Calcium Analysis of Eggshells

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Abstract

The chemical content of eggshells is important for tracking the health of fowl, as the content of their eggshells reflects aspects of their diet, environment, and behaviors. Due to this, eggshells can serve as an indicator for environmental contaminants and the conditions in which the birds are found. Carbonic anhydrase plays a role in the development of eggshells and is affected by many environmental impacts, such as heavy metals. Calcium carbonate makes up a large portion of eggshells, yet heavy metals can replace calcium in eggshells, leading to deformations and contamination to the egg that can affect those consuming it. However, the analysis of calcium content in eggshells has proven difficult. A common instrument used to determine elemental composition is the ICP-MS; however, calcium is difficult to analyze. The argon used to generate the plasma interferes as it has a similar mass to calcium, which leads to artificially high and inconsistent calcium concentrations. A new method for isolating the calcium from eggshell is being developed to analyze the environmental impacts on chicken health. Oxalate is used to precipitate calcium oxalate from the eggshells. Gravimetric analysis is done using STA and IR. This will be done in addition to a full characterization using ICPMS, XRD, and C-N analysis.

Introduction

Eggs are a major component of the life cycle of avian species and the environment. The composition of the egg reflects the diet consumed by the animal and can be used as an indicator of the environment it lives in[1]. Proper calcium intake is imperative for the success of offspring and the production of eggs. Improper calcium intake or presence of heavy metals, like strontium, can disrupt the hatching success of the eggs[2].

Calcium carbonate is the main chemical component of eggshells and makes up about 94% of the eggshells in poultry chicken eggshells[3,4]. Other trace metals like Sr, Ba, Mn, As, Cd, Cu, Pb, Hg, Se, V, and Zn could also possibly be found in the chemical composition of the eggshells[2,5]. The presence of some of these heavy metals can lead to egg malformation, embryo death, transfer of the metal to the yolk and consumption by other species.

While most metals can be quantified using techniques such as ICP-MS, Ca^{2+} is surprisingly difficult. When using ICP-MS, there are many interferences with the Ca^{2+} signal, including the argon gas used to generate the plasma. ICP-OES is the preferred method to quantify Ca^{2+} but not every lab has access to this instrument. To isolate the Ca^{2+}, the powdered eggshell is dissolved in acid and mixed with oxalic acid. The oxalate preferentially precipitates Ca^{2+} as $CaC_2O_4{\cdot}H_2O$ and is being investigated as a method to determine Ca^{2+} content in eggshells.

Methods and Materials

Sample Preparation

- Collected eggs and washed with a mild detergent.
- Divided eggs into sharp and dull ends and removed inner membranes.
- Ground eggshells using a McCrone Micronizing mill and 200 proof EtOH for 3 minutes.
- Slurry was dried at 105ºC in an oven.
- Dried eggshell was homogenized to a powder using an agate mortar and pestle.
- 0.15 g of eggshell reacted with 10 mL of 1 M hydrochloric acid (HCl) and 1.3 g of oxalic acid ($C_2H_2O_4$).
- Precipitate collected via vacuum filtration.
- Precipitate samples used for STA (Perkin Elmer STA 6000 coupled with Perkin Elmer Spectrum One FT-IR in nitrogen and air backgrounds), XRD (Bruker D8 Advance Instrument),
- 0.15 g of unprocessed eggshell samples were back-titrated with 5.0 mL of 1 M HCl and 0.1 M NaOH for $CaCO_3$ analysis.

Oxalate decomposition equation for STA

$$CaC_2O_4{\cdot}H_2O\ (g) \rightarrow CaC_2O_4\ (g) + H_2O\ (g) \rightarrow CaCO_3\ (g) + CO\ (g) \rightarrow CaO\ (g) + CO_2\ (g)$$

Results

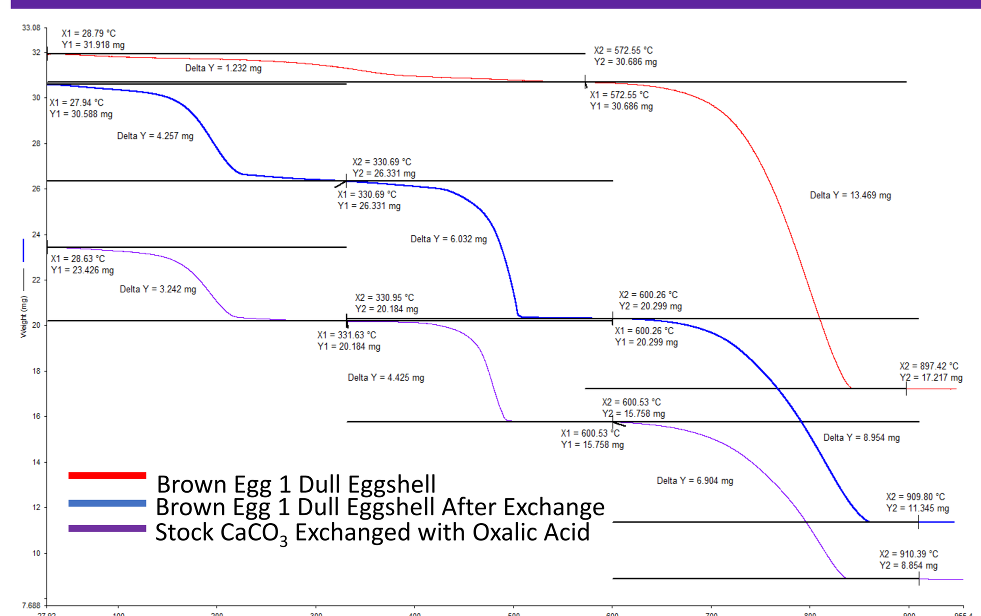

Figure 1. Decomposition of Brown Egg 1 Dull End eggshell, precipitate of Brown Egg 1 Dull End after exchange with oxalic acid, and $CaC_2O_4{\cdot}H_2O$ prepared using the same procedure from stock $CaCO_3$ using Thermal Gravimetric Analysis by TGA.

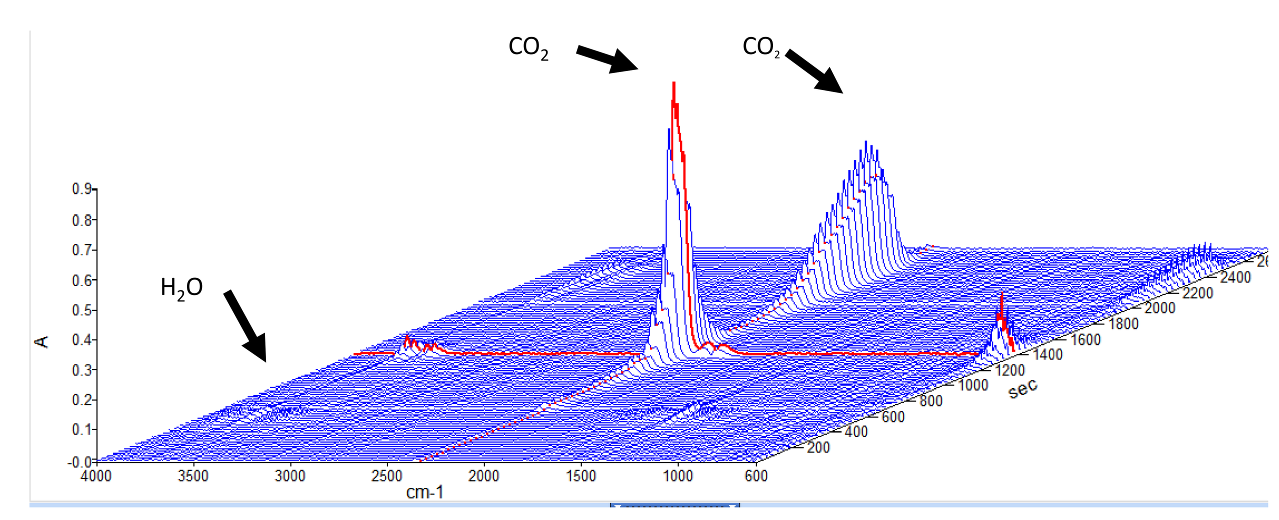

Figure 2. Stack plot of the accumulated FT-IR spectra of Brown Egg 1 dull end exchanged during STA analysis in nitrogen background collected over time. H_2O peak was seen during the decomposition of $CaC_2O_4{\cdot}H_2O$, first thermal event, CO_2 peaks were seen during the decomposition phases of CaC_2O_4 and during $CaCO_3$ decomposition, second thermal event.

Table 1. Percent $CaCO_3$ in unprocessed eggshell samples determined via back-titration.

Eggshell Sample	%$CaCO_3$	Standard Deviation
B1 Sharp	92.68%	0.4573
B1 Dull	N/A	N/A
LB1234 Sharp	92.13%	0.9248
LB1234 Dull	92.60%	0.4213
F123 Dull	93.69%	0.1270
F123 Sharp	94.24%	0.7046
WTG1234 Sharp	90.76%	0.6353
WTG1234 Dull	93.78%	0.7487
SB1 Dull	92.02%	1.1320
SB1 Sharp	89.79%	0.3691
CM1 Dull	93.17%	0.3769
CM1 Sharp	93.42%	0.6987
B2R Dull	95.01%	0.7358
B2R Sharp	95.26%	0.4821

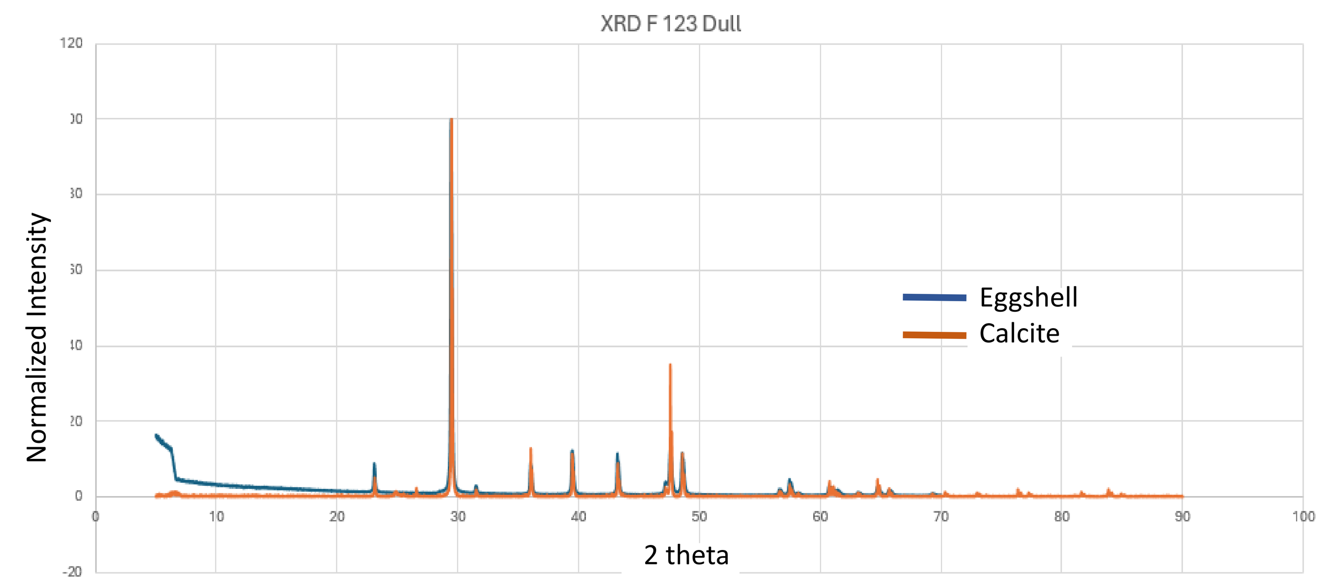

Figure 3. Comparison between calcite ($CaCO_3$) and the "F123 Dull" eggshell on XRD. Confirms the eggshell is mostly $CaCO_3$.

Results (cont.)

Table 2. Percent $CaCO_3$ by mass, percent recovery from exchange, percent of Ca^{2+} in exchanged sample, and percent Ca^{2+} in eggshell of Brown Egg (1) sharp and dull end, Brown Egg (2) (Rotten) sharp and dull end, and Large Brown Egg (1234) sharp and dull end using STA coupled with FT-IR in nitrogen background.

Sample	% $CaCO_3$ in Nitrogen	% Recovery from Oxalate Exchange	% Ca^{2+} in Exchanged Sample	% Ca^{2+} in Eggshell
Brown Egg 1 Sharp	95.463%	95.765%	26.355%	35.172%
Brown Egg 1 Dull	95.973%	95.645%	26.503%	35.514%
Brown Egg 2 Rotten Sharp	95.842%	94.551%	26.632%	35.230%
Brown Egg 2 Rotten Dull	95.909%	94.788%	26.660%	35.381%
Large Brown Egg 1234 Sharp	96.003%	95.107%	26.660%	35.535%
Large Brown Egg 1234 Dull	95.805%	95.976%	26.251%	35.236%

Discussion/Conclusion

- The % $CaCO_3$ found in the eggshells using STA was ~ 95%, which was slightly higher than expected. This is probably due to the diet of the chickens. The eggshells contained ~35% Ca^{2+}.
- When the eggshells are exchanged with oxalic acid, an ~95% conversion is seen. Any loss is due to transfer loss. Reductions in transfer loss are being investigated.
- There was no significant difference between the sharp and dull ends of the eggshells or between the different eggs using STA.
- The %$CaCO_3$ calculated using back-titration were smaller than expected (~94%). These values did not match STA data. This is likely due to samples not being 100% eggshell and containing some remaining membrane. Unlike STA, titration led to variations.
- From XRD it can be concluded that the eggshell samples were mostly $CaCO_3$ and the samples matched calcite from the database.

Future Work

- Analysis of digested eggshell samples using ICP-MS and ICP-OES instrumentation to determine the concentration of any trace heavy metals and contributing chemical components to the composition of the shell.
- Continue XRD analysis.
- Continue to run samples on STA and IR in air background.
- Data analysis and processing.

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References

- Butcher, G.; Miles, R. Concepts of Eggshell Quality. edis.ifas.ufl.edu. https://edis.ifas.ufl.edu/publication/VM013.
- Mora, M. A. Heavy Metals and Metalloids in Egg Contents and Eggshells of Passerine Birds from Arizona. *Environmental Pollution* **2003**, *125* (3), 393–400. https://doi.org/10.1016/s0269-7491(03)00108-8.
- Murakami, F.; Rodrigues, P.; Teixeira De Campos, C.; Antônio, M.; Silva, S. Physicochemical Study of CaCO 3 from Egg Shells Estudo Físico-Químico Do Carbonato de Cálcio Obtido a Partir Da Casca de Ovo. *Ciênc. Tecnol. Aliment* **2007**, *27* (3), 658–662.
- Malau, N. D.; Adinugraha, F. Synthesis of hydrokxyapatite based duck egg shells using precipitation method. *IOPscience*. DOI: 10.1088/1742-6596/1563/1/012020.
- Daniels-Abdulahad, M. NSUWorks NSUWorks All HCAS Student Capstones, Theses, and Dissertations HCAS Student Theses and Dissertations Heavy Metal Content in Eggs from Wild and Managed-Care Heavy Metal Content in Eggs from Wild and Managed-Care Humboldt Penguins Humboldt Penguins **2023**, https://nsuworks.nova.edu/cgi/viewcontent.cgi?article=1143&context=hcas_etd_all/.
- Lechtanski, V. L. *Inquiry Based Experiments in Chemistry*; Oxford: New York, **2000**; 159-165.
- Mohadi, R.; Sueb, A.; Anggraini, K.; Lesbani, A.; Calcium Oxide Catalyst Based on Quail Eggshell for Biodiesel Synthesis from Waste Palm Oil *J. Pure App. Chem. Res.*, **2018** 7(2),130-139. DOI: 10.21776/ub.jpacr.2018.007.02.390

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