Penetration of Sodium Lauryl Sulfate in the *Gallus Gallus Domesticus* Egg Shell

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Abstract

Washing chicken eggs before selling them to local stores is a common practice for egg companies, however washing them with chemicals could be more harmful than not washing them. The use of chemicals and sanitizers for washing eggs is used to clean the eggs from harmful substances that lay on the surface of the shell. However, the penetration of these chemicals may be just as harmful as not washing them with these sanitizers. In this study, sodium lauryl sulfate (SLS), which is a washing agent found in soaps was used to clean the eggs, then the eggs were tested to see if SLS penetrated the chicken egg shell. Twenty one eggs, divided into four groups were tested in this project. Half of the eggs in each group were first washed with tap water to investigate whether rinsing the eggs before being exposed to a chemical would weaken the shell and influence the amount of chemical which penetrates the egg. Each group varied in the length of exposure time of SLS. The high performance liquid chromatography (HPLC) instrument was used to detect if sodium lauryl sulfate penetrated the egg shell. Sodium Lauryl Sulfate was detected by the HPLC in the eggs treated for five minutes. The results of this investigation could further the scope of how egg production companies will wash their eggs in the future, and bring awareness to the egg industry on the type of chemicals that should or should not be used as washing agents.

Introduction

Eggs are a major contributor in the American diet which is why making sure eggs are properly cleaned before being eaten is crucial [16]. The practice of washing commercial eggs is controversial and the methods of egg washing differ in many countries. In some European countries egg washing is not permitted; in the United States, Australia, Japan, and Brazil, egg washing is routine for the production of commercial eggs. The European Union does not wash their eggs because they have concerns that once the eggs are washed the cuticle that surrounds the shell can be damaged which can lead to bacteria entering the shell [7]. Most countries are more worried about bacteria penetrating the eggs than other substances. However, other countries, including the United States, believe that washing the egg removes the bacteria and other harmful residues before cooking which is why we continue to wash our eggs. In fact, egg producing companies, as well as some household consumers, routinely wash their eggs before cooking. The amount of time an egg can be rinsed may affect the ability of harmful substances to penetrate the egg. Commercial egg washing has been known to be a benefit by removing harmful bacteria and other unwanted substances on the chicken egg which is why many studies have explored the penetration of bacteria.

Harmful bacteria such as *Salmonella* has been proven to penetrate the pores of the egg shell when they are laid [8,16]. According to a study found in the Public Library of Science, the *Salmonella* strain is a very important and common food poisoning pathogen that has been found to penetrate washed eggs more frequently than unwashed eggs. Many studies have been conducted with *Salmonella* because it is a common food poisoning pathogen that can cause serious sickness in those who eat a contaminated egg with this pathogen. In fact, washed eggs had a higher penetration rate than unwashed eggs according to a study of testing done on the penetration of *Salmonella* strains [3]. A bacteria strain called *Campylobacter jejuni* was able to penetrate the egg shell, however, the bacteria was not found in the yolk [2]. The ability for these bacteria to penetrate the egg shell raises the question of what other harmful substances can penetrate commercial eggs. Many studies have proven that a variety of bacteria strains can penetrate the egg's shell. Bacteria is able to penetrate the shell both during the process of being laid and after being washed. Besides bacteria penetrating the egg shell, other substances such as common chemicals found in soap may penetrate through the shell to the yolk as well. Few studies have been conducted on the penetration of chemicals and whether it can have an impact on the consumers who eat them.

Currently, there are studies being conducted to find ways of preventing harmful substances from penetrating the egg shell. According to The International Journal of Poultry, science experiments have been conducted on sealing the egg shell to prevent the loss of moisture [14]. Currently, there are studies seeking to identify ways to make the commercial egg shell stronger and less permeable to harmful agents. However, if more studies were directed towards exactly what penetrates the chicken egg shell, such as the types of chemicals, then avoiding the usage of those chemicals would also be beneficial knowledge to egg producing companies and consumers. The egg production companies would then be able to avoid using the chemicals identified as being able to penetrate the egg. Therefore, identifying which chemicals that can penetrate the chicken egg shell need to be explored. In this investigation, the penetration of one common chemical (sodium lauryl sulfate) found in cleaning detergents and sanitizers will be tested on commercial chicken eggs.

As said before, washing eggs is highly enforced in the United States; however what they use to wash the eggs is not. Any chlorine based solution or common sanitizing chemicals can be used for washing the eggs. Sodium lauryl sulfate is a common chemical found in sanitizing solutions used for washing eggs. It is also commonly found in hand soap and other cosmetic products such as shampoo and dish washing soap. Sodium lauryl sulfate commonly found in different soaps will be tested on family owned eggs that have not been washed to evaluate if there is penetration through the shell and to the yolk through a technique called high performance liquid chromatography. In the experiment, there will be a series of trials to test whether the amount of time the egg is exposed to sodium lauryl sulfate will affect the penetration of the egg shell. The high performance liquid chromatography technique is used to separate the sodium lauryl sulfate from the yolk and the albumen. Standard samples of SLS will be run on the instrument to obtain chromatograms and to find the retention time of sodium lauryl sulfate from the HPLC column. The result is depicted in a graph called a chromatogram. To determine if the experimental egg samples absorbed SLS during the washing process, the egg samples will be run through the HPLC and the resulting chromatogram will be compared to the standard retention time and chromatogram from the standard samples. Reverse phase with the C-18 column is the most accurate for detecting sodium lauryl sulfate in the HPLC. As seen in figure 1, sodium lauryl sulfate has a hydrophilic head and a hydrophobic tail [3]. The molecular composition of sodium lauryl sulfate affects the way the chemical compound binds to the column which is why it's important. The retention time of the sodium lauryl sulfate depends on how long the hydrophobic tail binds to the column.

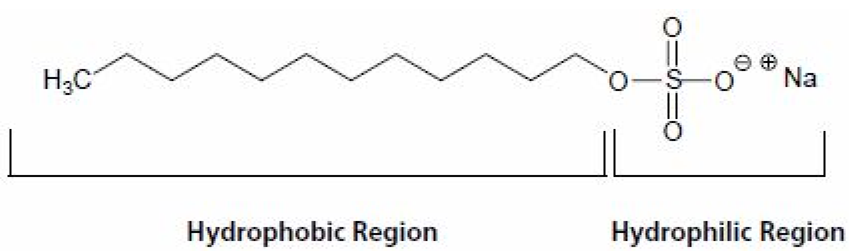


Figure 1. Sodium Lauryl Sulfate Molecule

Sanitizing foods with sodium lauryl sulfate at a high concentration may increase the chances of the chemical being introduced into the food from the washing process. This experiment is also testing whether the Environmental Protection Agency (EPA) set concentration limit for sodium lauryl sulfate is accurate for washing chicken eggs. Since some foods have a more permeable membrane than others, then that set concentration may not be accurate for chicken eggs. The findings of this experiment may further the scope of how countries across the globe will wash or not wash their eggs. The ability for bacteria to penetrate the egg shell raises the question of what other harmful substances can penetrate commercial eggs such as sanitizing agents that are used to wash the eggs before packaging and shipping to stores.

Research Questions

Does sodium lauryl sulfate penetrate the chicken egg shell when it is used as a cleaning sanitizer?

Does rinsing the eggs with tap water have an effect on the penetration ability of sodium lauryl sulfate?

Is the Environmental Protection Agency's set concentration limit for washing foods accurate for sodium lauryl sulfate for washing chicken eggs?

Purpose

The purpose of this experiment is to determine if sodium lauryl sulfate can penetrate through the chicken egg shell and into the egg itself. If it is found that it can, it may change the scope of how egg companies wash eggs and bring greater awareness to people who buy commercial eggs. Also finding if sodium lauryl sulfate penetrates the chicken egg shell might raise other questions about the permeability of the egg shell and what other substances that come into contact with the eggs could permeate into the yolk and egg white.

Hypothesis

The longer the eggs are treated with sodium lauryl sulfate, the higher the chance sodium lauryl sulfate will enter the eggs. Therefore, the eggs that are treated with sodium lauryl sulfate for 5 minutes will show traces of sodium lauryl sulfate. The HPLC will detect very little sodium lauryl sulfate in the eggs that were washed for 1 minute. In addition the eggs that were rinsed with tap water first will show a higher peak of detection of sodium lauryl sulfate.

Safety

The experiment was conducted at Thousand Oaks High School in a class lab. Safety precautions were taken while working with the high performance liquid chromatography machine and chemicals. All physical contact with the chemical was avoided and the chemical was stored in the appropriate place in the lab. A lab coat and goggles were worn throughout the stages of the experiment. If the chemical was spilled, all safety precautions were taken before proceeding with the cleanup process.

Materials

- High Performance Liquid Chromatography (HPLC)
    - Column C18 Reverse Phase
    - Solvent of 70% Acetonitrile and 30% Water (kept in flammable cabinet)

  - 7 of 0.45 µm syringe filters

- 21 unwashed chicken eggs from family owned chickens
- Liquid form (35% concentration) and solid form (pure) of sodium lauryl sulfate
- 21 Falcon tubes, 21 microtubes, and 15 HPLC vials
- Eppendorf Centrifuge 5418
- Stock solution of 100 mL of 1000 ppm sodium lauryl sulfate
- Bio Pippett Plus P1000 micropipette and tips
- Bio Pippett Plus P200 micropipette and tips
- Serological Pipettes
- 500 mL or 250 mL beakers
- Volumetric flask 100 mL
- Two 100 mL bottles (one glass and one plastic)
- Tap Water from faucet
- Deionized water
- Stop watch
- Spoon
- Gloves
- Lab coat
- Goggles
- Lab Refrigerator kept at a constant of 4°C

Procedure

Treating Eggs

Half of the eggs were rinsed with tap water to test whether rinsing the eggs before treating them with sodium lauryl sulfate had an effect on the penetration. Tap water was used instead of deionized water because deionized water would cause a disruption in the eggs' osmosis and it would also affect the penetration of sodium lauryl sulfate. The rinsed eggs were rinsed in potable water at 35 degrees Celsius and dried immediately after and stored in the lab refrigerator.

The concentration of sodium lauryl sulfate at 350 ppm was used because the U.S Environmental Protection Agency requires a maximum concentration of 350 ppm of SLS be used for sanitizing foods [13]. Using the maximum concentration increases the chances of receiving results. A stock solution of 100 mL of 1000 ppm SLS was made and stored in the lab refrigerator until further use.

The stock solution was serially diluted to make different concentrations to run through the HPLC. The dilutions were made with a P200 micropipette into microtubes. The standards made were 50 ppm, 100 ppm, 200 ppm, 300 ppm, and 400 ppm. The standards were filtered using 0.45 µm filters and put into HPLC vials and stored in the lab refrigerator.

A total of 21 unwashed eggs were used and categorized into one control group and three experimental groups. The control group consisted of 3 eggs. Each experimental group consisted of 6 eggs and all were soaked in the same concentration of sodium lauryl sulfate. There were 6 eggs in each group because treating the eggs in triplicates would improve results, 3 eggs for the rinsed category and 3 eggs for the not rinsed category. The eggs were kept in the lab refrigerator throughout the experiment to prevent the eggs from going bad. The control group consisted of three eggs that were not rinsed with tap water or treated with sodium lauryl sulfate. Also, Table 1 shows a more organized representation of how the eggs were grouped in the control and three experimental groups.

Experimental Group 1 contained 3 eggs that were rinsed with tap water, then soaked in a 400 mL beaker containing 350 ppm SLS for 1 minute. Also in Group 1 there were 3 more eggs that were not rinsed with water but were soaked in a 400 mL beaker of 350 ppm SLS for 1 minute.

Experimental Group 2 contained 3 eggs that were rinsed with tap water and then soaked in a 400 mL beaker containing 350 ppm SLS for 2.5 minutes. Also in Group 2 there were 3 more eggs that were not rinsed with water but were soaked in a beaker containing 350 ppm SLS for 2.5 minutes.

Experimental Group 3, contained 3 eggs that were rinsed with tap water, then soaked in a 400 mL beaker containing 350 ppm SLS for 5 minutes. Also in Group 3 there were 3 more eggs that were not rinsed with water but were soaked in a 400 mL beaker containing 350 ppm SLS for 5 minutes. All the eggs were stored in the lab refrigerator until the eggs were cracked and the yolk and albumen were mixed together. The mixture was put into Falcon tubes using a serological pipette. The mixtures were stored in the lab refrigerator at 4°C.

Table 1. Organizational Chart of Eggs

	Time of Treatment (minutes)	Eggs Rinsed	Eggs Not Rinsed
Control Group	0 min	none	3 eggs not treated
Experimental Group 1	1 min	3 eggs treated with 350 ppm SLS	3 eggs treated with 350 ppm SLS
Experimental Group 2	2.5 min	3 eggs treated with 350 ppm SLS	3 eggs treated with 350 ppm SLS
Experimental Group 3	5 min	3 eggs treated with 350 ppm SLS	3 eggs treated with 350 ppm SLS

Sample Preparation

Two milliliters of the egg mixtures were collected and transferred to 2 mL microtubes using a P1000 with tips. The microtubes filled with the egg mixture were centrifuged for 10 minutes at 9000 relative centrifuge force (RCF) units. The RCF is also measured as gravity. After the samples were centrifuged the supernatant was extracted using a P200 pipette. The samples were diluted to a half mixture and half water solution in order for the samples to be the proper consistency to go through the HPLC. After they were diluted they were filtered through a 0.45 µL HPLC filter and put in HPLC vials. It may be possible that not all of the sodium lauryl sulfate that could have been in the yolk was extracted and put through the HPLC. The vials were organized and ready to be put through the HPLC.

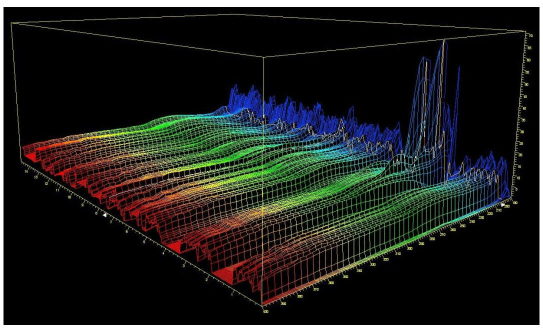
High Performance Liquid Chromatography (HPLC)

A method and sequence table was created and inputted into the computer for an isocratic method. The sequence table is for the computer and instrument to know what sample is in what order and when the instrument takes an injection of which sample. An isocratic method for the

HPLC means one solvent is used during the mobile phase. The mobile phase is when the solvent carries the yolk through the column and sodium lauryl sulfate sticks to the column then elutes off [4]. A solvent of 70% of Acetonitrile and 30% water was made in a glass bottle and stored in the flammable cabinet until use. The stationary phase consisted of 70:30 acetonitrile and water. The samples were then prepared and ready to put through the HPLC. Due to the time limit, the only egg samples that were run through the HPLC were the control group, the 1 minute rinsed and not rinsed, and the 5 minute rinsed and not rinsed. Reverse phase was used with a C18 column and a flow rate of 0.5 mL per minute with the thermostat set at a temperature of 35°C. The post time for all the samples was 20 minutes. The post time was set for 20 minutes because based on other papers the retention time of sodium lauryl sulfate is between 2 to 4 minutes so the post time won't be any longer than 20 minutes.

Results

According to the sodium lauryl sulfate standard chromatograms from the HPLC, the retention time for sodium lauryl sulfate is around 2.5 minutes. Comparing all the standard chromatograms of sodium lauryl sulfate, all standards shared one time: 2.5 minutes. Figure 3 shows a peak of 2.5 minutes in the chromatogram of the 400 ppm standard of SLS. This suggests that the retention time for sodium lauryl sulfate is around 2.5 minutes. The only samples that showed a retention time of 2.5 minutes were the egg samples that were not rinsed and treated for 5 minutes. Figure 4 shows the chromatogram of the sample of egg that was not rinsed and treated with SLS for 5 minutes along with peaks corresponding to other substances in the yolk that eluted off the column. The control group showed no SLS detection. The sample of eggs that were treated for 5 minutes and not rinsed results were inconclusive because no peaks were found in the chromatogram. Eggs that were treated with SLS for less than 5 minutes did not have SLS in the egg yolk or it was too low to be detected by the HPLC. The results from HPLC showed no correlation between eggs that were rinsed and eggs that were not rinsed. Figure 2 is a 3D plot graph of the 400 ppm standard of sodium lauryl sulfate generated by the HPLC computer; it shows the peak at which the sodium lauryl sulfate eluted off the C18 column.



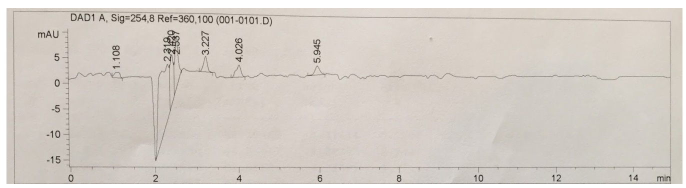


Figure 2. 3D Plot of 400 ppm standard SLS

Figure 3. Chromatogram of 400 ppm Standard SLS

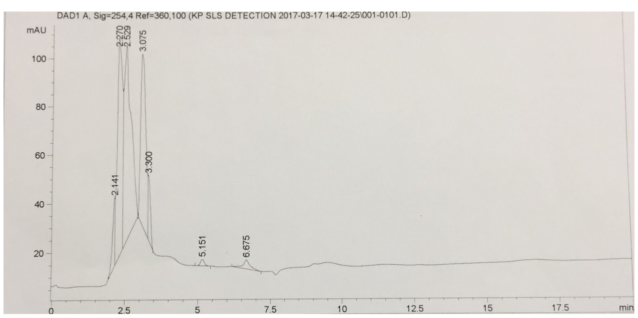


Figure 4. Chromatogram of Sample of Egg not rinsed treated for 5 minutes

Discussion

Based on these findings, using chemicals that are in common soaps such as sodium lauryl sulfate to clean eggs that humans consume should possibly be avoided. As predicted the control group showed no SLS detection in the egg yolk. However, the eggs treated with SLS for 5 minutes contained enough SLS to be detected by the HPLC. The chicken egg shell becomes weak after being treated with sodium lauryl sulfate for 5 minutes. These results support the hypothesis that sodium lauryl sulfate penetrated the eggs that were washed for 5 minutes. If egg companies are washing their eggs for 5 minutes or more with sodium lauryl sulfate or with a similar chemical, the eggs may contain that chemical in the yolk. It is still undetermined whether rinsing the eggs before treating them with sodium lauryl sulfate had an effect on the penetration.

With more time, the samples from the Experimental Group 2 could be run to see if sodium lauryl sulfate penetrated the egg shell after being treated for 2.5 minutes. If so, then the exposure time of sodium lauryl sulfate should be reduced to less than 2.5 minutes for egg treatment. If enough eggs are consumed that contain sodium lauryl sulfate, it may have an effect on the human's digestive system. Since not all of the egg samples were run through the HPLC, with more time, the additional samples and the samples that were inconclusive would be re-run. This data would further determine if rinsing has an effect on the absorption of sodium lauryl sulfate. This would add more to this current discussion. Further studies will have to be done to determine whether egg producing companies should stop washing their eggs with sodium lauryl sulfate or any similar chemical. For now, the egg producing companies should still clean the eggs, however, try to restrain from using chemicals to clean them for longer than five minutes. Based on these results, more possibilities of contamination of chicken eggs raises more questions that could be investigated in future studies.

Conclusion

Ultimately, common soaps that contain chemicals such as sodium lauryl sulfate can permeate the chicken egg shell; this could cause some concern when consuming eggs that have been exposed to these chemicals. Having the knowledge that sodium lauryl sulfate can permeate the egg shell should bring awareness to the egg producing companies and the consumers. According to these findings, treating eggs with sodium lauryl sulfate for 5 minutes or longer should be avoided since long-term ingestion of this chemical has not been studied. More studies should be done to expand the data and knowledge on the penetration of chemicals in chicken eggs. If more studies are conducted then the controversy over washing eggs could be solved and consumers and egg producing companies will know how to properly clean the eggs before being sold to stores and before they are consumed. Egg producing companies should use fewer chemicals and reduce exposure time of sodium lauryl sulfate and other chemicals when washing eggs. Reducing the exposure time of sodium lauryl sulfate and using lower concentrations of sodium lauryl sulfate could reduce the chances of the chemical penetrating the egg shell and being consumed by humans.

Sources of Error

As part of a scientific experiment, some sources of error have occurred. Many challenges were encountered when working with the high performance liquid chromatography. The samples were run overnight, and a power failure was discovered the next morning. This occurred two nights in a row. After the two power failures, the samples were only run during the day. Then, the pressure of the column got too high so it had to be discarded. The second column that was used was still a C18 however it was used already. Having some samples run on one column and others run on another may affect the retention time of sodium lauryl sulfate, however this was taken into consideration when analyzing the chromatograms. Running the samples a second time on one column and having no distributions might have better results. The only major source of error would be from having some samples run on one column and others runned on another however this was taken into consideration when analyzing the results from the HPLC.

Further Work

Since we know that sodium lauryl penetrates the shell, we could investigate if sodium lauryl sulfate affects an organism. To test if SLS affects an organism, an experiment could be conducted on the respiration rate of yeast. The control group for this experiment is a 1 mL eppendorf tube with yeast media. The experimental group will consist of three 1 mL eppendorf tubes with different amounts of concentration of the chemical. The tube with the highest concentration of the chemical will have a greater effect on the yeast respiration rate. The chemical that penetrates the chicken egg could affect the respiration rate of organisms

Other chemicals such as chlorine-based sanitizers could be tested for possible use in the egg washing process. Experimenting with other chemicals would add to the discussion of what types of chemicals could penetrate the egg shell. Other substances that could be tested on eggs are insecticides. Spraying insecticides near an egg producing farm could get on the eggs and permeate into the egg shell as well. For an additional experiment, a couple of commonly used insecticides could be tested on chicken egg shells to determine whether the insecticides permeate the shell using the HPLC machine.

Also, another possible experiment could be testing sodium lauryl sulfate on the properties of the yolk and egg white. Testing if sodium lauryl sulfate affects the yolk and egg whites properties. For example, it would be interesting to see if sodium lauryl sulfate denatured or destroyed certain proteins in the egg yolk or egg whites.

Refrigerated eggs versus unrefrigerated eggs is also very controversial. An experiment could be conducted between the difference between eggs that have been refrigerated for a certain amount of days before use and unrefrigerated eggs. The eggs will still be treated with a commonly used chemical. After the experiment, there may be a difference between the penetration ability between refrigerated and unrefrigerated eggs.

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