# Cleaning Power Comparison Between Citric Acid and Sodium Hypochlorite

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## Abstract

**Objectives:** The purpose of this research was to compare the effectiveness of sodium hypochlorite and citric acid at cleaning surfaces.

**Methods:** ATP bioluminescence assay was used to evaluate surface cleanliness pre- and post-treatment with active cleaning agents of choice. Independent two sample t-test statistical analyses were conducted to ensure both cleaning agents have similar load of contaminants. Paired t-test statistical analysis was utilized to evaluate efficacy of citric acid and sodium hypochlorite for cleaning surface.

**Results:** Sodium hypochlorite significantly reduce the number of ATPs on a surface (p-value = 0.00084). Meanwhile, citric acid efficacy could not be evaluated due to incompatibility with enzymes found in Hygiena UltraSnap.

**Conclusion:** Further research needs to be conducted to evaluate the cleaning efficacy of citric acid. While sodium hypochlorite significantly reduces ATPs number, following manufacturer's guideline and removing physical debris from the surface is necessary to achieve Hygiena's prescribed cleanliness standard.

**Keywords**: citric acid, sodium hypochlorite, bleach, cleaning, bleach, ATP

## Introduction

With so many cleaners' choices out there, are operators equipped with the knowledge to choose the most appropriate cleaners for the food premise and understand the safety aspect?

An article from NBC News informed that an accident occurs due to the cleaning agent's incompatible mixing (Li, 2019). This accident that resulted in death is preventable if the employees are educated on the cleaners' safety aspect. Furthermore, it will be beneficial for the business to fully comprehend what kind of cleaners they need. Besides saving cost, this will also reduce the likelihood of improper chemical storage and the possibility of incompatible chemical mixing. Common practices in the restaurant will be reviewed, and additional information on the cleaners' safety aspect will be provided. Educating and helping the public make an informed choice of cleaning products -- this paper will mainly compare the effectiveness of a natural surface disinfectant, namely citric acid, with a traditional surface disinfectant – sodium hypochlorite.

## Literature Review

In 2016, it was estimated that 4 million Canadians suffered from foodborne illnesses annually (Government of Canada, 2016). Out of those that were sick, 11,600 required hospitalization (Government of Canada, 2016). The treatments and medications administered, not to mention the number of deaths, will undoubtedly add to the country's economic burden. Canada spent 242 million Canadian dollars solely to manage the Maple Leaf Listeriosis outbreak's foodborne illness back in 2008 (Thomas, et al., 2015). Foodborne illnesses are preventable. One way of reducing the risk is by routine cleaning and disinfecting, both for households and food service establishments (WHO, 2008).

### Effectiveness of cleaning agents

There have been many pieces of research that compare the effectiveness of various active agents in cleaners. Testing the cleaners' efficacy in similar environments is crucial (Springthorpe, Grenier, Lloyd-Evans, & Sattar, 1985). For example, if it is a cleaner for a food-contact surface, one must conduct the test on a surface, at room temperature, at the manufacturer's recommended concentration.

There were multiple active ingredients tested. It included naturally derived and chemical ingredients. Some of the agents found in the studies were green tea extract (GTE) (Randazzo, Falco, Aznar, & Sanchez, 2017), plant hydrosols (thyme, black cumin, sage, rosemary and bay leaf) (Tornuk, et al., 2011), citrus extracts (Medina-Rodriguez, et al., 2020), organic acids (lactic acid, acetic acid, citric acid) (Bhatti, 2016) (Li & Wu, 2013), non-ionic surfactants (Restaino, Frampton, Bluestein, Hemphill, & Regutti, 1994). On the other hand, agents categorized as traditional include sodium hypochlorite, iodine, QUATs, aldehydes, alcohols, potassium bromide, strong bases, and acids (Springthorpe, Grenier, Lloyd-Evans, & Sattar, 1985).

The research paper above shows that a higher concentration of natural cleaners might be necessary to achieve the same cleaning goal as traditional cleaners. Additionally, the bitter orange extract, in addition to citric acid, contains naringenin as well – a type of flavonoid with antibacterial properties (Medina-Rodriguez, et al., 2020). Apart from possessing antimicrobial quality, citric acid is “not listed as a substance that causes asthma, reproductive or developmental harm, or skin sensitization” (Culver, Geiger, & Simon). However, since the citric acid’s pH ranges from 2-4, it may act as a “slight to moderate irritant” to mucous membrane and skin (Culver, Geiger, & Simon). Besides creating an unfavourable environment for growth due to its naturally low pH, citric acid also breaks the contaminants’ nucleic acid bonds and precipitates proteins (Dvorak, 2008). Citric acid is not toxic to aquatic life or another environment (Culver, Geiger, & Simon). Moreover, citric acid is registered as a disinfectant in Health Canada (Fong, Gaulin, Le, & Shum, 2014). All the same, citric acid has a narrow spectrum, which means it also has limited antimicrobial efficacy – unlike sodium hypochlorite, which has a broad spectrum (Fong, Gaulin, Le, & Shum, 2014). Moreover, citric acid may be corrosive and may react with bleach to produce a chlorine gas, a potent irritant (Fong, Gaulin, Le, & Shum, 2014). Meanwhile, the famous traditional active ingredients commonly used are sodium hypochlorite and QUATs (Culver, Geiger, & Simon). In 2011, sodium hypochlorite was crowned as the most widely used sanitizer (Pfuntner, 2011). Due to its effectiveness, broad-spectrum, low cost, and ease of manufacture, sodium hypochlorite is popular (Pfuntner, 2011). Sodium hypochlorite mechanism of work includes causing damage to the microorganism’s outer membrane – resulting in the “loss of permeability control and eventual lysis of the cell” (Pfuntner, 2011). Another way sodium hypochlorite can work is by cellular enzyme inhibition and DNA destruction (Pfuntner, 2011). Generally, sodium hypochlorite is used at a concentration of 5.25-6%. However, now the concentrated solution, 8.25% sodium hypochlorite, is also readily available at the market (Culver, Geiger, & Simon).

All the same, sodium hypochlorite efficacy is highly affected by temperature, pH, and the presence of organic materials (Pfuntner, 2011). In high temperatures or at pH that is less than five or more than 7, sodium hypochlorite is less effective (Pfuntner, 2011). Additionally, sodium hypochlorite's shelf-life is limited to two to three weeks before it degrades substantially (CDC, 2020). What is more, sodium hypochlorite is labelled as asthmagen, and it is also highly toxic to aquatic life (Culver, Geiger, & Simon). Furthermore, sodium hypochlorite may react with organic matter and form trihalomethanes, a carcinogen (Culver, Geiger, & Simon).

### Common Practices

Neal (2013) mentioned time constraints, forgetfulness, and the perception of inconvenience as the barriers to proper surface cleaning. Though this happened in foodservice establishments, there is a chance that this might happen in household environments. The public may not know that cleaning the soiled surface before disinfection significantly impacts the disinfectant's effectiveness (Government of Canada, 2020). Moreover, it is essential to read the label's instructions to know how to utilize cleaners. As written in the analysis above, different active ingredients have different working methods. Some ingredients work more rapidly than the other. For example, sodium hypochlorite may disinfect at 5 minutes dwell time, shorter than citric acid's dwell time at 10 minutes (Culver, Geiger, & Simon). Furthermore, it is wise to understand that some cleaner is more effective towards specific agent(s) when choosing cleaner. Therefore, it is unrealistic to expect one product to kill all viable microorganisms. For example, though citric acid only requires 60 seconds to sanitize non-food contact surfaces effectively, it is only effective against two specific bacteria (Culver, Geiger, & Simon).

### ATP Bioluminescence Assay

This method detects the number of adenosine triphosphates (ATP), which is an indirect measurement of organic residue on a surface that has the potential to support pathogen growth (AIB International, 2013). ATP bioluminescence assay's advantages include the simple method, high sensitivity, cost-effectiveness, and the tests' rapidity (AIB International, 2013). The monitoring system is paired with the ATP Bioluminescence Assay. The monitoring system works by enumerating the ATP and using the relative light unit (RLU) as its measurement unit (Hygiena, 2020a). The yield of RLU is proportional to the amount of ATP available (Hygiena, 2020a). Hygiena (2020a) prescribed the standard scores for a pass, cautionary, and fail results. A score of 0-10 is considered as a pass, 11-30 as cautionary, and 30+ as a failure (Hygiena, 2020a).

### Research Question

There is a limited amount of information regarding the effectiveness of citric acid on a soiled surface. Therefore, this research will focus on comparing citric acid's ability to clean a surface in the presence of physical debris. Similar steps will be done for sodium hypochlorite to find out whether there is any difference regarding the two active ingredients' cleaning power. The result can be translated on the acceptability of cleaning practice in either food service establishments or general household to ensure the foods' cleanliness and safety.

## Methodology

### Materials and Methods

ATP bioluminescence assay was used to measure the cleanliness of a surface. UltraSnap Hygiena ATP is used as the test swab and Hygiena SystemSURE Plus ATP as the monitoring system. ATP assay method is chosen because it measures surface cleanliness and give instantaneous results.

Medium ground beef is the choice of soil to be applied on the cutting board. It is chosen to mimic the soil commonly found in food premises. Fifty grams of the beef sample was diluted with 450 mL of distilled water to make up a $10^{-1}$ beef solution which will act as a contaminant. The beef solution was mixed by shaking the Ziploc bag containing the sample for one minute to achieve homogeneity.

### Procedures

#### Surface Preparation

For this study, one plastic cutting board was utilized. The cutting board was pre-washed with Palmolive orange detergent and patted dry with paper towels. The cutting board was then divided into two parts, one side for bleach and the other for citric acid. A dropper was used to drip 3-4 drops of the beef solution onto the cutting board. Next, a cotton bud was used to spread the solution on the cutting board evenly on an area of 10 cm x 10 cm. A paper with a hole sized 10 cm x 10 cm was used to measure the area. Paper was left in the original position to measure the same area for swabbing later. After division and the introduction of the contaminant, the cutting board was sprayed with the active ingredients of choice – citric acid or sodium hypochlorite. Paper towels were used to dry the cutting boards' surface by patting motion.

#### Swab Test

Using Hygiena UltraSnap and with the help of the measuring paper, an area of 10 cm x

10 cm on the cutting board was swapped (Hygiena, 2021). The plastic tube shall be placed in the monitoring system to obtain the readings. Calibration was done once at the beginning before obtaining the readings for the samples.

**Cleaners Preparation**

Concerning cleaners' preparation, food quality anhydrous citric acid from Xenex Labs was used. A concentration of 5% is chosen to mimic the concentration of citric acid in lemon juice (Hertzberg, Greene, & Vaughan, 2010). On the other hand, 200 ppm of bleach solution as per the procedure outlined in CDC's website for cleaning and sanitizing with bleach (CDC, 2020).

A digital thermometer with probe was left on the table amidst the room to measure the room temperature ensuring it is between 20-22C, in order to get an accurate result. The step above shall be done due to the reagents being sensitive to temperature, producing lower RLU at a lower temperature (Hygiena, 2021).

## Results

| Ho and Ha | Test used | p-value | Conclusion |
|---|---|---|---|
| Ho: there is no difference in the "before treatment" ATP readings between citric acid and bleach. Ha: there is a difference in the "before treatment" ATP readings between citric acid and bleach. | Independent two samples t-test | 0.974 | Do not reject Ho. Since p-value is higher than 0.05, there is not enough evidence to prove that the before treatment reading of both citric acid and bleach are statistically different from each other. However, power is at 62%, meaning that there's a high chance a beta error can occur. Increasing sample size is recommended to reduce the chance of beta error. |
| Ho: There is no significant decrease in the ATP readings after treatment with bleach. Ha: There is a significant decrease in the ATP readings after treatment with bleach. | Paired t-test | 0.00084 | Reject Ho. There is a difference between the reading from before treatment with bleach and after treatment with bleach. Power is at 99%, meaning there is very little chance for Type II error. Type II error is made when Ho is not rejected, meanwhile Ha is right. |

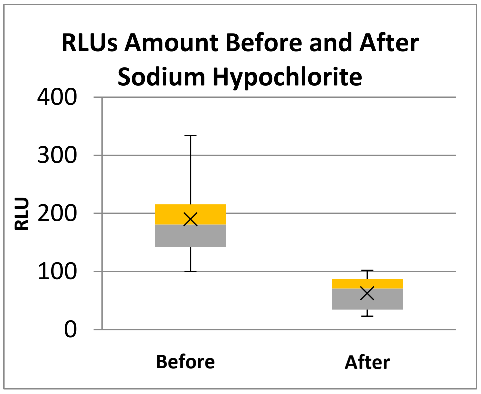


*Figure 1. Box plot of RLUs amount before and after the application of sodium hypochlorite*

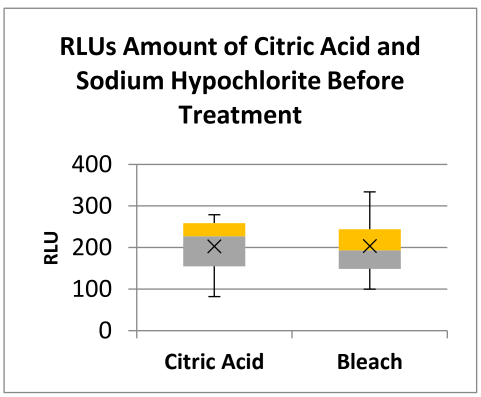


*Figure 2. Box plot of citric acid's and sodium hypochlorite's RLU amount before treatment*

Before treatment with sodium hypochlorite, the average of ATP readings is at 190 RLUs. In contrast, after application of sodium hypochlorite, the average of ATP readings is at 63 RLUs. The author checked the data's normality by utilizing Shapiro-Wilkinson normality test. The result showed that the data is normal. Hence, the inferential statistic reading is obtained from the one-tailed paired t-test. With a p-value of 0.00084221, the conclusion is to reject the null hypothesis. Meaning, the ATP reading after treatment with sodium hypochlorite is significantly lower than the ATP reading from before treatment. Supporting the conclusion above, the box plot in Figure 1 showed that there is a difference between the RLU's average, before and after treatment with sodium hypochlorite.

At the same time, citric acid and sodium hypochlorite has similar ATP readings before treatment, citric acid averaging at 202 RLUs and sodium hypochlorite averaging at 204 RLUs. As for the comparison between the ATP readings before treatment for both citric acid and sodium hypochlorite, normality test indicates that the data is normal. Next, F-test two-sample for variances is conducted to find out whether the data has equal or unequal variances. The test result informed that the data has equal variances. Hence, the inferential statistic read for normal data with equal variances is the two-tailed two-samples t-test. With a p-value of 0.9738, the conclusion is that we cannot reject null hypothesis. This means that there is not enough evidence to prove whether there is any difference between the ATP readings from before treatment for both citric acid and sodium hypochlorite.

Furthermore, the mean and median of both data is very similar to one another. These similarities are displayed on Figure 2.

The data for comparison between ATP amount from before and after treatment with citric acid was not displayed. Readings of samples after treatment with citric acid shows either 0 or 1 RLU. There is no way to know whether citric acid is really effective in removing contaminants.

## Discussion

Statistical analysis on the efficacy of sodium hypochlorite showed significant difference in ATP amount before and after application of sodium hypochlorite on a soiled surface. With a p-value of 0.00084, null hypothesis is rejected. Hence, alternative hypothesis – that there is a significant decrease in ATP reading after treatment with sodium hypochlorite is true. This means that sodium hypochlorite is effective at reducing contamination. However, the average of ATP readings after treatment with sodium hypochlorite is considered a failure based on Hygiena prescribed standard. Pfuntner (2011) mentioned about the efficacy of sodium hypochlorite being affected by the presence of organic soils. There is a possibility that the bits and pieces from beef solution reduced sodium hypochlorite cleaning power. With an average of 62 RLUs, it stands far above 30 RLUs, Hygiena's failure boundary. Therefore, although sodium hypochlorite may be effective at reducing contamination load, it is not enough to rely solely on spraying sodium hypochlorite cleaner to assure surface's cleanliness. As recommended by the Government of Canada (2020), removing the physical debris on the surface before using cleaning agents will boost the efficacy of the cleaners. This approach may be applied to achieve the standard for passing the cleanliness test from Hygiena which stands at 0-10 RLU. Additionally, allowing the sodium hypochlorite to sit at the recommended time by the manufacturer might help to improve its cleaning efficacy (Culver, Geiger, & Simon).

In order to ensure that the contamination load is similar for both agents to clean, an independent two samples t-test for ATP readings from citric acid and sodium hypochlorite before treatment is conducted. With a p-value of 0.974, the statistical analysis concludes that there is no significant difference between the ATP readings obtained from before treatment either with citric acid or sodium hypochlorite. This means that both citric acid and sodium hypochlorite have similar load of contaminants before treatment.

Meanwhile, citric acid's cleaning power could not be evaluated with the method used for this research. This was unanticipated and only uncovered upon further research. During the experiment, the ATP readings after treatment with citric acid show either 0 or 1 RLU at the most. This is because the incompatibility of the active cleaning agent chosen – citric acid – with the enzyme luciferin-luciferase found in the Hygiena UltraSnap (Mubiru, Coyne, & Grove, 2008). Citric acid caused a shift in the pH which should be balanced by luciferase, a buffer (Mubiru, Coyne, & Grove, 2008). This alteration to the optimum pH at 7.8 is causing a weaker emission to be detected by the monitoring system (Mubiru, Coyne, & Grove, 2008). Likewise, the hydrogen bonding of ATP by citric acid is quenching the light output, resulting in a lower reading and underestimation of ATP value (Mubiru, Coyne, & Grove, 2008). Hence, ATP bioluminescence assay cannot be used to measure the effectiveness of citric acid as cleaning agent. Additionally, citric acid used is not specifically for cleaning purpose but rather a food grade citric acid. Whether this modification affects the cleaning power of citric acid is unknown and shall be evaluated in the future.

## Limitations

Several limitations were present in this research project. First, due to budget restriction, not a lot of replications can be obtained as only a limited amount of Hygiena UltraSnap swabs were available. The small sample size increases the likelihood of type II error occurrence. Type II error means Ho is not rejected when Ha is true. Combined with the limited financial resource, only one concentration of sodium hypochlorite and citric acid is investigated. As a result, the most effective cleaning concentration for both sodium hypochlorite and citric acid could not be evaluated. Moreover, ATP bioluminescence assay is employed due to its rapidity which is an important trait considering narrow window of time. Unfortunately, the citric acid cleaning power could not be evaluated due to the incompatibility between citric acid and the enzyme present in the Hygiena UltraSnap.

As well, ATP bioluminescence assay is only an indicator of cleanliness which may or may not directly relates to the likelihood of foodborne illness occurrence, the main concern of public health. An alternative such as Aerobic Plate Count (APC) may be used to enumerate surviving microbes on the surface.

## Knowledge Translation

Though the use of sodium hypochlorite is effective at significantly reducing surface contamination, it does not necessarily mean that the surface is clean. Most people assume that spraying cleaners and wiping are enough to clean a surface, not realizing that without following the manufacturer's guide it might not achieve its intended purpose. Environmental hygiene monitoring is important to ensure the effectiveness of cleaning program in place. Presence of organic materials and microorganisms, left overtime may result in the formation of biofilm. When biofilm is present, higher concentration or longer sitting time may be required to clean the surface.

Publications for the media regarding the correct way to utilize surface cleaners can be broadcasted in the form of ads found in social media. The purpose of this action is to increase awareness, enabling the public to optimize the efficacy of cleaners.

## Future Research

The author recommends future research to explore:

- The effect of temperature on the cleaning efficacy of sodium hypochlorite
- Larger sample size obtained and various type of cutting board or food contact surfaces used, such as marble, metal, and wooden
- Utilizing Aerobic Plate Count (APC) to compare cleaning efficacy between sodium hypochlorite and citric acid

## Conclusions

The statistical analysis of the before and after treatment with sodium hypochlorite show that there is a significant decrease of contamination. However, although there is a significant decrease, the average number of RLU reading is still considered a failure according to Hygiena's standard. Therefore, steps such as following manufacturer's guide and removing physical contaminants before applying cleaner are necessary to properly clean a surface. The results on citric acid cleaning efficacy are inconclusive due to the incompatibility between citric acid and the enzyme found in Hygiena UltraSnap. Another method needs to be used to measure the cleaning power of citric acid. Nevertheless, the ATP readings for both the citric acid and bleach before treatment are not significantly different from each other, which means the method developed has successfully eliminated one confounding factor, the difference in initial load.

## Acknowledgements

The author would like to acknowledge Frederick Shaw, British Columbia Institute of Technology (BCIT) Laboratory Manager for the technical assistance given during the research project. The author would also like to acknowledge Dale Chen, BCIT Environmental Health instructor for patiently guiding the author to finish the research project.

## Competing Interest

The author declares that there is no competing interest for this research.

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