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Declaration: I declare that this is my own work and is the final version. I have given credit and is thankful to the use of words and ideas, whether in written or visual form.

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Personal engagement

Calcium is the basic raw material for bone development. In addition to directly affecting height, it also has other important physiological functions in the body to ensure the smooth progress of normal growth and development. Eggs are one of our most common foods, and the calcium content in egg shells is also one of the common organic matter with the highest calcium content, and it is also an important source of calcium in biomedicine. At present, egg shells are often used as calcium additives in livestock and poultry feeds. source. To this end, an experiment was specially designed to determine the amount of calcium in eggs.

Exploration

Research question:

What is the percentage of calcium (measured by complexometric titration) existing in raw egg shells of Duck eggs, Goose eggs, Quail eggs, Japanese bamboo eggs and Chinese three-yellow eggs compare with the whole weight of the egg shells?

Background information:

The eggshell is mainly composed of inorganic matter, which accounts for about 94%-97% of the eggshell, and organic matter accounts for about 3% to 6% of the eggshell. Inorganic substances are mainly calcium carbonate (about 93%), and a small amount of magnesium carbonate (about 1%), calcium phosphate, magnesium phosphate (about 2.8%) and Fe, trace Si, Al, Ba and other elements.[1] Organic matter is mainly protein, which belongs to collagen, which contains about 16% nitrogen and 3.5% sulfur. The eggshell is the CO_2 produced by the hen's own metabolism, which combines with water to form H_2CO_3. Then, H_2CO_3 under the action of carbonic anhydrase, it dissociates to produce CO_3^{2-}, which then combines with the Ca in the blood to form $CaCO_3$, which is evenly deposited on the eggshell membrane to form a hard eggshell.[2] At present, there are three main methods for determining the content of calcium in egg shells: acid-base titration, redox method, and complexometric titration. In the redox method, not only Ca^{2+}, but other metal ions such as Ba^{2+}, Mg^{2+}, Pb^{2}, etc. can form precipitates with oxalic acid, which will make the measurement result much larger. In the acid-base titration method, hydrochloric acid can not only dissolve calcium carbonate, but also magnesium carbonate. Therefore, the amount of hydrochloric acid measured by this method is the total amount of dissolved Ca^{2+} and Mg^{2+}, and the result will be too large. Therefore, neither of these two methods can accurately determine the content of Ca^{2+} in eggshells, and errors will result in errors. The EDTA complex method (complexometric titration) can shield the interference of other metal ions, and the measurement results are the most accurate[3]. This method is used in this experiment. Complexometric titration is also called complex titration. First, the egg shell is pretreated into a Ca^{2+} solution, which is added in the titration process, and the p^H

[1] https://doi.org/10.1152/ajplegacy.1921.57.2.264
[2] https://doi.org/10.1152/ajplegacy.1921.57.2.264
[3] https://byjus.com/chemistry/complexometric-titration/

value is adjusted to about 13 with sodium hydroxide solution. At $p^H=12$, Mg^{2+} will form magnesium hydroxide precipitation, thereby removing the interference of Mg^{2+}. Ca^{2+} can form a stable complex with EDTA. Then add proper amount of triethanolamine, triethanolamine can react with Fe^{3+} and Al^{3+} to form a firm complex. The degree of ionization of the complex is very small, which greatly reduces the concentration of Fe^{3+} and Al^{3+}, thereby reducing the error caused by Fe^{3+} and Al^{3+}, And then add a small amount of calcium indicator, the calcium indicator itself turns blue, and can form a red complex with Ca^{2+}, and then titrate with standard EDTA reagent. Stop the titration when the solution turns pure blue, and pass the consumption of EDTA. The content of Ca^{2+} can be calculated[4].

Null hypothesis $[\mathrm{H}_0]$**:** Using complexometric titration, the calcium content in eggshells of Duck egg, Quail egg, Japanese bamboo egg and Chinese three-yellow egg are all about 40% to 45% compare with the total weight of the eggshell. Except for Goose eggs with calcium concentration that is lower than 40% compares with the total weight.

Alternate hypothesis $[\mathrm{H}_1]$**:** Using complexometric titration, the calcium content in the eggshells of Duck eggs, Goose eggs, Quail eggs, Japanese bamboo eggs and Chinese three-yellow eggs are below 40% but higher than 30% compare with the total weight of the eggshell

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4 https://www.vedantu.com/chemistry/complexometric-titration.

Explanation and prediction: The calcium content in the eggshell should above 50% compare with the total weight of the eggshell. The chemical composition of eggshells: 94%-97% inorganic matter, 3%-6% organic matter. Inorganic matter calcium carbonate accounts for 93%, so calcium carbonate accounts for 87%-90% of the total because the calcium content in calcium carbonate is: 40/100*100%=40%, so the calcium content in eggshell is about 35%~36%.

Table 1 showing variables:

	Variables	How is it controlled?
Independent variables	● The types of eggs	● The eggs are selected from 5 different egg types.
Dependent variables	● EDTA titration final value measured in mL	● The color of the mixture is controlled from red to blue by dropping EDTA solution.
Control variables	● A mixture of sodium chloride and calcium purpurin in a mass ratio of 100:1(Calcium indicator) measured in mL ● The volume of EDTA solution in mL ● The volume of Hydrochloric acid solution measured in mL ● The p^H value of mixture ● Triethanolamine aqueous solution	● The volume of calcium indicator is controlled with dropper ● The p^H value of the mixture is controlled by dipping Sodium hydroxide solution with concentration of 1M/L. ● The volume of EDTA solution is controlled using burette. ● The volume of Hydrochloric acid solution is controlled with dropper.

Table 2 showing materials:

Item	Number of items
Analytical Balances	1
25mL burette	1
Plastic droppers that can hold 10mL	5
250mL volumetric flasks	2
Erlenmeyer flasks	2
25mL pipette	2
10mL measuring cylinder	1
200mL Beaker	2
Alcohol lamp	1
Tripod	1
Asbestos net	1
Mortar	1
Glass stirring rod	1
Dissolved Ethylenediaminetetraacetic acid disodium salt (EDTA)	200mL
Eggshell	10 g
Calcium indicator (a mixture of sodium chloride and calcium purpurin at a mass ratio of 100:1)	10mL
Triethanolamine aqueous solution	100mL
1M/L sodium hydroxide solution	100mL
6M/L hydrochloric acid solution	100mL
p^H test paper	25 pages
Duck eggs	2
Goose eggs	2
Quail eggs	2
Japanese bamboo eggs	2
Chinese three-yellow eggs	2
Distilled water	1L
Match	1 box
Filtration apparatus	1

Risk analysis:

1) During the experiment operation, the hydrochloric acid and sodium hydroxide solutions used are highly corrosive, so you must pay attention to safety when using them, and do not spray drips on the experimental bench. If you accidentally touch the

skin, you should wash it with plenty of running water as soon as possible. If a large amount of hydrochloric acid is sprayed, it should be neutralized with sodium bicarbonate solution in time. In case of spraying a large amount of sodium hydroxide solution, 3% boric acid solution should be used for neutralization in time.

2) There are many glass instruments in the experiment, please put them in place during use, so as not to accidentally touch them and break them.

3) When measuring reagents, take them according to the amount. If you take out too much, you can only discard them and do not put them back in the original bottle to avoid contaminating the reagents in the original bottle.

4) Due to the high accuracy of the analytical balance, a windshield should be used to isolate the load reading of the analytical balance.

5) When using the alcohol lamp, follow the rules for the use of the alcohol lamp, use a match to light it from the bottom up, and cover it with the lamp cap after use, and do not blow it out.

6) When using the funnel to filter, the filtered liquid should be transferred into the funnel along the glass rod to prevent the liquid from splashing.

Safe disposal of waste:

1) The solution used during the experiment is disposed in container designated for chemical waste.

2) The membranes of the egg and the remaining egg shell are disposed in the waste organic container.

3) Egg whites and egg yolks were consumed (cooked) before the egg shells were bought to the lab.

Procedure:

1) Removed and disposed the inner membrane of the Duck egg shell.

2) Washed the Duck egg shells, dry it, and grinded it into a powder in a mortar.

3) Used an analytical balance, weighed 0.25g of eggshell powder,

4) Putted it into a beaker, moisten it with a little water, and use a plastic dropper to take 5mL of 6M/L hydrochloric acid solution and drop it into the beaker until the eggshell is basically dissolved.

5) Used a match to ignite the alcohol lamp, heat the dissolved eggshell solution until the eggshell is almost completely dissolved, and turn off the alcohol lamp.

6) After the solution has cooled to room temperature and the foam in the beaker was basically dissipated, filtered the remaining insoluble residue with a funnel and put it into a 250ml volumetric flask.

7) Add water to the volumetric flask to make a constant volume, configure it as a sample solution, and shake it well.

8) Used an analytical balance to weigh out 1.26g of ethylenediaminetetraacetic acid disodium reagent, add it to a beaker, add a small amount of water to dissolve and stir thoroughly.

9) Transfer the solution in the beaker to a 250ml volumetric flask, add water to the volumetric flask to make the volume constant, configure it as an EDTA standard

solution, and shake it well.

10) Pour the solution in the volumetric flask into a beaker, use a 20mL pipette to measure 20mL of eggshell solution, pour the solution in the pipette into a 250mL conical flask, add a small amount of water to rinse the remaining sample in the pipette Pour the solution into an Erlenmeyer flask.

11) Measure about 5mL of triethanolamine solution with a graduated cylinder and pour it into a conical flask to remove interferences such as Fe^{3+} and Al^{3+}.

12) Measure about 5mL of 1M/L sodium hydroxide solution with a graduated cylinder, add it to an Erlenmeyer flask, dip a small amount of sample on the p^H test paper with a glass rod, test the p^H value, and use a plastic dropper to drop 1M/L Adjust the p^H of sodium hydroxide solution to between 12~13.

13) Use a balance to weigh 0.1g of calcium indicator and add it to the conical flask, the solution turns red.

14) Take an appropriate amount of EDTA standard solution into a 25mL acid burette, record the level reading of the burette before titration, titrate with the EDTA standard solution, wait until the solution turns from red to blue and does not return to red within a period of time, representing After the titration, record the reading of the burette after the titration;

15) Repeat the above steps, each time the experimental sample number, a total of 5 titrations, calculate the calcium content in the eggshell.

16) Repeat step 1 to 15 with Quail eggshells, Chinese three-yellow eggshells, Goose eggshells, Japanese bamboo eggshells.

Analysis

Raw data:

Table 3 showing the volume of EDTA titration in 5 different kinds of eggshells

Trial	volume of EDTA in mL ±0.1 mL									
	Duck		Quail		Chinese three yellow		Goose		Japanese bamboo	
	Initial	final	Initial	final	Initial	final	Initial	final	Initial	final
1	0.2	12.8	0.1	13.2	0.2	15.3	0.2	12.2	0.0	13.5
2	0.1	12.7	0.1	13.1	0.1	15.7	0.0	12.3	0.0	13.3
3	0.2	13.3	0.0	13.1	0.0	15.4	0.3	12.4	0.0	15.0
4	0.0	12.9	0.0	13.5	0.1	15.5	0.0	13.0	0.1	13.5
5	0.2	14.0	0.0	12.8	0.1	15.1	0.0	12.0	0.0	14.3
Mean	0.1	13.1	0.0	13.1	0.1	15.4	0.1	12.4	0.2	13.9

Data processing:

(The numbering of the equation is shown at the end of each equation)

The first is the calculation of the eggshell content in the eggshell solubilization solution. In the experiment, 0.25g of eggshell sample was taken and the volume was 250ml after being dissolved. Then take out 20ml of the eggshell solubilization solution each time the eggshell content is:

$$m_{eggshell} = \frac{0.25}{250} \times 1000 \times \frac{20}{1000} = 0.02g \text{ (1)}$$

The second is the calculation of the Mar concentration of EDTA salt in the preparation of EDTA standard solution. In the experiment, 1.26g EDTA disodium salt was taken, and its molar weight was 336.06g/M. After dissolving it, the Mar concentration of EDTA salt in the standard solution was c_{EDTA}:

$$c_{EDTA} = \frac{1.26 / 336.206}{250} \times 1000 mol / L = 0.014991 mol / L \text{ (2)}$$

EDTA solution has six coordination atoms. The complex formed is called a chelate. EDTA is often used in coordination titration and is generally used to determine the content of metal ions. The EDTA solution can be combined with a chelating agent that can combine with metal ions such as Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, and the chemical reaction formula for combining with Ca^{2+} is as follows:

$$Ca^{2+} + Na_2Y = 2Na^{+} + CaY \quad (3)$$

In the formula, Na_2Y -ethylenediaminetetraacetic acid disodium salt.

CaY -Chelating agent formed by EDTA and Ca2+.

It can be seen from the above formula that the EDTA solution reacts with Ca^{2+} in a 1:1 ratio to form a chelating agent. Then the mass of Ca^{2+} measured during the titration is m_{Ca2+} is:

$$m_{Ca^{2+}} = c_{EDTA} \times \frac{V_{EDTA}}{1000} \times M_{Ca^{2+}} \quad (4)$$

In the formula, $m_{Ca^{2+}}$ ——the mass of Ca^{2+} measured during the titration, the unit is g.

c_{EDTA}——The Mar concentration of EDTA, 0.014991M/L.

V_{EDTA}——The consumption of EDTA solution during titration, in mL.

$M_{Ca^{2+}}$——The relative atomic mass of calcium, 40.078g/M.

So, the content of Ca^{2+} in eggshell $w_{Ca^{2+}}$ is:

$$w_{Ca^{2+}} = \frac{m_{Ca^{2+}}}{m_{eggshell}} \quad (5)$$

The precision of each instrument used in the experiment is as follows: the precision of the analytical balance is 0.001g, the precision of the 25ml acid burette is 0.01ml, the precision of the 25ml pipette is 0.01ml, and the precision of the 250ml constant volume bottle is 0.1ml. According to the error synthesis formula:

$$y + \Delta y = f(x_1 + \Delta x_1, x_2 + \Delta x_2, \cdots, x_m + \Delta x_m)$$
$$\Delta y = \left| \sum_{i=1}^{m} \frac{\partial f}{\partial x_i} \Delta x_i \right| \tag{8}$$

In the formula: Δy ——measurement error of calculation result.

Δx_i ——Measurement error of each direct measurement quantity.

x_i ——Each direct measurement quantity.

f ——The functional relationship between the calculation result and each direct measurement quantity.

According to equation (8), the error analysis of equations (1) ~ (5) (except equation (3)) is as follows:

In formula (1), the error of the eggshell content in 25ml of the eggshell solubilization solution is 0.48% each time.

In formula (2), the error of the Mar concentration of EDTA salt in the standard solution is 0.12%.

In formula (4), the error of the mass of Ca^{2+} measured during the titration is 0.18%;

In formula (5), the error of the Mar concentration of EDTA salt in the standard solution is 0.66%.

Therefore, in the experiment, the measurement error of the content of Ca^{2+} in the eggshell produced by the accuracy of the experimental instrument was 0.66%.

Record and summarize processed data table are the following:

Table 4 showing the processed data in 5 different kinds of eggshells:

	V_{EDTA} /mL	$m_{Ca^{2+}}$ /g	$w_{Ca^{2+}}$ /%
Duck	13	0.007810521	39.052
Quail	13.1	0.007858586	39.29
Chinese three yellow	15.3	0.009192382	45.96
Goose	12.28	0.007377938	36.89
Japanese bamboo	13.9	0.008351249	41.754

Figure 1 showing the mass in grams of calcium in various eggshells:

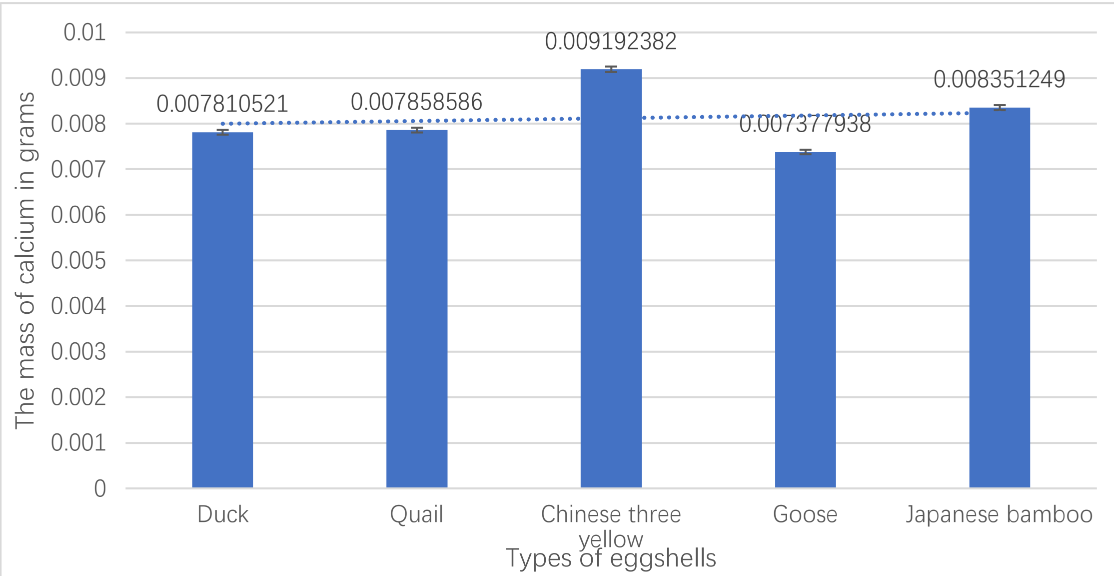
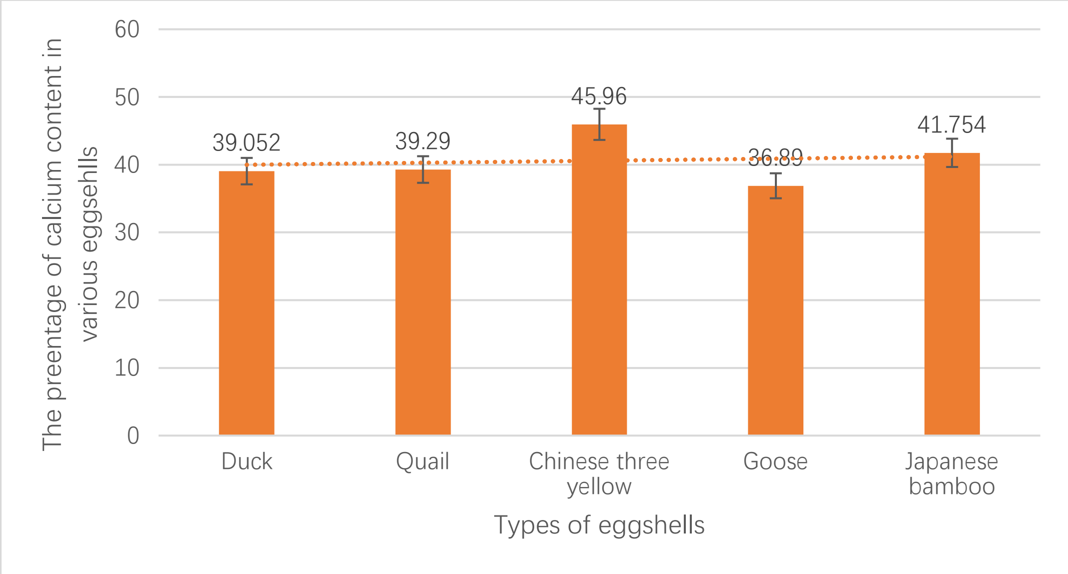


Figure 2 showing the percentage of calcium content in the various eggshells:



Analysis:

From the above experiment, the average calcium content in eggshells of Duck egg, Quail egg, Japanese bamboo egg and Chinese three-yellow egg are all about 40% to 45% compare with the measured total weight of the eggshell. Chinese three-yellow eggs have the highest average calcium content of about 45.96%. The exception is the goose egg, which has the lowest calcium content of about 36.89%.

Conclusion and Evaluation:

Conclusion:

According to the empirical data, null hypothesis is supported. The Goose eggs have the lowest concentration of calcium content compare with the measure weight. The rest of the 4 eggs have calcium content of about 40%-45%

Limitations and Experimental design:

Even through the experiment yields valid data and supported conclusion, this experiment has some limitations. First, the p^H value of the pre-titrated solution is not accurately measured with a p^H meter. On the contrary, the p^H value of the solution is measured with p^H test paper. Under some interferences of external light sources, the observation of the color of the p^H test paper maybe distracted. Hence, some trails could have unsatisfied p^H level, which yields error to the titration volume. Secondly, the eggshells were washed with regular water instead of distilled water. The regular water maybe too reactive which carried away some calcium content in the eggshell before the measurement. Thirdly, the eggshells were dried under the sun before the titration, which sunlight promoted the disintegration of calcium in the eggshells under UV light. With all the limitations, this experiment has some advantages over its measurement. Aqueous triethanolamine successfully blocked the Molecular interferences from iron ions, which decreased the error in the experiment.

Suggestions for improvement:

In the future, the p^H value of the pre-titrated solution can be measured with an electric p^H meter. Also, the eggshell can be washed with distilled water and dried in dryer.

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