# A Longitudinal Study Observing the Effectiveness of Conventional Preserving Methods through Mold Coverage and Rotting in Apple Slices

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**Abstract:**

Throwing out old, rotten fruits are thought to cost the average person upwards of $500 per year, often exacerbated due to lack of using effective preservation methods.[1] Currently, a lack of conclusive research exists within this area, as most research on fruit rotting or decay has been observed in habitats outside of pedestrian homes.[2] To remedy this lack of knowledge, the paper investigates fruit rotting by preserving apple slices in the kitchen with the following conventional methods: salted, vacuum sealed, boiled and unaltered for control. Four-day interval observations were used over a total of nine days, holding a consistent refrigerator temperature without significant alterations in placement of the samples within the fridge, allowing us to adequately observe the level of rotting and microorganism mould growth on the apple slices. Overall, the results show a statistically significant ($p < 0.05$) increase in mould growth in the salted group ($M = 76.7$, $SD = 15.28$) when compared to the other groups, while the three other treatment group's mould growth and fruit rotting efficacy were observed to not be significantly different ($p > 0.05$). In addition, while no conclusive method of fruit preservation is shown to be the most optimal in this study, several limitations are identified, emphasized, and addressed, and future directions in furthering this study are discussed.

**Introduction**

The Commission for Environmental Cooperation (CEC) has estimated that Canadian households waste 4.41 kg of food per person per week or roughly 229.32 kg annually, with fruits and vegetables comprising about two thirds of the avoidable waste.[3] Furthermore, fruit preservation is also closely associated with hygienes; unhygienic conditions can facilitate spoilage of the fruits that could ultimately lead to contamination of other food,[4] which in turn results in serious health consequences when ingested. A study from US Food Safety and Inspection Service (FSIS) discovered more than 97% of people don't wash their hands or use improper hand washing techniques before meals, which results in possible bacterial transfer from their hands to food.[5] As food waste being an important issue facing today's society, it has been shown that food waste can be reduced by six-fold when foods are refrigerated when compared with fresh foods.[4]

In order to meaningfully address this issue, this study furthers the research by proposing an additional level of food preservation techniques taken from conventional, traditional preserving methods in addition to refrigeration - to increase the longevity and sustainability of home food preparation while reducing food waste. The conventional food preservation methods that are used in this study are salting, boiling and vacuum sealing as they are the most readily available methods encompassing the common household kitchen[6]. Salting is a long-practiced preservation method for food that likely goes back further than any other preservation method, and will be included as even most modern variations of this technique still follow generally the same steps[7]. Boiling the food is also known to be a generally excellent method of killing pathogens due to the denaturing of proteins in high temperatures; following that sense of logic, if the proteins on the bacterium within the fruit are denatured, they will cease to function, resulting in the same effects as a conventional preservation method. Vacuum sealers are also considered as they are likely representative of the current and future population as projected growth and ownership of vacuum sealers (also known as

“Food Vacuum Machines”) are shown to be significantly increasing[8], likely due to an increase in demand for hygienic packaging solutions.

Accordingly, this study investigates the effectiveness of conventional food preserving methods: salting, boiling, and vacuum sealing by examining and comparing levels of rotting and moulding of apple slices that had close contact with unhygienic hands. Apple slices are used in the study to simulate average household fruit purchases because they represent one of the most commonly consumed fruits according to 2019 US Statistica [9].

The null hypothesis ($H_0$) states that there is no significant difference in the efficacy between the preservation methods, while the alternative hypothesis ($H_A$) states the opposite. Of the three preserving methods, salting is anticipated to be most effective in prohibiting bacterial growth due to the osmotic shock from the intense hypertonic environment created that drains water, incapacitating bacterial cells[10].

**Methods:**

Three similar sized and shaped apples (Royal Gala) are cut equally into a total of 12 pieces; cut in half first from the top, rotated 90 degrees and cut again to ensure roughly equal surface area. Each apple yields approximately four equal slices with very similar surface area. Each slice is handled with unwashed hands to ensure full contact between hands and every slice.

Each group (3 pieces each) of apples is processed with the corresponding preservation methods: for boiling: the apple is boiled for 1 minute in water (after water is brought to boil) in a pot. For salting, each “face” of the apple is smothered on a flat surface laden with salt. For vacuum, a vacuum sealer is used to thoroughly seal the ziplock bags. Subsequently, each apple slice is placed in plastic bags separately and the bags are labelled (eg. "Control #1", "Control #2", etc.) with masking tape. There should be 12 bags in total. The bags containing apple slices are weighed individually on a digital kitchen scale, and the weight is recorded as weight of fresh apple (Figure 1a). This allows for draining the rotten liquid at the end of the experiment and measuring the relative level of rotting (loss of mass). Then the bagged apples are placed in the fridge at around 4 degrees Celsius for 1 week, which mimics the environment of how people normally store their unfinished food in a fridge. The apples are observed and recorded every 4 days in the observation log (Figure 1). At the end of the roughly one week experiment (2 observations), the percentage of mold coverage is first recorded, then the bags are weighed again after having the bottom corner cut-off and having the rotten liquid drained and weighed again on the scale. Surgical masks and eye goggles were equipped to minimize the contact between rotten apples and experimenters for final weighing.

The level of rotting is compared by measuring the following: percentage of moldy(darkened) area on the observable surfaces and percentage of weight change. To mathematically measure the rotten level inside the level, we will use % weight change as a way to determine the level of rotting because we assume bacteria will be the main decomposers, and the decomposed tissues will be lost through gas and water (the reason why rotten food always have a puddle of liquid under it.)[11] Because most of the mold colonies (except for the salted group) are tiny individual black spots, each mold spot is counted as 1% of surface area if the moldy spot is smaller than 3mm in diameter; accordingly,  it would count as 2% if the moldy spot is more than 3mm (while the largest spot observed was 4mm).

The means, variances, standard deviations are calculated separately. Two way ANOVA is used to analyze the difference in means, if a significant difference is obtained, a post-hoc

Tukey's HSD test is used to determine which of the groups are significantly different from each other. After the Tukey post-hoc test,  subsequent paired t-tests are conducted between each of the significantly different groups, assumptions of independence are checked using a normal distribution, and results are taken to be significant at a 95% confidence interval (CI), holding alpha of 0.05.

**Figure 1.**

*Observation Tracking Table*

| | **Control** | **Salted** | **Vacuum Sealed** | **Boiled** |
|---|---|---|---|---|
| **Nov.1st** | Starting date, apple slices look normal, and feel firm. | Starting date, apple slices look normal. Surfaces are covered with salt crystals. Feel firm | Starting date, apple slices look normal and feel firm | Starting date, apple slices look normal and feel firm. |
| **Nov.5th** | Apparent rotting has occurred but very little colonial growth, the rotten areas have s light brown color that covered about 60-80% of apple's surface. The observation is very | Very badly rotten, almost the entire surface of the apple has turned dark brown, with very visible white and black colonial growth on top. Feels a little soggy and | No apparent rotting.<br>All 3 apples look fresh with most of its white/pale pulp unchanged. Looks a little | This is a rather confusing observation. All 3 boiled apple slices turned brown and looked very rotten. The rotting actually |

| | | | | |
|---|---|---|---|---|
| | similar in all 3 control bags.<br>Still firm | observed some liquid at the bottom of the bag. The observation is consistent in all 3 bags. | wrinkled.<br>Feels firm. | looks clean with no microbial growth.<br>Feels very soft. |
| **Nov.9th** | Doesn't seem to have changed much from Nov.5th's observation.<br>Control#1 has 1 small and subtle moldy spot (diameter of about 3mm).<br>Control #2 has 2 small and subtle moldy spot (diameter of about 2 and 4mm). Some of the palp turned a little darker compared to its surroundings.<br>Control #3 has 2 small and subtle moldy spots(diameter of about 2 and 4mm). No other apparent color change.<br>Feels firm with a little softness. | Badly rotten with an extensive amount of mold/bacterial growth.<br>Bag #1 has its entire surface covered in black molds that almost looks like it has a layer of fur. Some liquid pooled underneath.<br>Bag 2 has the surface covered in different coloured molds: green, white, black. With some liquid pooled underneath.<br>Bag#3 is not as bad. It has many small dark colonies covering about 60% of its surface, with some liquid pooled under. | Doesn't seem to have changed much from Nov.5th's observation.<br>Vacuum-sealed bag #3 has one black moldy spot that's about 6mm in diameter.<br>Still fresh with its white palps.<br>Feels firm. | Bag #2,3 has not changed since Nov.5th.<br>However,bag #1 has almost "melted" with a noticeable pool of brown liquid underneath. The back of the peels have lost its color and the apples are very soft to touch.<br>No molds or other colonial growth. |

**Results:**

In total, $N$ = 12 samples were collected, with three samples in each of the four groups, respectively. The following Equation (1.1) was used to calculate percentage weight change (i.e rotting) and Equation (1.2) was used to calculate percentage of mold coverage:

$$\left|\frac{Weight\ of\ Apple\ Slice\ After\ (Drained) - Weight\ Of\ Apple\ Slice\ Initial}{Weight\ of\ Apple\ Slice\ Initial}\right| \times 100\% \ = \ \%\ Rotting \qquad (1.1)$$

$$[x(\#\ of\ mold\ spots < 3mm) \times 1\%\ surface\ area]\ + [y(\#\ of\ mold\ spots > 3mm) \times 2\%\ Surface\ area]$$
$$= \%\ Mold\ Coverage\ on\ Surface\ Area \qquad (1.2)$$

As there were two factors potentially influencing the result, both rotting (Figure 2b) and microbial growth (Figure 2a), a two-way ANOVA was used to discern a potential difference between the four treatment groups with an alpha level of 0.05. The result of the two-way ANOVA test demonstrates that there are significant differences when comparing the amount of rotting and moulding in each sample $F(3,16) = 70.27$, $p < 0.01$. Furthermore, there also exists a significant difference between the four treatment groups $F(1,16) = 60.81$, $p < 0.01$; accordingly, the interaction effect was also significant, $F(3,16) = 71.27$, $p < 0.01$, indicating that there exists a significant difference between both the treatment groups and their respective amounts of rotting and moulding.

**Figure 2a.**

*Comparison of Mould Growth by Treatment Group*

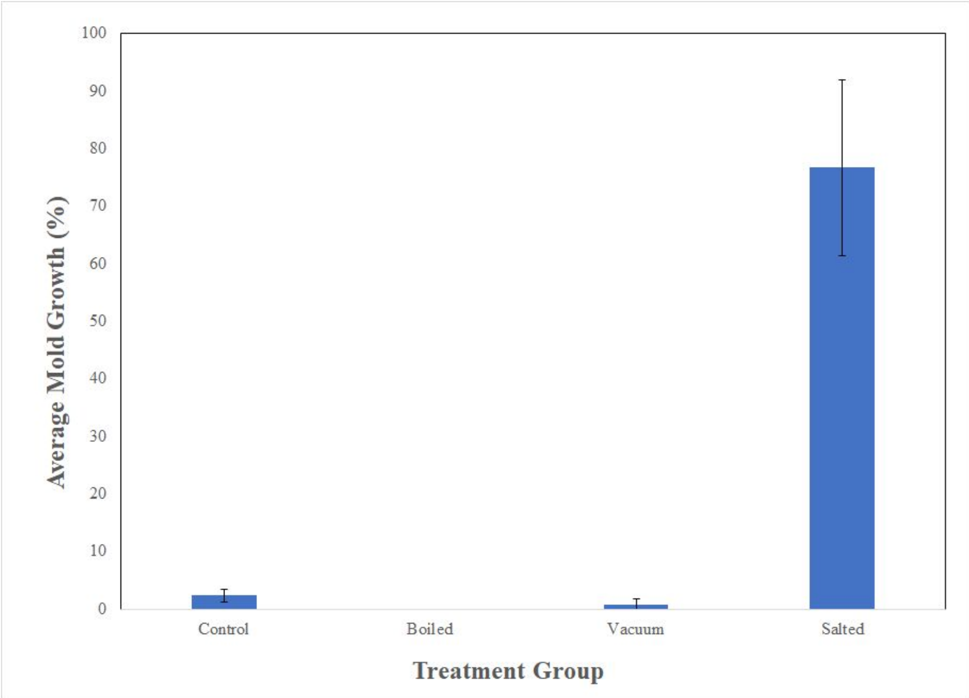
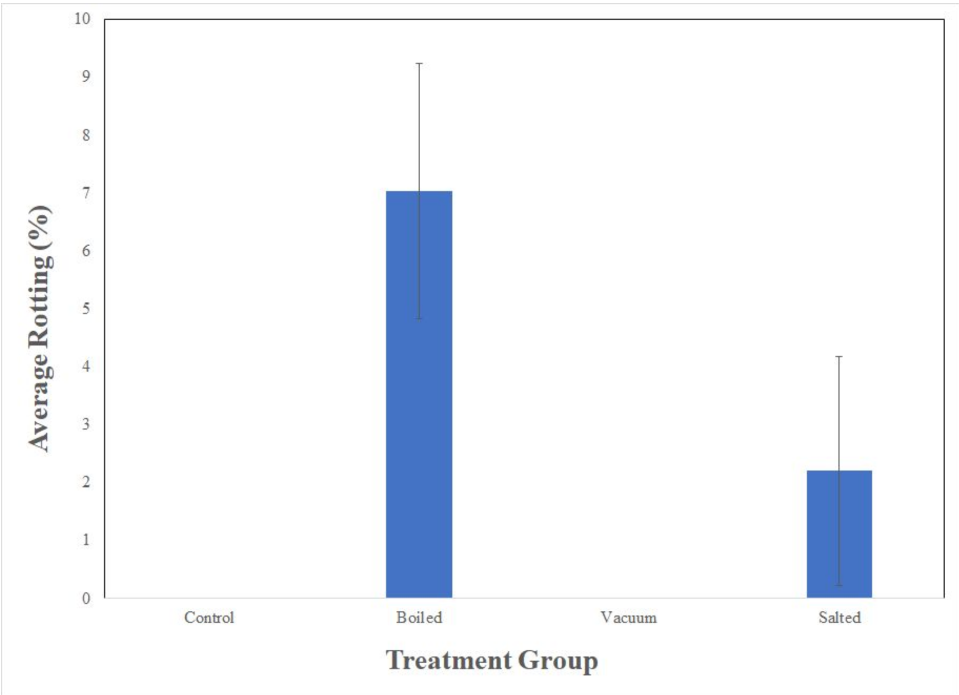



**Figure 2b.**

*Comparison of Rotting (Weight Change) by Treatment Group*




The post-hoc Tukey's test (Table 1) expands on the differences between the groups and indicates that the salted treatment group is the significantly different group in fruit moulding and rotting compared to all other groups - in line with the observed trend in both Figure 2(a) and 2(b).

**Table 1**

*Tukey's HSD Post-Hoc Test Results Table*

| Compared Groups | Absolute Difference | Critical Value | Result |
|---|---|---|---|
| **Control to Boiled** | 2.35 | 1.04 | Not Significantly Different |
| **Control to Vacuum** | 0.83 | 0.37 | Not Significantly Different |
| **Control to Salted** | 38.27 | 16.94 | Significantly Different |
| **Boiled to Vacuum** | 3.18 | 1.41 | Not Significantly Different |
| **Boiled to Salted** | 35.92 | 15.91 | Significantly Different |
| **Vacuum to Salted** | 39.1 | 17.32 | Significantly Different |

Using a paired t-test between the salted group and the three other treatment groups, the amount of microbial rotting is noted to not be significantly different in the salted group ($M$ = 2.2, $SD$ = 1.97), $t(2)$ = 2.61, $p$ = 0.12 when compared to the other groups. However, microbial growth ($M$ = 76.7, $SD$ = 15.28) is significantly greater in the salted group when compared to the other three groups: boiled $t(2)$ = 8.70, $p$ = 0.013, vacuum $t(2)$ = 8.60, $p$ = 0.001, control $t(2)$ = 7.964, $p$ = 0.0154. This result contradicts the null hypothesis (H0) that there exists no significant statistical difference in the preservational methods of fruit, and accepts the alternative hypothesis (HA) that there exists a difference in preservational ability between the four treatments. Additionally, this result contradicts our original hypothesis that the salted preservational method should yield the least amount of microbial growth, as the results indicate that the salted group, in fact, had significantly more microbial growth when compared to the other treatments; no treatment group had significantly less microbial growth or moulding compared to the others.

**Discussion**:

Based on the original statistical analysis, the differences in moulding and rotting between the four treatment groups arise solely from the salted group, which had the most extensive moulding and rotting compared to the other three groups.

The vacuum-sealed group, on surface analysis in Figure 1, showed the least rotting and moulding possibly due to the little available oxygen that's responsible for oxidative rotting and aerobic bacterial growth, only allowing anaerobic tolerant bacteria to survive. Also, the extreme negative pressure from the near vacuum environment was likely to implode the bacterial cells.[12] Accordingly, the vacuum-sealed group had the moulding and rotting on average, $0.67 \pm 1.15$ and 0, respectively.

The boiled group had extensive rotting activities ($M = 7.03$, SD = 2.20) with no microbial growth. It is possible that the boiled water not only denatured the bacterial cells, but also denatured the membrane proteins in the apple cells that resulted in the loss of structural integrity of the apple.

The salted group had the most extensive microbial growth ($M$ = 76.7, SD = 15.28) with minimal rotting ($M$ = 2.2, SD = 1.97). It was possible that the little fluid loss was due to the extensive bacterial metabolism with water. One possible explanation for the excessive moulding was that the salt on the superficial layer created a gradient of hypertonicity on the apple: the surface had the strongest hypertonic environment that possibly killed many bacterial cells along with apple cells; however, some bacterial cells could have infiltrated the surface palp on the apple and gotten deeper down to a level where it was more suitable for bacterial growth.[13] Then, the dead apple and bacterial cells on the surface layer start to decompose, which further feeds more nutrients to the bacteria deeper down. Perhaps due to this reason, salting the apple palps ends up facilitating spoilage and promoting microbial growth, as the result of the paired t-tests supported the conclusion that the salted group has significant statistical differences (alpha = 0.05) in moulding when compared to boiled ($t(2)$ = 8.70, $p$ = 0.013) , vacuum sealed ($t(2)$ = 8.60, $p$ = 0.001) , and control groups ($t(2)$ = 7.964, $p$ = 0.0154).

Although salting has been a historically proven method in food preservation, it did not work as expected when applied directly on the palp of the fruit. Perhaps it was due to most preservational methods also pairing with smoking, known as curing[6,14], altering the preservational process. The result may be different if that salting was done to fruits with their skins intact, which could be an effective layer of defense from bacterial infiltration.

Since the experiment primarily aims to investigate the level of fruit spoilage based on different preserving conditions, the results could be further improved by introducing and comparing more varieties of fruits, as well as including other popular preserving methods like freezing and drying.

On top of that, possible human errors could have impacted the observed results. For example, bacteria were transferred to apple slices by having the experimenter rubbing unwashed hands on the apple slices to simulate household conditions. As a result, bacteria from the experimenter's hands could have transferred on to the first few slices of the apples, leaving the rest of apple slices with slightly lower microbial counts to start with, which could be the reason for the surprisingly low microbial count on the control group if left last to package. A more even spread of bacteria on the apple slices could be achieved by mixing the apple slices together thoroughly before treating them, or having multiple experimenters each taking care of fewer numbers of slices.

Another factor that could affect the observed result is the inhibition of moulding from the pooling of the liquid. As seen in Appendix A, the part of the apple slice that was soaked in the rotting liquid had no moulding, despite its surrounding area having extensive moulding activity. This in turn, gave a lower molding surface area percentage that could lead to inaccurate results. This problem can be addressed by double-bagging (one big and one small Ziplock bags) the apple slices while cutting a small hole into the bottom corner of the inner bag to allow the liquid to drain, and the drained liquid could still be retained by the outer bag for measurement.

Finally, the experiment explored only three of many possible ways to preserve and store unfinished food. Although the result from the experiment indicates vacuum-sealing being the most effective method to preserve apple slices and salting being the most adverse method, we do not anticipate this result would apply to other food-products due to the complexity of different types of food. As aforementioned, salting might function as a good preserving method if fruit or food had its skin intact. Similarly, vacuum-sealing other food products like meat could produce a suboptimal result, as the low oxygen environment could facilitate the growth of anaerobic bacteria like Clostridium botulinum on meat [15].

**Conclusion:**

Conclusively, our study rejects the null hypothesis, and lends support to the alternative hypothesis, the salted treatment group's preservation efficacy being significantly different. Based on the findings of this paper, salting is the worst preservational method, while vacuum sealed, boiled, and just storing without treatment do not yield a significant difference in microbial growth and moulding. Furthermore, boiling and salting the palps will facilitate and enhance the rate of fruit spoilage compared to not treating the fruit at all.

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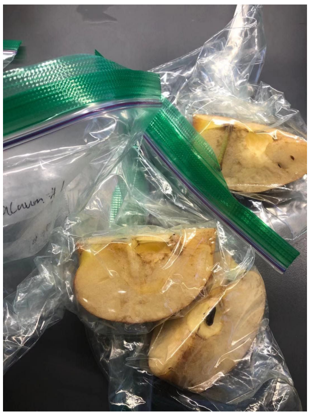
**Appendix.**

A. Photos of the observations:

| | |
|---|---|
| Nov 5th Boiled |  |
| Nov 5th Salted |  |
| Nov 5th Control |  |

| | |
|---|---|
| Nov 5th Vacuum |  |
| Nov 9th Control |  |
| Nov 9th Vacuum |  |
| Nov 9th Boiled |  |

| | |
|---|---|
| Nov 9th Salted |  |

B. ANOVA Results

| Anova: Two-Factor With Replication | | | | | | |
|---|---|---|---|---|---|---|
| SUMMARY | Mold Change | WC | Total | | | |
| Control | | | | | | |
| Count | 3 | 3 | 6 | | | |
| Sum | 7 | 0 | 7 | | | |
| Average | 2.33333 | 0 | 1.166667 | | | |
| Variance | 1.33333 | 0 | 2.166667 | | | |
| Boiled | | | | | | |
| Count | 3 | 3 | 6 | | | |
| Sum | 0 | 21.1 | 21.1 | | | |
| Average | 0 | 7.033333 | 3.516667 | | | |
| Variance | 0 | 4.853333 | 16.78167 | | | |
| Vacuum | | | | | | |
| Count | 3 | 3 | 6 | | | |
| Sum | 2 | 0 | 2 | | | |
| Average | 0.66666 | 0 | 0.333333 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Variance | 1.33333 | 0 | 0.666667 | | | |
| Salted | | | | | | |
| Count | 3 | 3 | 6 | | | |
| Sum | 230 | 6.6 | 236.6 | | | |
| Average | 76.6666 | 2.2 | 39.43333 | | | |
| Variance | 233.333 | 3.88 | 1758.471 | | | |
| Total | | | | | | |
| Count | 12 | 12 | | | | |
| Sum | 239 | 27.7 | | | | |
| Average | 19.9166 | 2.308333 | | | | |
| Variance | 1214.81 | 10.58629 | | | | |
| ANOVA | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Sample | 6449.25 | 3 | 2149.753 | 70.27249 | 1.98E-09 | 3.238872 |
| Columns | 1860.32 | 1 | 1860.32 | 60.81135 | 7.71E-07 | 4.493998 |
| Interaction | 6540.64 | 3 | 2180.214 | 71.26822 | 1.79E-09 | 3.238872 |
| Within | 489.466 | 16 | 30.59167 | | | |
| | | | | | | |
| Total | 15339.6 | 23 | | | | |

C. Raw Data Keeping Table

| | Control | | | Boiled | | | Vacuum Sealed | | | Salted | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Weight of the fresh apple (grams)** | 49 | 41 | 44 | 37 | 44 | 47 | 49 | 40 | 38 | 32 | 36 | 53 |
| **Weight of the rotten apple (grams)** | 49 | 41 | 44 | 34 | 42 | 43 | 49 | 40 | 38 | 32 | 35 | 51 |
| **Percentage of weight change (%)** | 0 | 0 | 0 | 8.1 | 4.5 | 8.5 | 0 | 0 | 0 | 0 | 2.8 | 3.8 |
| **Percentage of surface area (approx.) covered by mold** | 1 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 90 | 80 | 60 |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