behind the prediction, what type of solid protein will have the fastest and the lowest protein digestion rate?

Solid proteins/food samples tested

Steamed tofu

Boiled boneless skinless chicken breast

Boiled egg white

Cheddar cheese

Mixed nuts

Hypothesis

The protein digestion rate of different types of solid proteins will be different. The type of solid protein with the highest protein to fat concentration ratio will have the highest digestion rate as it takes more energy to break down lipids as one gram lipids gives off 9 calories compared to 4 calories from protein. Lipid is hydrophobic macromolecule which means it will water in the stomach thus decrease the digestion rate. Also the enzyme we will use to observe the digestion rate is pepsin, which is a polar substance. Another factor which will influence the protein digestion rate is the water concentration: solid protein with the highest water concentration will have the highest protein digestion rate as it allows the food to interact with water and polar pepsin in stomach more vigorously. Based on those factors and research about the macromolecule content in solid proteins which will be tested, I predict that boiled egg will have the highest protein digestion rate and mixed nuts will have the lowest protein digestion rate.

Background information

The chart below shows the protein concentration, fat concentration, water concentration and protein to fat concentration ratio of each solid protein being tested

Solid protein name	Protein concentration	Fat concentration	Water concentration	Protein to fat concentration ration
Fried tofu	17.2% +/- 0.05%	20.2% +/- 0.05%	50.5% +/- 0.05%	17.2 : 20.2 (0.851 : 1.00) +/- 0.05%

Boiled boneless skinless chicken breast	16.8% +/- 0.05%	0.390%	76.7% +/- 0.05%	16.8 : 0.39 (43.1 : 1.00) +/- 0.05%
Boiled egg white	10.5% +/- 0.05%	0.00%	87.8 % +/- 0.05%	- (fat-free)
Cheddar cheese	24.9% +/- 0.05%	33.1% +/- 0.05%	36.7% +/- 0.05%	24.9 : 33.1 (0.751 : 1.00) +/- 0.05%
Mixed nuts	17.3% +/- 0.05%	51.4% +/- 0.05%	1.75% +/- 0.005%	17.3 : 51.4 (0.336 : 1.00) +/- 0.05%

Human stomach:

   Stomach is part of the human digestive system. It has a saclike shape and is located between the esophagus and the intestines. The stomach is about 30.5 cm long and 15.2 cm wide. The capacity is about 0.94 liters in an adult. Food enters stomach and the gastric juices break down the food. Some substances are absorbed before entering the intestines.

   Gastric acid is hydrochloric acid secreted by the stomach. The highly acidic environment in stomach (pH of 2) enables stomach to kill bacteria before food entering intestines. However, it causes proteins from food to lose their characteristic folded structure. They denature. The stomach also secrets pepsin, an enzyme breaks down peptide bonds between amino acids, a process known as proteolysis. Thus the protein digestion mainly happens in human stomach.

   The temperature of human stomach corresponds to human body's temperature: around 37 Celsius degrees.

Enzyme-substrate reaction and substrate specificity of enzymes:

   The reactant an enzyme acts on is referred to as the enzyme's substrate. The enzyme binds to its substrate, forming an enzyme-substrate complex. While enzyme and substrate are joined, the catalytic action of the enzyme converts the substrate to the product of the reaction.

The reaction catalyzed by each enzyme is very specific; an enzyme can recognize its specific substrate even among closely related compounds. Only a restricted region of the enzyme molecule actually binds to the substrate. This region is called active site.

Pepsin has broad specificity with a preference for peptides containing linkages with aromatic or dicarboxylic L-amino acids. It preferencially cleaves C-terminal to Phe and Leu and to a lesser extent Glu linkages. The enzyme does not cleave at Val, Ala, or Gly.

We chose pepsin as the enzyme because this is the enzyme in human stomach and we believe it will work on most foods we test (substances).

Hydrophobic vs. hydrophilic

A hydrophobic molecule is repelled by water. They often cluster together when dropped in water, just as oil does. Hydrophobic molecules are also called lipophilic because they will dissolve in oils and other lipids. They tend to be electrically neutral and nonpolar and work better with neutral and nonpolar solvents.

Hydrophobic molecules include alkanes, oils, fats and other greasy substances.

Hydrophilic is typically used to describe a property of a molecule and refers to the likelihood of its bonding with the hydrogen molecule in water. A hydrophilic molecule is not just soluble in water but also in other polar solvents. It will dissolve less readily in oils and other hydrophobic solvents.

Variables

Independent variable: protein to fat concentration ratio in solid proteins tested

                Water concentration in solid proteins tested

Dependent variable: Protein digestion rate

Controlled variables:Temperature of the environment (corresponding to human stomach: 37.0 ^{o}C +/-0.01 ^{o}C)

pH value of the environment (amount of HCl: 0.10M)

Amount of pepsin (162 mg in every 200 ml 0.1M HCl solution)

Amount of solid protein being tested (10.0 g +/-0.01g)

Control of variables

-

-       Temperature: to keep the temperature in beakers as constant as possible (37.0 ^{o}C), I used hot water bath method. I put 5 beakers into a big cooking pot filled with 4 liters of water. By heating the water in cooking pot to 37.0^{o}C first, the solution in the beakers will keep the temperature rather constant because it takes longer time for larger amount of liquid to cool down. The temperature in the beaker is measured every 15 minutes from the beginning till the end of the experiment to keep constant.

-       pH value of the beaker: to mimic the human stomach, the HCl concentration in the beaker corresponds to the HCl concentration in human stomach: 0.10 moles/liter. So I added 20.0 ml of 1.00 M HCl to every 180 ml of water to make 0.10 M HCl solution.

-       Pepsin: in every beaker we put 162 mg of pepsin (1 pepsin capsule) and stirred it till all the powder completely dissolved in the HCl solution.

-       Solid protein: 10.0 grams of each type of solid protein is measured and to be tested.

Materials used

-

5 x 500 ml beaker

1 x 100 ml graduated cylinder

5 x Pepsin capsules (Solaray Dietary Supplement from Vitamin Shoppe)

1 x Scale (uncertainty: +/- 0.005g)

1 x Thermometer

2 x Bottle of 1M HCl solution (20.0 ml of 6.00M HCl with 100 ml tap water)

3 x Safety goggles

3 x Pairs of safety gloves

1 x Cooking pot (as big as possible)

1 x Chopstick

Procedure:

-

1) Measure 10.0 g of each of 5 solid proteins (tofu, chicken, egg white, cheese and nuts)

2) Put 180 ml of tap water into each of 500 ml beakers.

3) Put 20.0 ml of 1.00M HCl into each beaker (5 in total).

4) Labe the beakers with number 1 to 5.

5) Put 1 pepsin capsule into each beaker (only the powder inside the capsule)

6) Put the chicken breast into beaker #1, tofu into beaker #2, cheese into beaker #3, nuts into beaker #4 and egg white into beaker #5.

7) Put 4 liters into the cooking pot and start heating it until it reaches 37.0 ^{o}C.

8) Put all 5 beakers into the cooking pot and using thermometer check the temperature of the beakers every 15 minutes till the end of the experiment (7 times).

9) Measure the weight of the solid proteins in each beaker every 30 minutes for 4 times in total. Use chopsticks to transfer the food.

Data collection: see handout attached

Data processing:

-

The chart below shows the measurement of the temperature in Celsius degrees of the beaker #1 as sample every 15 minutes.

Time	Temperature of the beaker #1 (^{o}C) +/-0.01 ^{o}C
4:27	37.0
4:42	36.5
4:57	36.2
5:12	37.6
5:27	37.3
5:42	36.9
5:57	36.6

The chart below shows the weight of the solid protein in each beaker measured every 30 minutes.

Time	Weight of protein in beaker #1 (g) +/-0.05g	Weight of protein in beaker #2 (g) +/-0.05g	Weight of protein in beaker #3 (g) +/-0.05g	Weight of protein in beaker #4 (g) +/-0.5g	Weight of protein in beaker #5 (g) +/-0.5g
4:20	10.0	10.0	10.0	10.0	10.0
4:50	40.6	67.2	93.8	125	151
5:20	37.8	61.6	84.1	118	140
5:50	33.6	56.1	84.0	113	140

Data presentation:

Steps to calculate the protein digestion rate in one time period:

100% - (Mass (g) of protein after 30 minutes / Original mass (g) of protein in food sample)

The chart below shows the protein digestion rate of solid proteins in each beaker over a period of time

Time (p.m.)	Protein digestion rate of solid protein of beaker #1 (%)+/- 0.5%	Protein digestion rate of solid protein of beaker #2 (%)+/- 0.5%	Protein digestion rate of solid protein of beaker #3 (%)+/- 0.5%	Protein digestion rate of solid protein of beaker #4 (%)+/- 0.005%	Protein digestion rate of solid protein of beaker #5 (%) 0.05%
4:20-4:50	-306	-572	-838	1.15×10^3	1.41×10^3
4:50-5:20	6.90	8.33	10.3	5.60	7.28
5:20-5:50	11.1	8.93	0.00	4.24	0.00

The negative number indicates the increase in weight of the solid protein due to the water absorption. This is caused by diffusion of water from higher concentration (HCl solution) to lower concentration (food).

The line graph below shows the protein digestion rate of different proteins over period of time

From the graph we can observe that in the first half hour, cheese will have the highest protein digestion rate and nuts will have the lowest protein digestion rate. In the second half hour, the protein digestion rate of egg white and cheese will be stopped. Chicken breast will have the highest protein digestion rate and nuts will still have the lowest protein digestion rate.

Conclusion

The data supports part of my hypothesis. Different types of solid proteins have different digestion rates. The mixed nuts had the slowest digestion rate because nuts had the lowest protein to fat concentration ratio (0.336 : 1) and lowest water concentration (1.75%).

My hypothesis about egg white having the highest protein digestion rate because egg white has the highest protein to fat concentration (egg white is fat free) and highest water concentration (87.%) is not supported by the data. This is caused by the 54.0% of ovalbumin and 11.0% of ovomucoid in the egg white. Ovalbumin and ovomucoid are nourishments in egg white which are protease (digestive enzyme) blocking proteins. Although pepsin has broad specificity, in this case its activity is blocked by ovomucoid and ovalbumin.

Overall, boiled chicken breast has the rather constant and high protein digestion rate throughout the experiment. This supports the theories behind my hypothesis. Other than boiled egg white, chicken breast has the highest protein to fat concentration ratio (43.1: 1) and the highest water concentration (76.7%).

The solid proteins being tested can be put into two categories: egg white and cheese are the fast digesting proteins and nuts, chicken breast and tofu are the slow digesting proteins which the process last longer in human stomach.

Evaluation of experiment

Although the data supported our hypothesis, human errors, random errors and two important systematic errors occurred during the experiment.

First, only small amount of pepsin was mixed with the HCl solution thus it is important not to lose any of the chemical. We used the diameter of 0.5 cm chopstick to stir the solution to help the pepsin to dissolve faster but some of the pepsin powder was stick on the chopstick when we pulled it out. The surface area of chopstick which touched the water was around 7.85 cm^2 $_{+/- 0.005cm}$ (r = 0.50 cm and h = 10.0 cm, h) and some pepsin powder was stick on this surface area. This also caused unequal amount of pepsin in different beakers and since the amount of pepsin in each beaker is a constant variable, this error affected the result.

From 4:27 p.m. to 4:57 p.m., the temperature of the beaker wasn't kept exactly around 37.0 ^{o}C. The temperature dropped to 36.2oC which also affected the result in that period since the enzyme couldn't work efficiently in 36.2 ^{o}C environment. This can be considered as both human and random error. The researcher was unable to keep the temperature constant due to lack of proper equipment but the time when the experiment was carried out (12/5/2009, 4:00p.m. – 6:00p.m.)

the weather was cold and rainy and it caused the water in the cooking pot to cool down faster than the researcher expected.

The number in the second chart on raw data does not match the number on the chart in the processed data section because the researcher made a human error by ignoring the significant figures.

During the experiment, the researcher failed to measure the temperature of every beaker in the cooking pot. Instead, we took one beaker as a sample (beaker #1) and assumed that the temperature of other beakers would be the same. It was a wrong assumption because we failed to check whether the cooking pot was placed in the center above the stove and whether the fire came from the stove equally. It might cause that part of the water in the cooking pot has higher or lower temperature than other part. Although the temperature of the liquid in the cooking pot will be the same after a while, the smallest difference in temperature could affect the enzyme activity which could cause a systematic error in the result.

At the beginning of the experiment planning, instead of measuring the solid proteins of the same weight, I wanted to use samples of the same size (1 cm x 1 cm x 1 cm) but soon I realized that same size samples would have different weight (eg. same size cheese is heavier than egg white). But it is a controversial issue because the amount of surface area touching the enzyme is an important factor which decides the protein digestion rate. 10.0 g of cheese is much smaller than 10.0g of egg white thus have much smaller surface area and enzyme will work better with egg white than with cheese because egg white and pepsin will have more contact area. I failed to take this factor into consideration and it caused another systematic error in the result.

Although the data supported our hypothesis, we cannot say that the data is valid due to the errors we made during the experiment.

Improving the investigation

Many errors in our investigation were due to lack of equipment. The realistic suggestion of correction the error is to use hot plate to stir the solution instead of using chopstick. The hot plate does not touch the solution direction thus would not carry any chemical with it. The hot plate can also warm up the solution which can be more accurate than using stove. The temperature can also be more easily controlled by using the hot plate because it is easy to overheat the solution using the stove.

Regarding the surface vs. weight issue, it would be better to use the samples of the same surface area and record the weight of each one. Although the samples would have different

weights, researcher can use formula to calculate the protein digestion rate for each sample without leaving any factor into consideration.

Resources

Background information:

http://en.wikipedia.org/wiki/Stomach

http://www.mamashealth.com/organs/stomach.asp

http://answers.yahoo.com/question/index?qid=2008201133859AAjAb9r

http://www.iscid.org/encyclopedia/Hydrophilic

http://www.iscid.org/encyclopedia/Hydrophobic

http://www.bodybuilding.com/fun/nutrient.php?id=1124

http://wiki.answers.com/Q/How_does_ph_affects_the_rate_of_chemical_reaction

Biology, 7th Edition by Campbell Reece. Publisher: Benjamin Cummings, 2005

Picture:

http://www.trialsightmedia.com/exhibit_store/images/stomach.jpg

http://www.holistic-wellness-basics.com/images/how-enzymes-work.jpg