The Effects of Diffraction Grating Density of Ultraviolet Light: Impacts on the Reduction of Mesophilic Bacteria in Processed Mozzarella Cheese

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Abstract

Harmful pathogens, such as *E.coli* (a type of mesophilic bacteria) in processed cheese is a safety risk for the dairy industry. UV light is an effective tool that is used to sterilize dairy products, but is underexplored. Paired with diffraction grating, the intensity maxima of UV light is expected to increase, further reducing bacteria in cheese. Therefore, it was hypothesized that an increase in diffraction grating density results in an increase in mesophilic bacterial reduction in processed mozzarella cheese. The number of slits on the diffraction grating surface was increased to enhance the intensity maxima of UV light. Cheese samples were treated with UV light and then analyzed using the total viable count (TVC) method. The samples were then analyzed through the ANOVA (Analysis of Variance) test to measure the significance and reliability of the results. However, due to financial limitations and low slit range (50-600), insufficient evidence failed to reject the null. Despite failing to conclusively affirm or refute the hypothesis, collected data proved that UV light does have an effect, but the effect of diffraction grating density requires larger slit range, as the effects were potentially too small to produce visible effect. The study also suggests that the relationship between diffraction density and bacterial reduction may be weaker than previously assumed. Moreover, suitable equipment should be used in future experiments to ensure complete sterilization, homogenization, and use larger ranges of slits and dilution samples. Nonetheless, UV light should continue to be studied as a viable method of sterilization for pasteurized products, to ensure safety and prevent future foodborne outbreaks globally.

1. Introduction

One quarter of the world's food shortage has been attributable to microbial contamination: an ethical and economical problem worldwide (Losito et al., 2014). *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella spp*, are alarming pathogens within the dairy industry. Approximately 1,600 hospitalizations and 260 deaths have been reported every year in the United States due to listeria outbreaks (Kim et al., 2015). Other pathogens, such as *E.coli,* have caused multiple outbreaks and foodborne illnesses. Diarrheagenic *E. coli* (DEC) pathotypes, including enteropathogenic (EPEC) and Shiga toxin-producing (STEC) *E. coli,* have been a main health concern. The prevalence of these pathotypes was documented in mozzarella cheese microbiota and other cheese variants. Notably, the presence of *E.coli* can signal favourable conditions for enteropathogens, including salmonella and shigella (Ribeiro-Júnior et al., 2025).

According to the Food and Drug Administration (FDA), milk used to manufacture cheese must be pasteurized for at least 15 seconds at 72°C to destroy pathogenic or spoilage bacteria. Nonetheless, spoilage and heat-resistant bacteria may still exist in raw milk or equipment surfaces through cheese manufacture and storage. One of the pathogens produced by *E.coli,* stx shiga toxin, can withstand milk pasteurization and other equivalents (Ali & Elsherif, 2015). Other *E.coli* pathotypes (DEC), exhibit the same pasteurization-resistant trait, as multiple pathogens were reported in cheese and dairy products after pasteurization (Ribeiro-Júnior et al., 2025).

Within recent years, attempts have been made to sterilize dairy products, such as cheese, through technology. Based on a quantitative study examining the effect of UV light on cheese, Kim et al. (2015) found that the use of UV-LEDs to decontaminate foodborne pathogens on cheese has been effective and provides a new innovative insight to the dairy industry. Other studies agree, stating that UV light treatment has decelerated mold growth in cheese, resulting in a potentially efficacious impact on shelf life (Urgu-Ozturk, 2022). According to a survey involving industry professionals, governments, and academics, the team identified that UV radiation was noted as of higher quality (94% of participants agreed), safer (92%) and increased shelf life (91%) (Delorme et al., 2020). In sum, these sources conclude that UV light treatment on cheese had positive impacts on dairy products.

Moreover, the studies regarding UV light and cheese have similar results. Keklik et al. (2022) found that after pulsed UV treatment, the reductions ranged from 1.31 to 2.20 log cfu/cm^2 for *E.coli* and *S.aurus*. Likewise, Proulx et al. (2015) discovered that pulsed-light treatment reduced bacterial contaminates of less than 2 log reductions.

Nevertheless, Koca et al. (2018) describes how UV treatment on dairy products can decrease nutritional value, since dairy products are light sensitive. Koca et al. (2018) explains how treated UV dairy products produce a burnt odor, due to photodegradation, resulting in off-flavours. In essence, Koca et al. (2018) introduces the negative impacts of UV light treatment, disagreeing with previous sources.

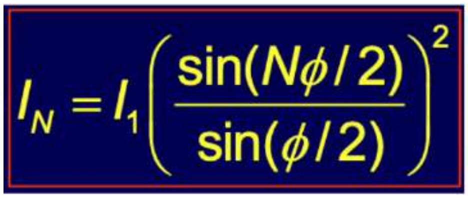
The majority of all bacterial reduction studies have measured total viable count (TVC) in dairy products. This method is commonly used to test the microbial quality of the cheese through the plate count method. Sulieman et al. (2013) and Ganesan et al. (2012) took samples of mozzarella cheese and carried out the plate count method by incubating agar plates mixed with media (cheese and peptone water) and counting the colonies present in the dish (Sulieman et al., 2013).

There are also other ways to measure bacteria, such as using a spectrophotometer. The method consists of light scattering (where light hits a cell), to measure cell density. However, the method cannot distinguish the difference between alive and dead cells (O’Connor, 2025).

Therefore, in order to measure bacterial reduction, the total viable count must be used.

The intensity of UV light plays a crucial role in exterminating bacteria in cheese. According to a study investigating the effectiveness of UV-C light for inactivating bacteria, Tirono (2023) found that treatment with higher intensity resulted in a large reduction of bacteria. Similarly, another study described that the relationship between the intensity of light and efficacy for reducing bacteria (such as *E.coli* and *S.aurus*), was a linear regression (Liu et al., 2024).

By utilizing the intensity of UV light, bacterial reduction can be maximized to ensure safety, and in theory, can be done through diffraction grating. As light shines through a diffraction grating surface, the result is an interference pattern, which redistributes light by separating it into many closely spaced slits, producing a sharper and narrower intensity maxima. Diffraction grating favors the ultraviolet region, where it reflects best. It does not alter the total power of the light source, rather it concentrates light in specific areas (Hecht, 2016), potentially increasing UV exposure. A diffraction grating surface is similar to a surface of a CD, with many parallel lines (Lasky, 2022). Based on lecture from the University of Illinois Urbana-Champaign, the formula that represents this is (Kwait, 2012):



$$I_N = I_1\left(\frac{\sin(N\phi/2)}{\sin(\phi/2)}\right)^2$$

The formula represented is the N-slit interference formula. It relates the intensity maxima of light (I(N)) to the number of slits (N) on a diffraction grating surface. It also demonstrates a proportional relationship between the intensity maxima of light and the number of slits: as the number of slits increases, the intensity maxima increases, as light is focused towards the center. Optical theory implies that the peak intensity of interference maxima is directly proportional to N^2, representing a distribution of energy that can be optimized to maximize safety.

This formula suggests an interesting relationship between the number of slits and how it can be manipulated to reduce bacteria. None of the previous research has examined this pattern; therefore, this study will focus on the specific gap: how UV light and diffraction grating can be applied for the reduction of bacteria. This leads to the question: “What is the relationship between the diffraction line grating density of ultraviolet (UV) light and the reduction of mesophilic bacteria in processed mozzarella cheese?”

Processed mozzarella cheese was chosen because it is underexplored. Hard cheeses require hazardous UV pulsed lasers to penetrate through the cheese. On the other hand, UV treatment on soft cheeses degrades the quality of the cheese. Because it requires lactic acid bacteria (LAB) through the fermentation process, it is crucial for developing flavour and nutritional value (Coelho et al., 2022). Exploring processed mozzarella cheese would shed light on the effects of UV sterilization on different cheese variants. This gap was addressed in “Possibilities of using the continuous type of UV light on the surface of lor (whey) cheese: impacts on mould growth, oxidative stability, sensory and colour attributes during storage”, where the author noted “Further studies are required for different dairy products” (Urgu-Ozturk, 2022). Various studies investigating UV light and bacterial reduction appear to focus on fresh and traditional cheese (Keklik et al., 2022), while affordable cheeses, such as processed mozzarella, are underexplored. These cheeses are more likely to be consumed by a larger community, compared to the expensive cheese. Therefore, processed mozzarella cheese was chosen as food safety should be ensured for everyone.

Mesophilic bacteria was chosen because *E.coli* is predominantly mesophilic. Mesophiles are microorganisms that thrive between 20°C to 40°C and are found in cheese and yogurt. Pathogens such as *E.coli, L.monocytogenes,* and *S. spp* belong to the mesophilic group (Shrestha, 2024).

My hypothesis is that as the number of slits increases, the bacterial reduction in processed mozzarella cheese increases. Due to the proportional relationship represented by the formula above, as the number of slits increases, the intensity maxima of light increases. As UV intensity increases, the bacterial reduction should also increase, based on the findings previously mentioned.

The importance of this study is how it can be used for the betterment of the future. By intertwining physics with food science, new ways to improve food safety and quality can be found. As a result, these improvements can prevent foodborne bacterial disease outbreaks, decelerate mold growth, increase shelf life, and improve food security globally.

2. Materials and Method

2.1. Preparation

To treat the cheese using UV light, a UVA light source (365nm) was used. Four 3D printed stands were used: UVA light, pinhole, diffraction surface, and cheese stand, designed on Shapr3D. The pinhole was created to enhance the coherence of the light. The UVA flashlight was placed on the UV stand, facing the pinhole, 0.6cm apart. The diffraction grating surface was inserted into the surface holder, 2.8cm from the pinhole. The surface was 9cm away from the cheese stand (see Figure 1). The UV light stand and pinhole was lifted 1.5cm and 2cm respectively. These distances and heights were selected to guarantee that the light passed through the pinhole and diffraction grating surface. The entire setup was placed inside a cardboard box with fluorescent paper, ensuring no light interfered with the experiment, while the fluorescent paper was used to visualize the UV light (see Figure 2). Additionally, the experiment was done in a dark room.

Figure 1

Experiment Design Set Up (Side View)

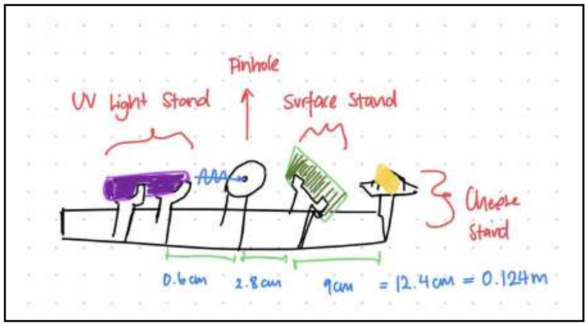
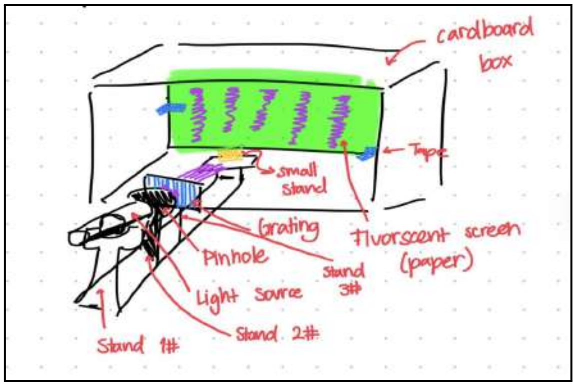


Figure 2

Experiment Design Set Up (Front View)



To ensure lab safety, UV safe goggles, gloves and a lab coat were worn at all times. The experiment required 15 mozzarella cheese samples (Caroline Mozzarella Cheese 200g), each 10g. The sample size was 3.5cm x 3.5cm and the cheese was 2mm thick. Before UV treatment, the cheese was stored in the school laboratory fridge at 1°C. The cheese was wrapped in parchment paper, inside a plastic container, and sealed inside a plastic bag to avoid cross contamination.

To conduct the bacterial analysis, peptone water and nutrient agar plates were prepared. These were prepared according to the instructions on the nutrient agar and peptone water bottles from HiMedia (reproduced below). Since autoclaves were not available, microwave sterilization and boiling were used instead. Therefore, the following methods were altered to fit the available materials at the laboratory. These sterilization methods were commonly used in the field, according to my expert advisor. Further, microwave sterilization was approved by ISO 11133-2 (International Organization for Standardization for microbiology of food) (Chongfuengprinya et al., 2021), making it suitable for sterilization.

Nutrient Agar Plates

1. 28g of nutrient agar powder was dissolved in 1000mL of deionized water in a 1000mL beaker (with enough space above the 1000mL mark to prevent overflowing). The solution was mixed using a glass mixing rod.
2. After the solution reached uniform concentration, 250mL of the solution was microwaved at medium high for two minutes. As the solution started bubbling, the microwave was turned off and the solution was set aside using gloves.
3. Sterilized petri dishes were placed on a weighing scale. The solution was constantly mixed with a stirring rod to ensure that the agar did not solidify. This process was done until the mixture was cooled.
4. 35g of the solution was poured onto each petri dish, covering half of the plate. The petri dish was covered with its corresponding lid, then set aside to solidify.
5. After solidification, the plates were turned upside down and placed in the laboratory fridge at 1°C. The plates were sealed inside one large plastic bag and labeled to avoid cross contamination.

Peptone Water

1. 7.5g of peptone powder was dissolved in 500mL of deionized water in a 1000mL beaker. The solution was mixed using a glass mixing rod.
2. After the solution was fully mixed, the solution was poured into a pot and heated until the solution started boiling.
3. Then, the pot was taken off the heat and set aside to cool before pouring it into a glass laboratory bottle (500mL) using a funnel. The bottle was kept in the fridge at 1°C.

The peptone water and nutrient agar plates were stored in a refrigerator at the laboratory preparation room, to which only informed teachers and lab technicians had access to. To ensure no unintended interactions with these chemicals, the plates were sealed in one large bag, and both the bottle and bag were labeled.

2.2. Treatment Time Calculation

The treatment time was calculated using two formulas: the intensity of light and the microbial inactivation dose formula. Instead of using UV meters, due to their high expense, to support the inactivation time calculation, two formulas were used. The equation was rearranged and combined to find the treatment time. I is noted for intensity (W/m^2), P for power (W), t for time (s), and D for dosage (J/m^2). A is for area (m^2) and is equal to $4\pi r^2$, where r refers to the distance that the light travels in meters, which is 0.124m from the UV source to the cheese. The intensity of light calculation represents an estimation of UV intensity, as it assumes isotropic emission of light (radiation of light uniformly in all directions) (UNSW Sydney, n.d.), because UV flashlight emits directional light.

Intensity of Light Formula

$$I = P/A$$
$$A = 4\pi r^2$$

Microbial Inactivation

$$D = I \times t$$

By substituting the intensity in the dosage equation, and rearranging for time, the final equation was:

$$t = D4\pi r^2/P$$

In "UV Light Application as a Mean for Disinfection Applied in the Dairy Industry", Chawla et al. (2021) states that the doses used for processed cheese ranges from 1.02 to 12.29J/cm^2, and reduces 3.37 to 5.41 log cfu. Due to time constraints with expert advisor supervision and to ensure that the bacteria could still be visibly counted, 0.34J/cm^2 was used, aiming to reduce 1.123 log cfu (one third of 1.02J/cm^2 reduction). This translates to 3,400J/m^2.

By substituting the dose, distance, and power of the UV light, the time translates to 218.983s, approximately 3 minutes and 38.94 seconds.

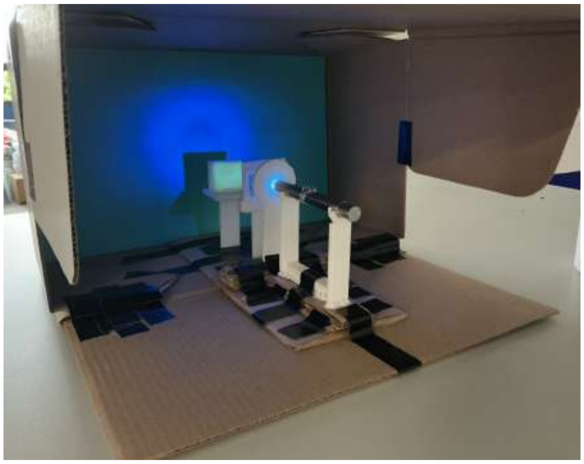
$$\frac{3400 \times 4\pi(0.124)^2}{3} = 218.983s$$

2.3. UV Treatment Method

According to studies published by the *Applied and Environmental Microbiology* and *Journal of Dairy Research*, UV light on cheese was conducted at room temperature (Kim et al., 2015; Urgu-Ozturk, 2022); therefore, the UV treatment was completed at room temperature. The experiment was done in a dark room, to ensure that no light interference disrupted the treatment.

To begin the UV treatment, the UV light, diffraction surface, and cheese sample were placed onto their respective stands (see Figure 3). The cheese sample was handled using tongs. The UV light source was turned on and treated the cheese for 3 minutes and 39 seconds. Then, the cheese was taken out and placed into a labeled container until analysis. After three trials were completed, the cheese was immediately analyzed using the plate count method. The experiment was then repeated for the remaining three slits. There were four types of diffraction grating surfaces: 50, 100, 300 and 600 slits. Each slit had three trials, which required three cheeses each: a total of 12 cheese samples. The remaining three samples were the untreated samples, used to analyze the change in bacterial reduction.

Figure 3
Photo of UV Light Treatment



To maintain safety throughout the experiment, gloves, laboratory coats, and goggles were worn at all times.

2.4. Microbiology Test: Plate Count Method

The procedure was based on the microbiology procedures from Keklik et al. (2022), Laila et al. (2025), and Ruiz et al. (2025) and modified to suit the experiment. In the studies by Keklik et al. (2022) and Ruiz et al. (2025), the cheese samples (10g) mixed with peptone water (90mL and 99mL, respectively) were homogenized using a stomacher, resulting in a 1:10 ratio of cheese and peptone water. However, a vortex was used instead, due to budget constraints. Then, serial dilutions were carried out for six different dilutions, by taking 1mL of the previous solution to 9mL of peptone water (Ruiz et al., 2025). However, only three dilutions were used in this experiment because the incubator available could not fit more than three dilutions for three trials (nine plates). Then, 100μL of the mixture was inoculated and spread evenly on the nutrient agar plates (Laila et al., 2025). Lastly, the plates were incubated at 37°C for 48 hours. For mesophilic aerobes, nutrient agar was selected as the growth medium for this study (Ruiz et al., 2025).

<u>Plate Count Method</u>

1. Using a strainer and a small plastic cup, the cheese sample was strained into a 150mL beaker.
2. 75mL of peptone water was poured into a 200mL erlenmeyer flask using a funnel. Then, using a 25mL pipette, an additional 15mL of peptone water was added to the flask. The 10g strained cheese was added to the flask, creating a 1:10 ratio (10g cheese, 90mL peptone water).
3. The flask was sealed with a stopper and homogenized using a vortex for two minutes at medium speed.
4. After homogenization, serial dilutions were performed using peptone water in test tubes: taking 1mL of previous solution and adding it to 9mL of peptone water using a pipette. Three tubes of 9mL peptone water were prepared for three dilutions (10^{-1}, 10^{-2}, 10^{-3}).
5. Using a 20μL micropipette, 20μL of the dilution solution was pipetted on a nutrient agar plate five times (100μL).
6. An inoculation loop was sterilized using an alcohol burner by waving it over the flame for three seconds. The alcohol burner was

activated by dropping a few drops of alcohol into the rope of the burner and was torched using a lighter. The inoculation loop was used to spread the solution over the agar in straight lines across the plate.

7. Then, the plates were labeled and covered with a lid. The plates were placed upside down and incubated at 37°C for 48 hours.
8. After incubation, the plates were taken out and counted for CFUs (colony forming units).

Based on the images from various studies (Laila et al., 2025), colonies are usually distinct, circular, and in uniform shape. Therefore, these CFUs are identified using the following criteria:

- Round, uniform shape
- A distinct colony

To ensure safety, my expert advisor was present for the entire experiment.

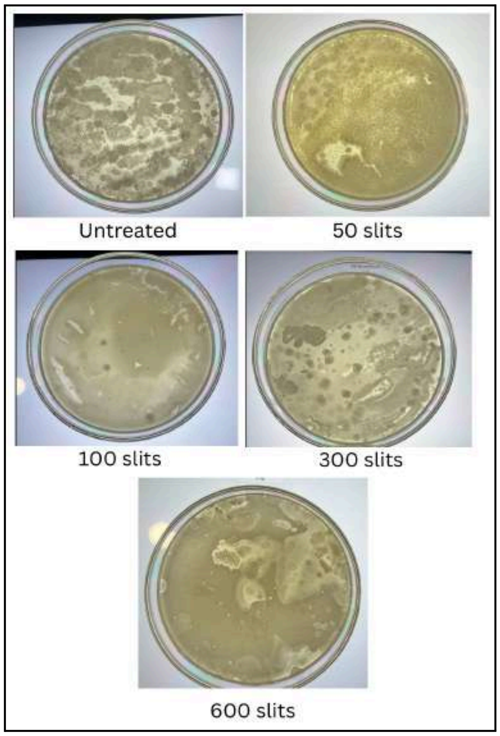
Every plate was documented and photographed. CFUs (colony forming units) are estimates of alive bacteria. Each photo was annotated and recounted for further accuracy.

Most of the untreated samples are denoted as TNTC (Too Numerous To Count), implying that the samples contained over 300 CFUs (refer to Table 1 and Appendix A).

Below (Figure 4) is an example of the colonies (unannotated) from each slit. The untreated sample is an example of an overcrowded plate (TNTC).

Figure 4

Photo of Plate Colonies



3. Data Analysis

3.1. CFU Calculations

After all the plates were counted, the colonies were calculated to CFU/mL because dilutions were used. Dilutions are used to decrease the amount of CFUs that form on a plate, thus creating a plate that is clearer to count. Accordingly, smaller dilutions (such as 10^{-2} and 10^{-3}) are bound to have less CFUs on plates. The following formula is used to calculate the CFU/mL, which allows the dilutions to be calculated.

Formula for CFU/mL

$$\text{CFU/mL} = \frac{\text{Number of Colonies}}{\text{Dilution Factor} \times \text{Volume Plated (mL)}}$$

The plated volume used in the experiment was 0.1mL, however, for the 50 slit trials, the volume plated was 1mL. The material used for plating (micropipette) was unavailable and a 5mL pipette was used instead. All the other samples had a 0.1mL plated volume. Still, the calculation corrects the volume differences mathematically; the CFU counts were standardized by converting it to CFU/mL.

After the calculations, the data was calculated by taking the log of each value. Most sources report CFUs as log; it is easier to analyze because the CFU/mL usually results in a value multiplied by 10^x, which creates room for errors. The data was averaged within each trial (dilutions) and then averaged across all trials.

Table 1
Processed Data Table: log CFU/mL Calculation Within Trials

Cheese Samples	Average CFUs in Trials (CFU/mL)		
	Trial 1	Trial 2	Trial 3
Untreated	TNTC (Invalid)		
50 Slits	5.139	4.153	3.968
100 Slits	3.150	3.876	4.784
300 Slits	5.812	5.065	4.930
600 Slits	5.055	4.143	5.491

3.2. Analysis of Variance Test (ANOVA)

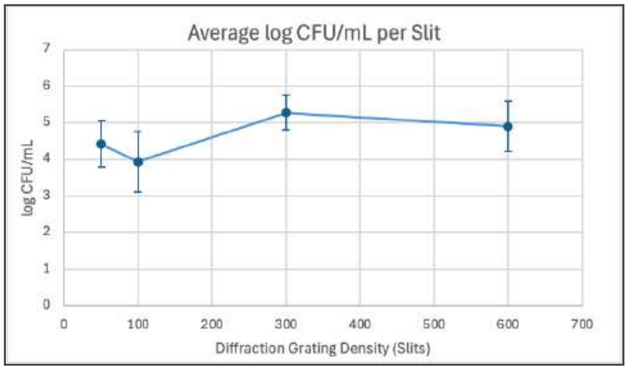
In studies investigating UV sterilization on cheese, the analysis of variance test was performed to test the effectiveness of the data (Keklik et al., 2022; Laila et al., 2025). In this experiment, the ANOVA test (single factor) was performed on Google Sheets, using the XL Miner Analysis ToolPak extension. The average log CFU of three trials for each of the slits was inputted into the program, and the p-value was determined.

It is important to point out that the untreated samples were not included in the ANOVA test as the data proved TNTC—a number cannot be assigned (>300 CFU).

3.3. CFU Analysis

A graph (Figure 5) was produced to represent the change in log CFU/mL across slits. The error bars are taken from the standard deviation of each value, given in Table 2.

Figure 5
Graph: Average log CFU/mL over Slit



4. Results

4.1. Descriptive Statistics

The primary outcome of the study was the CFU count for each group. The data used from the CFU study was analyzed through methods such as mean, standard deviation, effect size, and test statistic to understand the reliability between the differences in data. The mean and standard deviation can be found in the table below (Table

2). The mean was obtained from the ANOVA test, whereas the standard deviation was calculated using the standard deviation function on Google Sheets.

Table 2

Processed Data Table: Mean and Standard Deviation of Treatment Groups

	Mean (log CFU/mL)	Standard Deviation
50 Slits	4.420	0.6295
100 Slits	3.937	0.8187
300 Slits	5.269	0.4751
600 Slits	4.897	0.6879

4.2. ANOVA Results

Based on the ANOVA test (see Appendix B), the reported p-value was 0.1568680459. The effect size was calculated using the results from the ANOVA test: SS between was divided by SS total to find the effect size. The effect size was $\eta^2=0.46037262215$. The test statistic was also evaluated from the ANOVA test, the F value (2.275014901).

5. Discussion

5.1. Summary of Key Findings

Based on the proportionality between the intensity maxima of light and the number of slits on a diffraction grating surface, it was hypothesized that by increasing the amount of slits, the amount of mesophilic bacteria reduced on mozzarella cheese would increase.

Initially, in Figure 5, the data shows a downward trend in log CFU/mL from 50 to 100 slits. However, from 100 to 300 slits, there was an increase in CFU. From 300 to 600 slits, the CFU value decreased. Even though the trend is inconsistent, UV light creates an impact nonetheless. In Table 1, the untreated samples were mostly denoted as TNTC, indicating a CFU count of over 300 colonies, which was consistent over the three trials. UV light reduced the bacteria in the samples: the untreated samples were overcrowded with CFUs, whereas when treatment was applied, the plates became countable and measurable.

5.2. Interpretation of Findings

The p-value, effect size, and test statistic were used to analyze the reliability of these results. According to the ANOVA test, the p-value was 0.156868045, thus failing to reject the null.

The null hypothesis assumes that a relationship/effect does not exist. A result rejecting the null suggests that an effect exists. Conversely, a result failing to reject the null implies that there was not enough evidence to suggest an effect. Because the p-value was statistically large (>0.05), the null hypothesis fails to be rejected.

Next is effect size, which measures the magnitude of the differences. Effect size is independent of sampling size and indicates that the effect can be meaningful. Statistical significance (the p-value) only indicates that the effects exist in the study and is dependent on sample size. Therefore, the two must be used to determine the reliability of the experiment. The value is $\eta^2=0.46037262215$. The large magnitude of the value (>0.14) generally indicates that the difference in values is substantial and practically significant.

Lastly, test statistic (F-statistic) was used to determine if the null hypothesis should be rejected. Based on the ANOVA test, the F value equals 2.275014901, whereas the F crit is 4.066180557. In general, if the F value is larger than F crit, the null hypothesis can be rejected. In this case, the F crit value is greater than the F value, failing to reject the null.

5.2.1. Interpretation of Hypothesis

Both the p-value and test statistic fail to reject the null, whereas the effect size indicates a significant difference in data that could be meaningful in real world circumstances. In essence, this study has failed to reject the null hypothesis. Although the study had insufficient data to support the hypothesis, the effect size could be potentially meaningful as it suggests a difference in the control groups. Yet, this difference could also be due to chance, as suggested by the test statistic and p-value. Nonetheless, the study did not directly affirm or refute the hypothesis, as there was insufficient evidence to do so.

5.3. Comparison with Literature

The effect size suggests that the trend between the number of slits and the bacterial reduction could be significant. In another study, the observed trend was found. Kim et al. (2015) found that UV-LED lights are more efficacious compared to UV lamps at reducing bacteria on pasteurized cheese, as LED lights lose less intensity compared to UV lamps. In fact, 99.99% of pathogens were inactivated and quality was not affected. When the team applied UV sources of higher intensity, it was found to be more effective at reducing bacteria.

Despite the relationship between the number of slits and the effect on bacterial reduction being inconclusive, this study had similar results to other studies: that UV light does indeed have an effect on bacterial reduction. All the plates for the untreated samples were too numerous in CFUs, uncountable, but the treated samples were countable. UV light did have an effect on the cheese, but the effect of the slit density remains weak. Studies with such trend were found in “Pulsed-light inactivation of pathogenic and spoilage bacteria on cheese surface”, “Surface decontamination of white cheese by pulsed UV treatment”, and “Ultraviolet Light Applications in Dairy Processing”, where UV light treatment on cheese has been found to be effective (Proulx et al., 2015, Keklik et al., 2022, Koca et al., 2018).

5.4. Limitations of the Study

Though there were financial limitations, available resources were leveraged to allow accessibility for technology advancement among communities that need the research. Hence, analysis can be produced in a cost-effective way, within budget constraints.

According to the microbiology tests done by experts in the field, the cheese and peptone solution are homogenized in a stomacher to reach uniform concentration (Keklik et al., 2022). In this study, a vortex was used instead and as a result, this created a semi-homogenized solution, with some visible particles of cheese floating in the peptone solution. The fluctuations in data could have been attributable to the partial homogenized solution. In addition, the sterilization methods also contributed to data fluctuations. The laboratory does not have an autoclave; therefore, other sterilization methods were employed. The sterilization method for nutrient agar was microwave sterilization and boiling for peptone water. These sterilization methods differ from using an autoclave, which is standard in microbiology tests. Additionally, more dilutions could contribute to easier and more clearer CFU counting. Of all three dilutions and trials, untreated samples were noted as TNTC, and could not be used for statistical analysis. The incubator could not fit more than three dilutions. In consequence, the data fluctuations could be due to inaccessibility of advanced equipment.

A low slit range (50-600) could have limited the bacterial effect of UV light, causing potential insignificances in trends. A clearer trend could potentially be achieved by using a wider range of slits. Initially, this slit range was not considered low, as small changes in the number of slits can influence light redistribution (University of Tennessee Knoxville, n.d.). In addition to a low slit range, UVA light (365nm) was used to treat the cheese because of safety concerns, despite studies in the field typically using UVC wavelengths (254nm). Compared to UVA radiation, UVC wavelengths are more effective for inactivating bacteria; this can explain the absence of observable bacterial reduction (Koca et al., 2018).

For future studies, stomachers and autoclaves or a suitable equivalent, should be employed. Furthermore, larger incubators should be used to increase the number of dilutions, increasing clarity. It is also important to consider the size of the study. Clear changes between small slit ranges may be imperceptible, as the study only focused on 50-600 slits. This could have potentially limited the effect between the different slits, resulting in an unclear pattern between bacterial reduction and diffraction density.

5.5. Significance and Future Implications

This study suggests that the relationship between diffraction grating density and bacterial reduction could be weaker than previously assumed, as a clear and definite relationship between the two was not achieved. The study only focused on four different slit densities, hence, a stable relationship was not established. While the data was insufficient to affirm or refute the hypothesis, UV treatment does have an effect on bacteria in mozzarella cheese, and should be utilized to ensure food safety. Pathogens such as *E.coli, L.monocytogenes,* and *S. spp* are main health concerns that must be addressed. To prevent pathogen outbreaks, UV light should be used to minimize environmental contamination and should be applied to the manufacturing environment to ensure safer products. Sterilization of dairy products can help improve food security through increased product life and lower risk of microbial contamination, preventing food and economical losses, while creating access to safe food for people across the globe.

Future studies regarding UV light diffraction density should use a wider range of diffraction grating slits for potentially clearer and stable results of the patterns between diffraction grating density and bacterial reduction. Future research may focus on increased dilutions and complete sterilization and homogenization of chemicals through standard tools. Then, the clarity of the study can be improved. Studies in this field could also investigate different food products to understand the role of diffraction grating and UV sterilization, advancing the safety of a broader range of food groups accessible to different communities.

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Appendix A

Raw Data Table 1: Counted CFU (from photos)

Cheese Samples	CFU count								
	Trial 1			Trial 2			Trial 3		
Untreated Sample	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
	TNTC	54	TNTC	TNTC	TNTC	78	TNTC	TNTC	TNTC
50 slits	TNTC	TNTC	138	TNTC	107	19	144	TNTC	60
100 slits	5	4	TNTC	27	21	TNTC	49	7	66
300 slits	TNTC	0	65	TNTC	26	52	59	77	136
600 slits	92	169	95	8	8	42	TNTC	TNTC	31

Appendix B

ANOVA Summary: Single Factor

ANOVA						
Source of Variatio	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.012193667	3	1.004064556	2.275014901	0.1568680459	4.066180557
Within Groups	3.530753333	8	0.4413441667			
Total	6.542947	11				