Permeability of Free-Range and Caged Eggs in Different Solution Types

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Abstract:

In our research, we sought to assess the osmosis rate of free-range and caged eggs in water (control), salt solution, and sugar solution. For de-shelling, a dozen of free-range and caged eggs were soaked in vinegar for two days and washed with warm water. All the de-shelled eggs were then submerged in water before placing them in their respective treatment groups. Weight measurements were taken before and after treatments as indicators to assess the osmosis rate of eggs. We hypothesized that the greater presence of monounsaturated fatty acids in free-range eggs will result in higher membrane permeability in comparison to caged eggs. In our results, we found both free-range and caged eggs absorbed water and gained mass when submerged in water. We also discovered that both sugar and salt treatments withdrew water from the egg which resulted in decreased egg mass in free-range and caged eggs. We discussed the osmosis rate of free-range and caged egg membranes in response to the same concentration of hypertonic environment of various substances. Overall, our results showed a higher rate of osmosis in caged eggs compared to free-range eggs in different treatment environments. The membrane permeability variations can enhance our knowledge about the transport mechanism of the membrane and the nutritional properties of eggs. This understanding can offer significant perspectives on food safety and human health.

Introduction:

Osmosis is a type of passive diffusion in which molecules move from an area of higher concentration to an area of lower concentration until an equilibrium is reached on each side of the membrane. In our experiment, we aimed to employ de-shelled eggs as our model to assess the osmosis properties in water, salt, and sugar solution. Specifically, we sought to compare the

osmosis rates and membrane permeability properties in free-range and caged eggs purchased from Vancouver local Superstores at Save-On-Foods and Pricesmart. Many previous literature studies have highlighted the chemical difference between free-range and caged eggs. In terms of chemical composition, free-range and caged eggs (conventionally-farmed eggs) have similar protein quality but differ significantly in fatty acid composition and cholesterol content (English, 2021). In addition, free-range eggs have comparatively enriched vitamin D than caged eggs because free-range farming hens have access to sunlight which elevates the vitamin D3 content in eggs (Kühn et al., 2014). Furthermore, the diets of free-range eggs and caged eggs vary in composition, with free-range eggs having a more diverse diet that includes cultivated and wild vegetation, supplemented grains, and insects. In contrast, caged eggs are limited to consuming chicken feed that is based on either wheat or corn. (English, 2021). Moreover, based on Jones et al.'s study (2010), free-range eggs have slightly lower vitelline membrane strength than caged eggs. Strong vitelline membrane strength in eggs may impede the diffusion of water via the membrane thus caged eggs are less porous than free-range eggs. Additionally, free-range eggs have a comparatively larger proportion of monounsaturated fatty acids than non-free-range eggs which in turn will have a more permeable membrane to allow solutes or water to pass through (English, 2021).

Based on these chemical mechanisms and properties of chicken eggs, we hypothesized that osmosis rates will be higher in free-range eggs compared to caged eggs because free-range eggs have a larger proportion of monounsaturated fatty acids, which have kinks in their structures that make them more permeable to water. In our study, we predicted that higher osmosis rates indicated by larger mean weight differences will be observed in free-range eggs than in caged eggs.

Methods:

Twelve free-range eggs were de-shelled by being submerged in a bucket of white vinegar for two days before the day of the experiment. The bucket was covered with a lid and stored at room temperature during the soaking period. The eggs were de-shelled by rinsing them with warm water. Upon de-shelling, the eggs were placed in a clean bowl and carefully rinsed with water to remove any residue. The twelve de-shelled free-range eggs were kept in a sealed plastic container overnight and stored at room temperature, as observed in Figure 1. The aforementioned steps were repeated for the preparation of caged eggs.

On the day of the experiment, the eggs were exposed to two rounds of treatments: for the first treatment, all twelve eggs were submerged in beakers filled with distilled water for an hour, and for the second treatment, the twelve eggs were split into three experimental groups, which were distilled water (control group), salt, and sugar solution. The experiment was carried out with a one-week interval. During the first week, only free-range eggs were subjected to the two treatments, while during the second week, only caged eggs were subjected to the same treatments. The control group was implemented to allow for a better comparison of results across the three groups and to determine whether there is a relationship between egg permeability and the type of solution they are being suspended in.

Before submerging the twelve free-range eggs in distilled water during the first treatment, the eggs were numbered according to the beaker they were placed in, and their initial weights were measured using OHAUS weighing scale. After placing the de-shelled eggs into the beakers for one hour, the eggs were dried with two to three Kim wipes and weighed again. The latter measurements were used to calculate the change in egg weight due to water intake through the

permeable membrane. The mean difference in weight of the eggs was calculated by taking the average weight changes (final weight - initial weight) and reporting it to three decimal places.

To prepare our salt solution for the second part of our experiment, we added 233.8g of salt into two liters of water (2M of NaCl solution), heated the solution on a hotplate, and used a magnetic stirrer to facilitate dissolving. Similar steps were taken for preparing the sugar solution, where we added 684.6 g of table sugar ($C_{12}H_{22}O_{11}$) into 1L of water (2M of sugar solution).

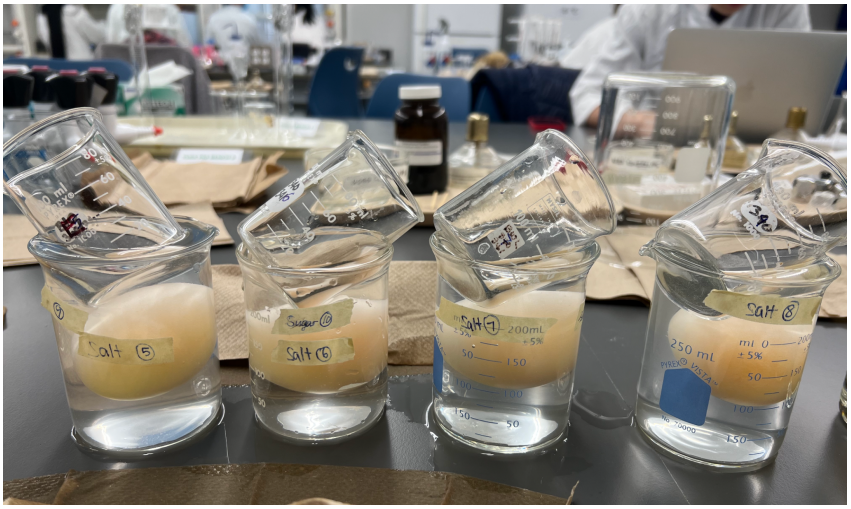

Figure 2. De-shelled caged eggs submerged in salt water solutions.

Next, we divided the twelve free-range eggs into groups of three: four eggs per water, saline, and sugar solution treatment. Eggs 1-4 were placed in beakers containing distilled water, just enough to cover the top of the eggs. Eggs 5-8 (as observed in Figure 2) and 9-12 were submerged in salt and sugar solution, respectively. To ensure the eggs were covered by the solution we utilized metal spatulas and small beakers to keep the eggs stationary and below the solution level in the beaker, as observed in Figure 2. The eggs were allowed to soak for an hour before we took them out. Upon removing the eggs from the solution, they were once again dried with Kim wipes and weighed on the weighing scale to determine if there were changes in egg weight. The eggs submerged in the sugar solution were dabbed with wet Kim wipes to ensure the

residue was removed and would not impact the weight measurements. Once again, the change in weight of the eggs was calculated (final weight - initial weight) and recorded to three decimal places. The aforementioned steps were repeated for de-shelled caged eggs.

The statistical significance of the weight difference between two types of eggs was evaluated using a two-sample t-test with a significance level of 0.05. The p-value for each treatment was calculated under the assumption of a null hypothesis stating that there is no significant difference in weight between the two types of eggs. A Two-Way ANOVA test with an alpha of 0.05 was also conducted in addition to the two-sample t-test for the second treatment to determine whether the weight means of two types of eggs are significantly different under the same treatment.

Results:

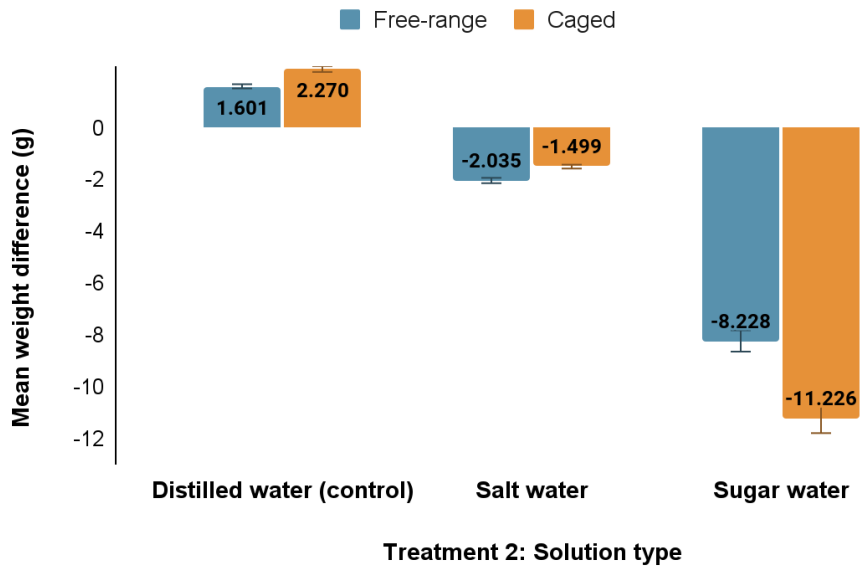

Figure 4. Mean weight difference across free-range and caged eggs after submersion in different solution types. The two-sample t-test result (n=4) obtained for distilled water was not statistically significant as the corresponding p-value was 0.064, which was greater than alpha (0.05). The salt and sugar solution results were significant with a p-value of 0.0145 and 0.011, respectively. A Two-Way ANOVA test produced a p-value of 0.02955.

The bar graph demonstrated in Figure 3 shows the mean weight difference in grams observed across free-range and caged eggs when they were submerged in water for an hour. Caged eggs have an average rate of absorbing 4.832g of distilled water per hour and free-range eggs have an average of 2.565g of water absorbed per hour. Overall, the weight of both types of eggs increased upon submersion in water. The p-value obtained for treatment 1 was 0.001 which was lower than alpha (0.05) and thus the results were statistically significant. The bar graphs represented in Figure 4 show the mean weight difference in grams across the three experimental treatments: distilled water (control), salt, and sugar solution. The mean weight difference of free-range and caged eggs for distilled water was 1.601g and 2.270g, respectively. For salt and sugar solution, the mean weight difference for free-range eggs was -2.035g and -8.228g, respectively. Similarly, for caged eggs, it was -1.499g (salt solution) and -11.226g (sugar solution). The negative sign indicates water lost by eggs whilst a positive value indicates water gained by eggs through the process of osmosis.

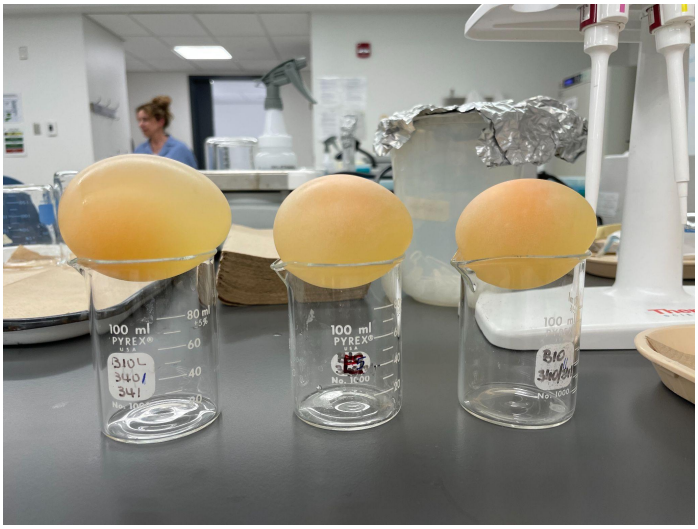

Figure 5. Comparison of de-shelled caged eggs soaked in distilled water, sugar water, and saltwater - from left to right.

A visual comparison among three treatment groups is indicated in figure 5. Water lost by both types of eggs was greatest when placed in a sugar solution, and caged eggs lost more water in the sugar solution than free-range eggs with a difference of 2.998g. A two-sample t-test was conducted for water, salt, and sugar solution and the p-values obtained were 0.064, 0.0145, and 0.011, respectively. The p-value for distilled water was greater than alpha (0.05) indicating the results were not statistically significant whilst the p-value for the salt and sugar solution was lower than alpha, indicating the results for the salt and sugar solution were statistically significant. A Two-Way ANOVA for the three experimental solutions which produced a p-value of 0.02955, indicating there is significant interaction between living conditions (free range vs. caged) and osmosis rate.

Discussion:

In the first round of water soaking, within the same time limit, on average the caged eggs gain more weight indicating a more rapid rate of absorbing water, suggesting the membrane of these specific caged eggs is more porous than the free-range eggs. At the treatment step, the weight still increases with a second hour of water soaking. The eggs continued to absorb water suggesting they have not reached an equilibrium point within one hour and osmosis is still happening. In contrast, when both egg types were treated with high concentration (2M) of salt and sugar water, the water was drawn out of the membrane leading to a decrease in weight. In particular salt treatment, the caged eggs demonstrate slower water movement compared to free-range eggs while in other groups the caged group shows faster water movement. With the exception of the salt-water treatment group, the observed inconsistency of caged eggs showing

lower osmosis rates may be attributable to sampling error due to the small weight difference. Alternatively, caged eggs may have higher permeability particular to sodium and chloride ions, resulting in reduced water movement.

As several studies suggest sucrose (table sugar), is a disaccharide that constitutes glucose and fructose, which is hard to cross the vitelline membrane via simple diffusion as it is larger (Nakamura et al., 2019; Chen & Huang, 2014). In our experiment, there is a significant difference between salt and sugar water treatments on both types of eggs shown in Figure 4, the water moves at a faster rate in a sugar environment compared to salt. Therefore, when the eggs were put into a hypertonic environment that is permeable to water but barely any sucrose, the water moves at a faster rate from inside to outside of the membrane. However, the membrane permeability for sodium and chloride ions has not been extensively studied. According to Liu et al. (2019), the typical salt concentration in eggs ranges from 0.2% to 0.4% of their weight. When exposed to a 2M salt solution, a hypertonic environment is also established where the concentration of solutes outside the membrane is higher than inside. This concentration gradient creates a driving force for sodium and chloride ions to move across the membrane. As a result, water molecules do not need to move as far to balance the osmotic pressure, since the movement of sodium and chloride ions across the membrane can also contribute to the osmotic equilibrium.

Many factors can affect egg membrane permeability, including the genetic information of chicken species, nutrition content when feeding, as well as environmental factors such as temperature and humidity. The experiment conducted in this study involved such factors that were difficult to control in the laboratory setting. As there is limited information on the type of nutrients the chicken was given and the environments the chicken was raised in - these variables may have differed our results. However, within the consideration of these variables, our results

showed a significant difference in the rate of water movement across the egg membrane between free-range and caged eggs.

On the other hand, if the living condition is affecting the membrane permeability for a variety of substances, it is possible that the underlying mechanisms or expression levels of transport channels are also being affected. Additionally, such a factor is changing the chemical properties such as nutritional content of the eggs, it is essential that the living conditions in poultry farming is carefully evaluated and taken into account to ensure the production of safe and healthy food products. Additional research could be conducted to evaluate the impact of various concentrations and durations of treatments, as well as to examine the composition of cell membranes and the expression level of water-transporting channels in different egg types. Specifically, investigations into the membrane permeability of eggs with regard to ions are warranted, given the scarcity of prior research in this area. Furthermore, dietary composition may play a role in egg size and permeability, and thus further studies could evaluate and assess the specific diets of each laying hen. Such experiments may necessitate a more comprehensive understanding of the laying hens that produced the eggs and limit the sample to eggs from the same breeder.

Conclusion:

Our results failed to support our hypothesis as we observed a higher osmosis rate (indicated by greater mean weight differences) across water, sugar solution, and salt solution treatments. The slightly higher osmosis rate in the salt solution of free-range eggs may be attributed to sampling error as discussed above. Overall, our results showed that caged eggs have a faster water diffusion rate than free-range eggs when submerged in water, suggesting the

potential difference in membrane composition or the mechanism of aquaporins among these eggs. Observing such differences in membrane permeability contribute to another variation in chemical properties between free-range and caged eggs.

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