SALMONELLA INACTIVATION METHODS IN BLACK PEPPER: THROUGH THE LENSES OF PREDICTIVE MODELING, SYSTEMATIC REVIEW, AND META-ANALYSIS

by

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(Under the Direction of Abhinav Mishra)

ABSTRACT

Black pepper is the most commonly used spice in the world. Unfortunately, black pepper is frequently contaminated with *Salmonella* during farming or processing. With spices being the vector for several foodborne outbreaks over the past two decades, it is crucial to ensure that inactivation methods are effective and well-understood. Therefore, we conducted a systematic review and meta-analysis of available literature that provides data for *Salmonella* survival when facing different inactivation treatments. We also developed secondary predictive models when sufficient survival data were available. Water activity, temperature, and the sample matrix were shown to have a strong influence on the outcome of most treatments, but in many cases, their impact has not been thoroughly investigated. Relative humidity (RH) was a relevant predictor for chlorine dioxide-based (ClO_2) treatments, but concentration was not. This work provides critical information for optimizing existing methods, developing new technologies, and formulating risk management strategies.

Keywords: Black Pepper, Peppercorn, *Salmonella*, Inactivation, Systematic Review, Meta-Analysis, Predictive Model

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DEDICATION

I would like to dedicate this to the Lord Almighty and my wonderful mother, Andrea Csuti, who has been an immense blessing and incredible friend through the good and the difficult.

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CHAPTER 1- INTRODUCTION

Black pepper is the most widely consumed spice in the world and can be used as a seasoning in both home cooking and food processing. However, black pepper is very frequently contaminated with *Salmonella*, which can cause serious foodborne illnesses. There have been several *Salmonella* outbreaks since 2000 where the identified vector was a spice. *Salmonella* can survive within black pepper and can even grow in the spice, as observed in a study by Xie et al. (2022). While dangerous when found in packaged seasoning, adding *Salmonella* to a processed food can cause the bacterium to grow if proper precautions are not taken. Therefore, ensuring that *Salmonella* is inactivated within black pepper is crucial to maintaining the safety of the foods to which it is added.

However, there are currently many available methods for inactivating *Salmonella*, and to date, no study has systematically reviewed and compared the overall effects of these treatments. A combination of predictive modeling and meta-analysis can show us how different inactivation treatments compare in terms of Decimal Reduction time (D–value), identify factors that influence how *Salmonella* survives these treatments, and pinpoint possible research and knowledge gaps regarding factors and potential confounding variables when designing and evaluating effective treatments.

A meta-analysis is a method of synthesizing and interpreting data where results from multiple studies are combined through statistical modeling. These results can be used to identify new patterns within study results, understand challenges in experimental design, and maximize the statistical power in estimating the effect size of an intervention (Gonzales-Barron & Butler, 2011).

While meta-analyses have long had a presence in the domains of public health and the social sciences, their application in food science has been relatively new.

After identifying methods and critical factors that may influence treatment efficacy, we decided to develop predictive models for methods where sufficient data was available to have statistically viable predictive models. It is well known that several factors can influence the effectiveness of inactivation methods, including temperature, water activity, and humidity. This systematic review and meta-analysis can provide valuable information for future researchers and policymakers to inform risk assessment and risk management strategies. The predictive models generated in this study can also be used to identify key factors when predicting *Salmonella* inactivation from available interventions. While *Salmonella* poses a significant risk in black pepper, a well-informed risk management program can effectively minimize the probability of a serious outbreak.

For this study, we examined various inactivation methods for controlling *Salmonella* in black pepper. We also analyzed *Salmonella* inactivation within infected black pepper samples with no treatment. We hypothesized that the overall effect of interventions would be a significant reduction in *Salmonella* and that these effects would be influenced by environmental factors, as well as the characteristics of the treatment process and the black pepper matrix. Our second hypothesis was that *Salmonella* populations would decline within infected black pepper samples, as black pepper is a low-moisture environment with innate natural antimicrobial properties. While evaluating different methods for inactivating *Salmonella* in black pepper, we developed secondary inactivation models for methods with sufficient data. A suitable method requires at least 10 experiments, allowing for one covariate to be added for every 10 independent experiments. These were analyzed for goodness of fit. Our third hypothesis postulated that a predictive model could

be developed for microbial inactivation with sufficient goodness of fit after taking relevant factors into account.

Therefore, we had two objectives. For the systematic review, we collected and sorted through all available literature in three scientific databases (ScienceDirect, PubMed, and Web of Science). Papers with relevant data were selected, and data were extracted for statistical analysis. A meta-analysis of available data was conducted using a random effects model. We compared effect sizes and heterogeneity between studies. We evaluated our findings to determine if any significant variables explain the variance in the experiment results.

We developed primary models for all experiments (2-phase linear model with tail). We also developed secondary models for methods where sufficient data and at least one suitable covariate were available, using the "one-in-ten" rule (Chowdhury & Turin, 2020) to select suitable methods. In short, the treatment category or survival data needed at least one suitable covariate and 10 independent experiments to qualify, with an additional predictor added for every 10 studies. We characterized the inactivation models based on goodness of fit, specifically by reporting the root mean squared error (RMSE), R-squared (R^2), and Akaike Information Criterion (AIC) of the primary models, and the residual standard error (RSE), R^2, and AIC for the secondary models, where applicable. We identified and analyzed various factors that may influence the inactivation process, discussing possible reasons for their influence, the limitations of our study and current knowledge, and possible prospects.

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CHAPTER 2- LITERATURE REVIEW

2.1 *Salmonella*– a global threat

Salmonella species are among the most significant causes of foodborne illnesses worldwide. The bacterium is responsible for ~155,000 deaths and 94 million infections annually worldwide. While extremely harmful to adults, children, the elderly, and immunocompromised individuals are much more likely to experience life-threatening illnesses from a *Salmonella* infection. Malnourished populations are also much more susceptible to *Salmonella* infection (Soltan Dallal et al., 2024). While the effect of *Salmonella* is most severe in developing countries (Soltan Dallal et al., 2024), the bacterium is one of the leading causes of death from foodborne illness in the United States; estimates suggest that over 1.2 million illnesses, 26,500 hospitalizations, and 420 deaths occur each year. Moreover, the annual cost of salmonellosis ($4.1 billion) accounts for approximately 23.6% of the total impact of foodborne illnesses on the US economy (Scallan et al., 2011; Medalla et al., 2021).

Making the problem even worse is the emergence of antimicrobial-resistant (AMR) and multi-antibiotic-resistant (MAMR) strains of *Salmonella*, which may not be readily treatable with available antibiotics. Occurrences of clinically relevant AMR *Salmonella* spp. were estimated to have increased by 40% between the 2015-2016 timeframe compared to the period between 2004-2008 (Medalla et al., 2021). MAMR *Salmonella* infections have also been observed worldwide over the last ten years (Alvarez et al., 2023; Mola et al., 2021; Patra et al., 2021; Zhang et al., 2024). This underscores the importance of making sure that *Salmonella* is controlled within food products.

2.2 A persistent foodborne pathogen

Salmonella is a genus of rod-shaped, predominantly motile, Gram-negative bacteria in the family of *Enterobacteriaceae*. They are facultative anaerobes and can quickly adapt to environments with widely significant changes in pH, a_w, temperature, and oxygen concentration. Their resilience and near ubiquity in the environment make them functionally impossible to eradicate. Therefore, proper food safety protocols must be implemented and followed to minimize the risk to human health (*Food microbiology*, 2019). Depending on various factors, such as prior exposure and available nutrients, *Salmonella* can survive in high-acid environments with a pH<3.85, including within fermented dairy products (Álvarez-Ordóñez et al., 2012). Moreover, studies have confirmed that *Salmonella* can survive at high salt concentrations (4% (w/V), as well as under temperatures as low as 2°C and as high as 54°C (D'Aoust & Maurer, 2007; Spector & Kenyon, 2012).

One of the most dangerous characteristics of *Salmonella* is its ability to survive in low-a_w conditions. Studies show that *Salmonella* spp. can survive in foods for extended periods with water activities below 0.2. Given that one of the food industry's main strategies for microbial control is desiccation, industry professionals must consider *Salmonella*'s resilience even within hostile environments (Morasi et al., 2022). The characteristics of the food matrix can also contribute to *Salmonella*'s ability to survive. A high fat content may protect *Salmonella* from the extreme pH environment within the stomach. Sugar has also been shown to protect *Salmonella* within low-a_w environments by partially replacing water within the cell membrane and stabilizing membrane proteins (Wason et al., 2021). There have been several *Salmonella* outbreaks within low-a_w food in the past two decades, with numbers increasing each year. Most of these outbreaks have been associated with foods high in sugar content, such as chocolate, almonds, and peanut

butter. However, a wide range of low-a_w foods have been linked to *Salmonella* outbreaks, from sesame products to infant formula. Improper sanitation and inactivation can also enhance the stress tolerance of *Salmonella* species. Some of the bacteria's strategies for surviving low-a_w environments are osmoregulation, dormancy, changes in membrane structure, and biofilm formation. Moreover, exposure to a stressful environment, such as a low water-activity food, can also increase thermoresistance within *Salmonella* species. Therefore, *Salmonella* can become more resilient to heat inactivation if it is exposed to a low-a_w environment before the treatment (Morasi et al., 2022).

2.3 *Salmonella* in Herbs and Spices

Herbs and spices, as well as seasoning blends, pose a challenge to *Salmonella* growth given their low-a_w and innate presence of antimicrobial phytochemicals (Sulieman et al., 2023; Zweifel & Stephan, 2012). Nevertheless, the European Union reported over 200 notifications of *Salmonella* in spice and herb imports between 2015 and 2019. Additionally, the number of notifications for *Salmonella* found in seasoning blends available on the European Market has been increasing as of 2019 (Śmiechowska et al., 2021). In the US, the CDC recorded 11 outbreaks, 81 hospitalizations, and 558 illnesses involving *Salmonella* from herbs since 2000. However, a series of 3 outbreaks between 2005 and 2011 caused 446 illnesses and 60 hospitalizations (CDC, 2022). According to the Rapid Alert System for Feed and FOOD (RASFF), the European Union has reported 352 notifications since 2020 (with one notification included from 2019) involving *Salmonella* when searching for the subject "*Salmonella*" while using the "pathogenic microorganism" and "herbs and spices" categories as filters (*RASFF WINDOW*, 2025). According to a 2022 study by the World Health Organization, *Salmonella* is one of the most significant

pathogens of concern, involved in the top 15 highest-risk scenarios during the overall risk assessment process.

Vectors for infection varied but mainly depended on water quality and proximity to the soil. Methods of inactivation also showed significantly different efficacies. While *Salmonella* may have difficulties growing in some herbs, spices, or seasoning blends, surviving cells can still grow and infect foods flavored with the infected spices. The study's findings underscore the importance of adhering to good hygienic practices, good manufacturing practices, and establishing a robust Hazard Analysis and Critical Control Points (HACCP)-based food safety plan when working with spices (FAO, 2025),

2.4 *Salmonella* in Black Pepper

Black pepper (*Piper nigrum* L.*)* is the world's most commonly and widely used spice, representing approximately 40% of the retail value generated by spices (Spence, 2024). The frequent contamination of black pepper by *Salmonella* is a significant public health concern; outbreaks in Norway, the United Kingdom, Canada, and the United States have resulted in more than 520 cases of infections, 108 hospitalizations, and two deaths. However, the worst outbreak from *Salmonella*-infected seasonings occurred in Germany, where infected paprika was added to potato chips, resulting in salmonellosis in over 1,000 people. This illustrates how contaminated spices can lead to outbreaks with serious consequences, as they are not consumed directly but are added to other foods (M. B. Vinha et al., 2025). While black pepper contains innate antimicrobial compounds, such as piperine (Butt et al., 2013; Shityakov et al., 2019), studies have shown that *Salmonella* can survive for up to one year when the black pepper is not treated (Keller et al., 2013).

Black pepper is usually harvested by hand, followed by threshing to remove the spike (Korikanthimath, 2003). While mechanical threshers are used, the traditional method —trampling

on the harvested berries via feet —is still widely used, despite significant hygiene concerns (Biju et al.; Korikanthimath, 2003). The peppers are then dried to reduce the moisture content from 67-70% to below 10%. During the drying process, the phenolic compounds within the spice are oxidized, resulting in the peppercorn's characteristic black color. However, blanching can be used before drying to speed up the process, while also accelerating color development, reducing foreign contamination, and the microbial load. The product is then cleaned and graded. Cleaning is done by hand, and then the mixture is run through an aspirator, which blows away the lighter, foreign matter, thereby separating it from the weightier peppercorns. Other elements, such as stones, metal particles, and stalks, are then separated mechanically. The peppercorns are then graded by sieving, though this can be combined with the cleaning step by including an aspirator. The product must then be stored away from moisture, heat, and light due to its hygroscopic nature, and many volatile and/or sensitive flavor compounds (Korikanthimath, 2003; Nisha et al., 2009).

Black peppercorns can then be ground, which results in a black pepper powder that is graded and sieved (Korikanthimath, 2003). Black pepper can be contaminated in all steps from harvest to processing. As seen in a study by Vinha et al. (2025), *Salmonella* was recovered from environmental samples, waste, and the product itself when sampling Brazilian farms and processing plants. The authors of the study emphasize the importance of good agricultural practices and good manufacturing practices, supported by HACCP, in protecting consumers from *Salmonella* when producing black pepper. A summary of their findings is presented Table 2.1. (Mariana Barboza Vinha et al., 2025).

As shown in Table 2.1., many of the black pepper samples contained *Salmonella,* with an average of 16.7% of packaged products testing positive for the bacterium. Black pepper accounted for 80% of notifications for *Salmonella* contamination of herbs and spices in Europe in 2021

(Mariana Barboza Vinha et al., 2025). Therefore, more information is needed to improve the safety of the world's most widely consumed spice.

2.5 Methods for inactivation– all studies

There are several methods for inactivating *Salmonella* within black pepper; conventional methods include steam sterilization, gamma irradiation, and Ethylene Oxide fumigation. The challenges with steam, the most widely used method, and gamma irradiation are the need for expensive, specialized equipment with high energy consumption, as well as the stigma among consumers associated with gamma irradiation. Ethylene Oxide fumigation has been effective but was banned in the European Union due to the toxicity of the gas (Mariana Barboza Vinha et al., 2025). However, several other methods exist, each with its own benefits and drawbacks, such as Radiofrequency heating (Jeong & Kang, 2014; Kim et al., 2012) Chlorine Dioxide fumigation (Chai et al., 2022; Wei et al., 2023), and various plasma treatments (Song, 2023; Sun et al., 2014). Moreover, many studies use combined techniques (Bang et al., 2021) or novel gases such as hydrogen peroxide (Song & Kang, 2022) to inactivate *Salmonella* within black pepper. However, to the best of our knowledge, no comprehensive overview has been published that systematically reviews and compares the results of available data. Therefore, we conducted a systematic review and meta-analysis of all published studies that provide inactivation data for different interventions designed to control *Salmonella* in black pepper.

2.6 A brief introduction to Meta-Analysis and Systematic Review

The earliest meta-analysis in food safety was conducted in 2004 to understand consumer behavior regarding food safety practices (Patil et al., 2004). The first direct application of meta-analysis to understand microbial growth in a food product was published in 2005 (Vialette et al., 2005). Ever since, meta-analytical techniques have been used to understand the effect of interventions on reducing microbial growth, beginning with Gonzales-Barron et al. (2008), who analyzed the effect of carcass chilling on *Salmonella* prevalence within pig carcasses. Some of the most recent publications include the studies by Rana et al. (2024) and Silva et al. (2021), who compared thermal interventions for killing *Salmonella* in low-moisture foods and inactivating pathogens in cheese using essential oils and lactic acid, respectively. However, very few meta-analyses have been conducted on the inactivation of pathogens within herbs and spices. Through our search, we were only able to find one such study: Arcos-Limiñana et al. (2025) explored the use of ultraviolet (UV) irradiation for a variety of spices.

2.7 Types of meta-analyses

Meta-analyses typically employ one of two types of models: fixed-effect models and random-effects models. Fixed effect models assume that there is a single, true effect size. This typically limits the model to be used only in cases where the sample comes from a single, homogeneous population (Dettori et al., 2022; Schwarzer et al., 2015). For this reason, fixed-effects models are seldom used in meta-analyses, as the data must come from multiple studies. Random effects models account for the assumption that effect estimates are more variable when considering multiple studies (Schwarzer et al., 2015).

Subgroup analysis can be helpful when comparing multiple interventions, treatment categories, or individual methods (Golden et al., 2019; Kateh et al., 2024; Leone et al., 2024).

Various measures of heterogeneity can explain the degree of variation between reported effect sizes. Two standard methods of showing heterogeneity are Cochran's Q-test, which is based on the distribution, and the I^2 index, which estimates the percent of variability that cannot be explained by chance alone. Therefore, a larger I^2 index indicates greater variability among results. Another widely used method is meta–regression, which is typically a linear or nonlinear regression model used to predict which of the analyzed study-level characteristics is responsible for the variability (West et al., 2010). Variability between studies can also be reported by calculating the τ^2 value; when closer to zero, this value indicates less variability, whereas higher values indicate more significant differences between study effect sizes (Ariel De Lima et al., 2022).

While random effects models are the most common meta-analytical models in food safety, other variations also exist. Multivariate meta-analysis can be used when there are several different ways to measure an outcome. This can be particularly helpful when different studies report outcomes on different scales. Network meta-analysis is a technique that seeks to compare different treatments to solve a single problem. This technique is widely used in medicine since it is capable of measuring direct and indirect evidence for the efficacy of an intervention (Schwarzer et al., 2015). However, we were unable to find any studies on microbial inactivation in the food industry that utilize network meta-analysis as a way of collating data.

2.8 Predictive models- a benefit for effective food safety

Predictive microbiology focuses on utilizing statistical models to describe microbial growth, survival, or inactivation within a food (Perez-Rodriguez & Valero, 2012). It assumes that microbial behavior in food- survival, growth, and death- is reproducible by controlling key factors that can be used to fit a statistical model (Łobacz et al., 2022). The basic formula of a statistical model can be seen in Equation 1:

Equation 1. General formula for a statistical model

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon$$

Where Y is the response variable, β_0 is the intercept, X_i the explanatory variables, β_i the regression coefficients, with ε being the error (Perez-Rodriguez & Valero, 2012).

The explanatory variables and regression coefficients are used to estimate a given outcome. These variables are also known as the deterministic part of a regression equation. The error is a term that explains data variability and is referred to as the stochastic part of the model. The type of model used for a dataset depends on the stochastic variable. In some cases, transformation techniques can be used to improve the model's ability to predict a likely outcome and to minimize error (Perez-Rodriguez & Valero, 2012).

Predictive models are frequently used to develop food safety plans. They may also be used to estimate a product's shelf life and to optimize the production and handling process. During risk assessment, predictive models can help identify microbial hazards from production to consumption. When developing an HACCP system for a production process, predictive models can help determine critical control points and critical limits. They can also be used to verify the effectiveness of existing HACCP protocols. However, it is important to be mindful of limitations inherent to predictive models: there is always a degree of error, models cannot be extrapolated to conditions that were not present when collecting the data used for prediction, and real-life applications frequently contain variables unaccounted for in most models (Taiwo et al., 2024).

Predictive models in microbiology are often evaluated by determining the accuracy and bias factors. Both are multiplicative factors that show the average amount of disagreement between the predicted and observed values. The bias factor can be interpreted as an average ratio of predicted and measured generation times. A perfect agreement would provide a bias factor of 1.

In a survival model, a bias factor over 1 would indicate overprediction, thereby making the model fail dangerous. In this case, bacterial inactivation may be less than what is predicted by the model, potentially leading to more surviving cells than anticipated. Fail-safe models occur when there is no danger for the model predictions to underestimate the microbial hazard. The accuracy factor predicts the spread of results within a prediction. An accuracy factor of 1 indicates perfect agreement, while larger values indicate the magnitude of spread (Ross, 1996). The equations for the bias and accuracy factors can be seen below:

Equation 2. Formula for calculating the bias factor.

$$b = 10^{(\Sigma \log\left(\frac{GT_{predicted}}{GT_{observed}}\right)/n)}$$

Equation 3. Formula for calculating the accuracy factor.

$$a = 10^{(\Sigma \frac{|\log\left(\frac{GT_{predicted}}{GT_{observed}}\right)|}{n})}$$

Where b and a correspond to the bias and accuracy factors respectively, $GT_{predicted}$ and $GT_{observed}$ refer to the predicted and observed generation times, and n refers to the number of observations (Ross, 1996).

Overall, a bias factor of less than 1 is needed, but a low bias factor can result in excessive food waste. Therefore, it is important to make sure that model predictions are reliable while the prediction error does not threaten food safety (Ross, 1996).

Given the high risk of *Salmonella* contamination from the improper handling of black pepper, it is essential to ensure that proper food safety systems are in place during processing. More than 50 studies and 155 independent experiments are available today that describe methods for inactivating *Salmonella* in black pepper while providing direct or indirect reports of log reduction. However, to the best of our knowledge, no comprehensive overview and analysis of

these findings has been conducted. Therefore, our work aims to conduct a systematic review and meta-analysis of all available literature and to develop predictive models for various inactivation methods that can be used in food safety plans, provided there is sufficient data.

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Table 2.1: Prevalence of *Salmonella* from harvest through processing (Vinha et al., 2025).

Sample	Process Method	Location	Average Positive (%)
Soil	Sun drying location (outdoor)	Farm	33.50
Soil	Greenhouse (drying)	Farm	16.67
Soil	Rotary Dryer	Farm	5.57
Drying Waste	Sun drying location (outdoor)	Farm	50.00
Drying Waste	Greenhouse (drying)	Farm	0.00
Drying Waste	Rotary Dryer	Farm	5.57
Fallen black pepper berry	Sun drying location (outdoor)	Farm	0.00
Fallen black pepper berry	Greenhouse (drying)	Farm	11.10
Fallen black pepper berry	Rotary Dryer	Farm	0.00
Threshed Berry	Sun drying location (outdoor)	Farm	16.67
Threshed Berry	Greenhouse (drying)	Farm	15.00
Threshed Berry	Rotary Dryer	Farm	0.00
Stored Peppercorns	Sun drying location (outdoor)	Farm	27.80
Stored Peppercorns	Greenhouse (drying)	Farm	27.77
Stored Peppercorns	Rotary Dryer	Farm	11.10
Raw Material	NA	Processing Plant	11.10
Packaged product	NA	Processing Plant	16.67
Processing Waste	NA	Processing Plant	16.67

CHAPTER 3- MATERIALS AND METHODS

3.1 Research Question and Relevance Screening

The first phase of our project commenced with a systematic review of the available literature on the survival of *Salmonella* in black pepper and the effect of various inactivation treatments on the *Salmonella* population. We began our work by formulating the research question: how do various inactivation methods compare when eliminating *Salmonella* in black pepper? The review was conducted following the guidelines of the PRISMA 2020 statement (Page et al., 2021). To begin our search, we selected three major databases of scientific literature: Clarivate's Web of Science, Elsevier's ScienceDirect, and the National Institute of Health's PubMed database. We then used the following search query to find relevant documents: Title, Abstract, Keywords: ("black pepper" OR "peppercorn" OR "Ground Pepper") AND *Salmonella* AND (Growth OR Inactivation OR Reduction OR Survival OR Fate).

We found 87 relevant documents within the three databases after removing duplicates. Afterward, we screened our publications according to the chosen criteria: the studies had to provide sufficient CFU or survival data to calculate the log reduction. The experiments had to be conducted with either whole or ground black pepper (and exclusively with black pepper). *Salmonella* had to be the subject of the inactivation and survival studies. The article had to be an English-language, peer-reviewed journal article. The article had to contain temperature and/or other treatment parameters or provide survival data based on time. No parameters were required for the no–treatment category, but water activity and temperature were recorded. After screening, we were left with 40 sources, from which we extracted data for 155 independent experiments.

We used Endnote 21.5 (Bld 20846) to download and organize references. We did not conduct further quality assessment to minimize bias when interpreting results (Leone et al., 2024; Stone et al., 2019).

3.2 Data extraction

After screening, we manually extracted data from the remaining sources and recorded all information within a spreadsheet (Microsoft Excel for Mac, version 16.93.1 (25011917), Microsoft Office 365). We determined 11 inactivation treatment categories that we used to group our studies: heat, UV, radiofrequency (RFH), plasma, indirect plasma, chlorine dioxide fumigation, chlorine dioxide with storage, gamma irradiation, steaming, electron beam irradiation, and no treatment. Methods that were only used in a single study were placed in the "miscellaneous" category. Within this category, some studies included multiple experiments, allowing us to conduct a meta-analysis; therefore, we were able to compare the independent experiments of phosphine fumigation (PH_3), ethylene oxide fumigation (EtO), Acetic Acid fumigation, UV-irradiation with heating (UVH), and combined Heat/Humidity treatments.

The following data were extracted for all (where provided): authors, log reduction, matrix (ground or whole), treatment type, treatment time (min), and temperature. Where possible, data on water activity were also extracted. Moisture content was converted to water activity using a moisture-sorption isotherm (MSI) for black pepper (Yogendrarajah et al., 2015).

Further data extraction was determined based on the type of treatment. A summary of specialized data is presented in Table 4.1. We extracted the data from graphs by using PlotDigitizer (PlotDigitizer), while the data found in tables was recorded in Microsoft Excel.

3.3 Data collection and primary models

We used the same data collected in our systematic review to develop primary models for each category determined inactivation or survival. Each individual experiment was modeled with the log reduction as the dependent variable (y) and time by as the independent variable (x). We selected a 2-phase linear model with a tail for primary modeling since it consistently showed the best fit, as determined by the AIC and RMSE. The primary models were generated by the Integrated Pathogen Modeling Program (IPMP) from the USDA (2018). The formula for the primary model used can be seen in Equation 4.

Equation 4. The formula for linear function with tail.

$$y = y_0 - \frac{t}{D}, t \leq t_L$$

$$y = y_{tail}, t > t_L$$

Where y_0 and y_{tail} represent the initial and final bacterial counts, respectively, t represents time, and D represents the D–value (USDA, 2018).

For survival studies, an analogous concept to the D–Value called D–90 was used. D–90 refers to the elapsed time in days needed for a 1-log reduction in total colony forming units (CFU) in a non-treated sample. To evaluate the fit of the models, the D–value (and its standard error), AIC, and RMSE were recorded from IPMP. The temperature for each experiment was recorded, when provided. In some cases (particularly within the no–treatment category), there was no temperature recorded; in this case, the temperature of the black pepper samples before inoculation was used. When no temperature data were discernible, we assumed that the experiments were carried out at room temperature based on the temperature of the black pepper samples before the experiment. We also extracted the water activity data for each experiment. Where only moisture content was provided, we used a moisture-sorption isotherm found within published literature to

estimate the water activity (Yogendrarajah et al., 2015). Tables 4.2. and 4.4. show the extracted data for thermal inactivation and no–treatment groups respectively. The inoculation procedure was also extracted where it was used to distinguish two treatments. In the case of ClO_2-based inactivation, studies varied on holding time constant while modulating concentration or changing time with constant concentration. To make sure that our effect sizes (D–values) were comparable, we fitted a 2-phase linear model with tail from IPMP with the dependent variable being log reduction and the independent variable being a product of time and concentration. The relationship can be described by a linear function. Therefore, we were able to estimate the equivalent time needed for the variation in concentration used to achieve a 1-log reduction.

Equation 5. Formula for calculating decimal reduction time (D–value).

$$D = \frac{x}{log_{10}N_0 - log_{10}N_F}$$

Where D is the decimal reduction time, x is time or dose, and N_0 and N_F are the initial and final bacterial counts (in CFU).

Equation 6. Estimation of time equivalent to variation in ClO_2 concentration

$$x_1 = \frac{y - \beta_0}{\beta_1 x_2}$$

Where x_1 is the estimated time equivalent, y is the log reduction (reference was -1), β_0 is the intercept, β_1 is the slope of the linear inactivation curve, and x_2 is the concentration.

To make sure that the results were comparable, a reference concentration had to be chosen; for this the mode of all concentrations used in all ClO_2-based inactivation studies was chosen: 10 mg/L. To calculate the time needed for a 1-log reduction, a value of -1 was chosen for y.

3.4 Meta-Analysis

We conducted a random-effects meta-analysis using the "meta" package in R (Schwarzer et al., 2015). Our effect size was the $\log_{10}$-transformed D–value and it's respective standard error after following the rules of error propagation (Lindberg, 2001). The τ^2 was estimated using the Restricted Maximum Likelihood (REML) method since we were working with relatively small sample sizes (Tanriver-Ayder et al., 2021). For treatment categories with more than 10 independent experiments, we conducted a meta–regression analysis. Predictors were chosen according to the best fit for our secondary models. Outliers were determined by Dixon's Q-test using the outliers package (Lukasz, 2005). Additionally, we performed a subgroup analysis for each inactivation method, choosing possible covariates that were common across most studies. We divided the heat, ClO_2, plasma, EtO, PH3, RFH, and Survival Studies– that is the No Treatment (NT) and Survival in storage after ClO_2 fumigation (ClO_2/Storage)– into subgroups.

To create a subgroup, we used the following criteria: a minimum of two experiments had to be conducted, and the subgroup needed to be a potential confounding variable. Moreover, sufficient data was needed for a subgroup to be formed- a majority of experiments needed to report the subgroup variable. Additionally, the ClO_2, heat, miscellaneous, and survival studies were evaluated by considering multiple subgroups based on the possible covariates. Where an experimental factor was not varied across studies, we did not consider it a possible covariate. For cases with more than 10 studies (the ClO_2, RFH, and heat treatment, as well as the NT categories), we selected several predictors according to the 1-in-10 rule to conduct meta–regression (Chowdhury & Turin, 2020). The model equations used during secondary modeling were employed in the meta–regression analysis. Our meta–regression results were evaluated using AIC, RMSE, and bubble plots, which reported our meta–regression findings.

3.5 Test for publication bias

We evaluated all treatment methods with more than 10 experiments (ClO_2, heat, no treatment, and miscellaneous treatments) for publication bias by generating funnel plots and conducting Begg and Mazumdar test for publication bias (Van Aert et al., 2019) via the meta package in RStudio (Schwarzer et al., 2015). For all other methods, there was not enough data to evaluate publication bias.

3.6 Secondary models

Choosing the most optimal secondary model meant we had to consider the following factors: overall fit (as described by AIC), number of predictors (to prevent overfitting), and the normal distribution of residuals (to ensure the validity of model predictions). Therefore, to select the optimal model, a full second-order polynomial global model was fitted, and all possible reduced second-order polynomial models were generated using the MuMIn package in R (Bartoń, 2010). The models were then screened based on the number of predictors and the normality of residuals (Shapiro-Wilkes test). We used the one-in-ten rule to determine the maximum number of predictors: For every 10 datapoints, we would allow one predictor to be fitted (Chowdhury & Turin, 2020). This effectively restricted secondary models to RFH, Heat and ClO_2 fumigation treatments, as well as the non-treated samples. Predictors were chosen by relevance and correlation to the dependent variable. A black pepper matrix (ground or whole) could not be used as a predictor for any category due to an insufficient sample size or lack of variation within the category. After screening the reduced second-order polynomial models based on residual distribution and number of predictors, we determined the most suitable model was the one with the highest AIC value. Where more than one predictor was viable (this was only in the case of heat-based inactivation), we used a reduced second-order polynomial model to conduct response surface analysis.

Equation 7. Full second-order polynomial equation used for global model (for 2 predictors)

$$log_{10}D = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2 + \varepsilon$$

In the case of models with multiple predictors, we fitted a response surface model using the rsm package in R (Lenth, 2008). When only one predictor was available, we used linear regression with the most suitable predictor, as determined by the screening method described above. Results were exported using the writexl package (Ooms, 2017). Since there were significant differences between radiofrequency treatment methods, no suitable secondary model could be established.

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CHAPTER 4- Results and Discussion

4.1. Systematic Review

After a systematic review and screening, we identified 40 studies that met our inclusion criteria with a total of 155 independent experiments. We categorized our studies into 11 methods, including no–treatment and miscellaneous, which comprised four methods with multiple, independent experiments and 6 individual methods providing a single D–value. Summaries of the data for the investigated covariates, categorized by treatment method, are presented in Table 4.2 to 4.17. The subcategories seen under "miscellaneous" are further explained in the meta-analysis chapter.

We extracted the D–values and their standard errors from each study using IPMP. Fitting the linear model with the tail proved to be the most suitable for each experiment. However, in some cases, the software was unable to fit an appropriate primary model; in these cases, we used R to run a simple linear regression to predict the most suitable D–value. Moreover, in some cases, IPMP was unable to provide an estimate for AIC or RMSE. Summary of the data gathered from our primary modeling are also presented in Tables 4.2 to 4.17.

The primary models that calculated D–values had AIC values ranging from -29.46 to 38.99, with the average value being 13.05. Meanwhile, the RMSE values ranged from 0.00 to 3.09, with an average of 0.44. In the case of D–90 values, the AIC values ranged from a minimum of -39.00 to a maximum of 34.12, with an average of -5.54. In the case of RMSE, values fell between 0.00 and 1.12, with a mean of 0.26. This indicates that the primary models exhibited significant variability in terms of their goodness of fit. The Standard Error of the effect sizes ranges from not

being measurable to a high of 366.26 for D–values, and 0 to 7777.38 in the case of D–90 values. Some studies in the NT category reported *Salmonella* growth in black pepper (Xie et al., 2022). One experiment had to be removed from the analysis of D–90 values following Dixon's Q-test because it was a significant outlier.

4.2. Secondary Models, Critical Variables, and Goodness of Fit

After screening the quadratic and all possible reduced quadratic models for each method, we found that the best inactivation method for heat was a reduced second-order polynomial model. In contrast, linear models provided the most suitable predictions for inactivation in the NT and ClO_2–treatment categories. We used the Shapiro test to select models with normally distributed residuals, ensuring that our model can predict accurately. The 1-in-10 rule was used to set the maximum number of predictors to minimize the chance of selecting an overfit model (Chowdhury & Turin, 2020). We selected AIC as our final evaluation criterion, since it can be applied to both linear and nonlinear models. RMSE was reported as well, but R^2 was only considered for linear models, since it has little to no validity when evaluating nonlinear regression models, and can provide highly misleading results (Spiess & Neumeyer, 2010). A summary of our goodness-of-fit statistics is presented in Table 4.18.

Overall, the secondary models for heat treatment and ClO_2–fumigation showed a much better fit compared to the best model available for the non-treated samples, even after removing outliers using Dixon's Q-test. To provide the best goodness of fit for all investigated methods, D–values and D–90 values had to be log-transformed.

Modeling **Salmonella** *Inactivation During Heat Treatment*

As shown in Figure 4.1, two key factors in predicting the D–value of a heat treatment are temperature and water activity. Higher temperature treatments result in a faster log reduction of *Salmonella* CFUs. However, higher water activities also tend to result in a more intense heat treatment, even at lower temperatures. *Salmonella* is one of the most critical pathogens that need to be controlled in low-aw foods. They have a strong tendency to adapt to stressful conditions, increasing their durability to hostile environments, including the temperatures used during heat treatments (Dawoud et al., 2017). These bacteria can survive in low-moisture, high-solute settings via osmoregulation, increasing the concentration of solutes within their cells to maintain turgor pressure. *Salmonella* can also modulate its membrane composition to adapt to environments with low water activity. Another adaptation mechanism is dormancy, whereby *Salmonella* spp. enter a viable but non-culturable (VBNC) state. If conditions improve, these *Salmonella* cells can return to a normal metabolic state and continue to reproduce. However, when *Salmonella spp.* reduce their metabolism, they also gain increased thermoresistance. The absence of water can increase the heat resistance of *Salmonella* cells by preventing protein denaturation (Dawoud et al., 2017; Morasi et al., 2022). Low water activity in general tends to increase the thermal resistance of *Salmonella* spp. This is in line with what Jin et al. (2018) found for low-moisture foods with high fat and high protein contents and what Wei et al. (2020) found in milk powder samples. The model residuals are normally distributed ($p = 0.924$). The Quantile-Quantile (Q–Q) plot is shown in Figure 4.2.

Modeling **Salmonella** *Inactivation During Chlorine Dioxide Treatment*

Chlorine dioxide (ClO_2) is a gas at room temperature that is highly soluble in water. It's primary antimicrobial properties come from being a potent oxidizing agent- specifically, the

unstable Cl-O double bond can easily break and generate free radicals that damage the cell membrane, destroy nucleic acid structures, prevent protein synthesis, and inhibit enzymatic systems responsible for basic metabolism In gaseous form, it can penetrate the food surface and has even stronger biocidal properties (Zhang et al., 2023). The relative humidity (RH) used during a ClO_2 treatment is a critical factor to consider when inactivation is being evaluated. This is likely because water solubilizes ClO_2, dissolves, and increases its contact with the food matrix (Park et al., 2018). Several studies have found that the efficacy of ClO_2 is conditional upon exposure time, RH, and gas concentration (Han et al., 2001; Wason & Subbiah, 2023). However, our results suggest that concentration has a negligible effect on the log D–value within the investigated ranges (5 mg/L to 15 mg/L). When predicting the log D–value from concentration, we found that concentration was not significant ($P = 0.799$) and had a low correlation ($R^2 = 0.068$). However, considering our small sample size (13 experiments), further studies should be conducted to more accurately measure the concentration's effect on the log D–value when using gaseous ClO_2 treatments for black pepper. RH had by far the most significant influence on the log D–value ($P<0.001$, $R^2 = 0.747$), with higher RH (%) indicating smaller log D–values. A graphical representation of our model is shown in Figure 4.3. The errors for our model are normally distributed, as indicated by the Shapiro-Wilkes test, which yields a p-value of 0.756. This suggests that there is no pattern in prediction errors that would cause substantial deviation within the data range studied in our analysis (Fig. 4).

Modeling* Salmonella *Inactivation During No Treatment

With *Salmonella*'s ability to survive in low-water activity settings, multiple studies have evaluated the pathogen's survival kinetics within black pepper. Many studies have examined how *Salmonella* survives within foods with low water activity. Studies have shown that *Salmonella* can

be recovered from black pepper even after a year of storage. However, survival curves appear to depend on the temperature and relative humidity of the environment (Keller et al., 2013). In some instances, such as the study by Xie et al. (2022), *Salmonella* was found to be able to grow within black pepper, with a 0.18 log CFU increase after 56 days. This experiment examined a *Salmonella* cocktail with a water activity of 0.3 and storage at room temperature.

This was in line with our overall findings; higher water activity led to more rapid reductions in *Salmonella* cell counts, while lower water activities increased their overall survival (Xie et al., 2022). While black pepper contains innate antimicrobials, such as the alkaloid piperine, some Gram-negative bacteria, including some *Salmonella Typhi*, have been shown to be more resistant to black pepper's antimicrobial effects. The method of exposure to antimicrobial compounds in black pepper (such as the alkaloid piperine) seems to be critical when predicting the antimicrobial properties of black pepper (Abd El-Hack et al., 2022; Ashish Singh et al., 2023; Shityakov et al., 2019). Considering that grinding black peppercorns would likely increase the cells' exposure to back pepper's antimicrobial phytochemicals, future studies could investigate the growth of *Salmonella* in ground and whole peppercorns directly. Our subgroup analysis, as presented in the following section, also indicates that *Salmonella* generally dies much faster on ground black pepper; however, with only two experiments conducted on whole black pepper, further research is needed. Moreover, cross-protection as a result of low-water activity stress may also explain the durability of *Salmonella* in black pepper (Morasi et al., 2022).

4.3 Limitations for Predictive Modeling

Our study has several limitations that highlight knowledge gaps in the existing literature. Heat-based inactivation had the most considerable amount of datapoints. However, as seen in our meta-analysis results, the heterogeneity for studies in all three categories was moderate to high.

Studies were most similar in the case of ClO_2–fumigation, but heterogeneity was still moderate (I^2 = 39.6%). This shows that study-based variability was significant for all groups, and our small sample size (13 experiments for ClO_2–fumigation) suggests that more data is needed. In the case of NT, the high variability of studies and a limited choice of predictors resulted in a low R^2 compared to the other methods. Based on the reviewed literature, RH and temperature variation may be critical factors to examine when considering *Salmonella* growth during black pepper storage (Keller et al., 2013). Given the relationship between relative humidity (RH) and water activity, ensuring that water activity in black pepper remains high during processing may be critical for preventing the survival of *Salmonella*.

4.4 Meta-analysis results

Meta-Analysis for Heat Treatment

Our random-effects meta-analysis suggests that heat-based inactivation results in an average log D–value of 0.81, with variability arising from differences in a_w, temperature, and matrix. Matrix and water activity result in the most significant variability when considering subgroup differences. A summary of our results is presented in Tables 4.19-4.21.

The between-study heterogeneity when evaluating heat treatments by matrix was 99.2%, with τ^2 values for the subgroups ranging from 0.1 (Whole) to 0.16 (Ground). The τ^2 for all experiments was 0.21, indicating low variability between experiments. It should be noted that both experiments used for whole black pepper in this category were from a single study (Song, 2023), and the I^2 between the experiments examining whole black pepper was still 64.2%. The difference between the whole and ground subgroups appeared to be significant ($P < 0.0001$), suggesting that matrix plays a strong role during heat inactivation. Specifically, the average log D–value for whole black pepper was 1.9 as compared to 0.8 for experiments on ground black pepper.

When analyzing the subgroups by temperature, the overall subgroup difference appears to be less significant than that of the matrix or water activity. All subgroups exhibit high between-study heterogeneity. Low-temperature studies (45-60°C) show significant variance within studies ($\tau^2 = 0.41$), whereas medium-temperature studies (65-75°C) have a τ^2 of 0.17. Low-temperature experiments showed an I^2 of 0.0% and an undetectable τ^2, although it should be noted that only two study experiments, both from the same study, were included in this category. High-temperature studies (80-85°C) had a τ^2 of 0.41, indicating higher variability within the studies.

When evaluating subgroups by water activity, two experiments (Song, 2023) were excluded since they did not provide water activity data. The mean log D–value in the case of high a_w (0.5) was the lowest, though all confidence intervals overlap. High water activity (0.65–0.75) experiments yielded an I^2 of 66.4, indicating more moderate heterogeneity, whereas the medium (0.45-0.55) and low (0.25-0.40) a_w subgroups showed high I^2 values (>98%). The τ^2 for high and low a_w experiments were somewhat lower than τ^2 for medium a_w studies. Our findings indicate that, among the three subgroup analyses, heterogeneity appears to be strongly related to differences in study design, rather than to sampling error alone. Table 4.22. presents the results from our meta–regression analysis, which considers both temperature and water activity. The matrix data could not be analyzed alongside water activity, since the only study using whole black pepper (Song, 2023) did not report water activity results.

Our QE and QM statistics confirm that both water activity and temperature are critical predictors of log D–value ($p < 0.0001$). The I^2 shows that 95.21% of the heterogeneity between studies is not due to sampling error, while the R^{2*} (83.09%) indicates that water activity and temperature explain most of the observed heterogeneity. Our findings suggest that, within the range of our data (45-85°C), both water activity and sample matrix are clear covariates for heat

treatment efficacy. Higher water activities result in lower log D–values. Possible reasons for this may be the increased exposure of cells to antimicrobial components in ground black pepper or that *Salmonella* cells can hide within the crevices of whole black pepper. However, to the best of our knowledge, no study has investigated the effect of the matrix on inactivation efficacy. Therefore, future studies may want to compare the effects of heat treatments on both whole and ground peppercorns.

As seen in Figure 4.11, plotting our effects and standard error results in a highly asymmetric and skewed funnel plot. Studies appear to report lower log D–values and measurements with lower standard errors disproportionately (Carlson et al., 2023). This seems to indicate the presence of noticeable publication bias. Begg and Mazumdar's Rank Correlation Test appears to support this, yielding a z-value of 1.39 ($p = 0.016$).

The key statistics to note here are τ^2, I^2, R^2*, and the p-values of QM and the z-value of the Rank correlation test. τ^2 describes the variance between studies while I^2 describes the heterogeneity that is not directly related to sampling error (Rücker et al., 2008). R^2* shows the percentage of I^2 that is explained by the moderator variables, while the p-value of QM shows the significance of the moderator variable as it predicts the response variable (the effect). The p-value of the rank correlation test z shows the probability of publication bias, with higher p-values indicating a probable absence of bias. However, it is essential to consider that meta-analyses with small sample sizes may yield false negative (Begg & Mazumdar, 1994). Therefore, it is also best to examine the funnel plot symmetry.

Meta-Analysis for ClO_2–treatment

Through the course of our systematic review, we found two main categories of ClO_2 treatments: ClO_2 fumigation is a simple gaseous ClO_2 treatment, while ClO_2 with storage also

evaluates continued microbial decline after ClO_2 treatment. We performed two subgroup analyses on ClO_2–fumigation: we grouped studies into 60,70, and 80% RH and 5, 10, 15 mg/L by concentration. Moreover, we conducted a meta–regression analysis on the ClO_2 studies. A summary of our findings is presented in Tables 4.23. and 4.24. When evaluating findings by RH, ClO_2–treatment shows an I^2 of 39.6% (0.0% to 69.4%), a τ^2 of 0.02 (0.0 to 0.2), indicating that while there was substantial heterogeneity between studies, individual studies themselves had low variability within them. The subgroup I^2s all had overlapping and wide confidence intervals (from 0 to >80%) while the predicted mean log D–value was 2.0. When dividing ClO_2–treatments by concentration, I^2 values had similarly wide confidence intervals, with lower bounds ranging from 0% to 17.3% and upper bounds ranging from 85.6% to 89.6%. τ^2 was low for all subgroups (the highest being 0.06). When analyzing subgroups by RH, the differences seemed more significant ($p = 0.077$) compared to subgroup analysis based on gas concentration ($p = 0.416$). The highest log-D–value was 2.7 (2.06 to 3.25) for the 41.5% RH subgroup, and the lowest was 1.8 (1.62 to 1.98) for the 80% RH subgroup. Simultaneously, log-D–values range from 1.9 (1.63 to 2.12) for studies with a 15 mg/L gas concentration to 2.1 (1.81 to 2.31) in the case of a 10 mg/L concentration.

Overall, these results align with our findings that ClO_2–inactivation is more dependent on humidity than concentration within the range of our considered variables. Our meta–regression results (Table 4.25) also confirm that RH has a better capacity at explaining residual heterogeneity, as the R^{2*} of 39.90% when grouping by RH completely disappears when grouping by concentration. Moreover, the I^2 is noticeably lower when analyzing the groups by relative humidity (RH) as opposed to concentration. However, the relatively low number of datapoints ($k = 13$) suggests that further research is needed to evaluate the impact of concentration and relative

humidity properly. The bubble plots generated from our meta–regression analyses also demonstrate the strong significance of RH compared to concentration.

In the case of ClO_2-based inactivation, the results appear relatively evenly distributed, suggesting lower amounts of publication bias. The Rank Correlation Test result also indicates some publication bias as seen in Table 4.26.

Meta-Analyses for Direct and Indirect Plasma Treatment

Plasma-based inactivation is a relatively new method for *Salmonella* inactivation in black pepper. Throughout our systematic review, we found nine experiments that used this method to treat black pepper. However, there were two methods for plasma-based inactivation: Direct plasma methods generate plasma that directly contacts the spices for decontamination, whereas indirect plasma methods are used to treat air to generate charged particles that then contact the microbes within a given food. Reactive oxygen and nitrogen species, charged particles, and UV radiation appear to all play a role in microbial inactivation within the atmospheric-pressure cold plasma method (Šimončicová et al., 2019). Specifically, UV-radiation may damage cellular DNA, lipid peroxidation, protein modulation, electrostatic membrane disruption, and electroporation have been proposed as mechanisms for non-thermal plasma's bactericidal properties (Liao et al., 2017). Remote or indirect plasma techniques are used to treat a gas, generating charged particles and reactive oxygen species that are then used to inactivate microbes on the target surface. One advantage of remote plasma technologies is that the apparatus can have flexible construction and may be used for foods of different shapes and sizes (Obileke et al., 2022). Nevertheless, both methods have been used for black pepper as seen in Figures 4.17 and 4.18. The results of the meta-analysis can be seen in Table 4.27.

Since only two indirect plasma methods were used for indirect plasma treatment, we were unable to conduct a subgroup analysis on the methods. The mean log D–value was 1.1 (0.83 to 1.36) with an I^2 of 17.5% and a τ^2 of 0.00159, indicating low heterogeneity within-study variance.

In the case of direct plasma methods, we divided our methods by the carrier gas: modified methods used either argon or an argon/air combination, whereas all other methods just used air. Modified methods had a mean effect size of 0.93 (0.45 to 1.42), whereas methods using air had a mean log D–value of 0.5 (-0.63 to 1.63). Given the breadth of the confidence intervals, we were unable to find any significant differences between the subgroups. Both subgroups had an I^2 over 90, indicating substantial heterogeneity. However, modified methods had a τ^2 of 0.23, compared to 0.96 in experiments using air, suggesting a much greater variance between studies. Many variables can affect the efficacy of plasma-based inactivation, from apparatus design to energy dose to flow rate to carrier gas (Obileke et al., 2022; Šimončicová et al., 2019).

Meta-Analysis for Gamma, UV, and Electron Beam Irradiation Treatment

Irradiation is a method used to control pathogens in food products, aiming to prevent foodborne illnesses. This technique has become more and more accepted by consumers in recent years. The main mechanism of Irradiation rests on destroying microbial DNA via direct ionization and generating reactive species that damage cell components by generating free radicals (Mshelia et al., 2023). Our systematic review identified three methods of irradiation: Gamma, electron-beam, and UV irradiation. Electron beam irradiation offers the benefits of not requiring a nuclear irradiation source, lower cost, and being less hazardous to humans than gamma irradiation. Electron beam treatments come in two forms: high-energy electron beams (HEEB, >300 keV) and low-energy electron beams (LEEB, <300 keV). Electron beam treatments usually have shallow penetration, which limits their uses in foodstuffs (Mshelia et al., 2023; Sani et al., 2025). UV

irradiation is also a popular form of irradiation since it can effectively preserve a food's nutritional and physical microstructure. The meta-analysis results of the reported irradiation-based treatments can be seen in Tables 4.28 to 4.30.

The two primary forms of UV irradiation are UV-C and UV-A, which have different mechanisms of inactivation. The UV-C wavelength range encompasses the maximum absorbance of RNA and DNA (265-275 nm), whereas UV-A (365 nm) destroys cells by generating free radicals (Arcos-Limiñana et al., 2025).

Gamma irradiation is a treatment that can penetrate deep within the food matrix. Gamma rays are a form of ionizing radiation with frequencies above 1019 Hz. Generating gamma rays typically requires radioactive isotopes, and the equipment typically has high initial costs. However, gamma irradiation is highly effective in eliminating pathogenic microbes (Shahi et al., 2021).

We used two subgroups to characterize UV treatments: black pepper matrix and radiation intensity. Overall, the low–intensity (0.57–1.7 mW/cm^2) and high–intensity (1110–2320 mW/cm^2) methods did not appear to have significant subgroup differences ($p = 0.844$), with mean effect sizes of 1.47 (0.06 to 2.89) and 1.31 (0.50 to 2.12), respectively. The I^2 index was above 95% for both the low- and high-intensity groups. The τ^2 was 0.658 for high-intensity studies and 1.002 for low-intensity studies, indicating that the latter had much higher variability between studies. It should be noted that the low-intensity subgroup consisted of only two studies: Park et al (2020) and Gabriel et al (2020) with intensities of 0.57 mW/cm^2 and 1.7 $mW/cm^{2,}$ respectively. In the high-intensity group, all studies had an intensity of 1110 1.7 mW/cm^2 except for one (2320 1.7 mW/cm^2).

When evaluating the differences by matrix, the test between the subgroups resulted in a p-value of 0.0729. The mean effect sizes were 0.80 (0.63 to 0.96) for whole black pepper and

1.61(0.73 to 2.48) in the case of ground black pepper. No heterogeneity or variance between studies was detected for experiments on whole black pepper (conducted by Gabriel et al (2020) and Kim et al (2023) respectively), while the subgroup evaluating ground black pepper showed high heterogeneity and variance between studies ($I^2 = 98.5\%$, $\tau^2 = 0.761$).

We could find only two inactivation methods that used electron beam technology to eliminate salmonella from black pepper; Gaba et al. (2022) used a low energy electron beam whereas Murdoch et al used a high energy electron beam for inactivation, which is reflected in the high I^2 (99.4%), the τ^2 (0.292). The effect sizes were 0.66 (0.57 to 0.75) for LEEB inactivation and -0.11 (-0.17 to -0.04) in the case of HEEB inactivation (Gaba et al., 2022; Murdoch et al., 2022). Due to the lack of available subgroup data, the studies exploring gamma irradiation could not be divided into subgroups. The mean effect size of the random effects model was 0.28 (-0.44 to 1.01) with high heterogeneity (I^2= 92.3%) and a τ^2 of 0.483.

Meta-Analysis for Radiofrequency Heating Treatments

Radiofrequency (RF) waves are electromagnetic radiation with frequencies of 300 kHz to 300 MHz. However, only frequencies of 13.56, 27.12, and 40.68 MHz can be used for industrial applications, to avoid interfering with communication signals (Qiu et al., 2022). RF heating relies on electromagnetic field oscillations that increase the temperature within food by generating friction from the rapid movement of charged particles (ionic migration) and rotating dipole molecules. This means that the efficacy of RF treatments depends on the inherent characteristics of the food matrix, such as moisture content, electrical and thermal conductivity, specific heat capacity, material density, ion concentration, as well as the RF radiation parameters, including power and frequency. The primary mechanism of inactivation stems from its thermal effect on cells, which damages ribosomal DNA, depletes Mg^+ ions, and denatures proteins. The oscillating

electromagnetic field can also rupture the cell membrane, leading to cell death (Bermudez-Aguirre & Niemira, 2023). A crucial consideration is that hot and cold spots can form within the treated food product, potentially posing hazards if not properly managed. However, RFH has several benefits, including energy efficiency and speed (Qiu et al., 2022). When RFH is conducted, steam is generated that can impact bacterial inactivation.

In our meta-analysis, we examined various implementations of RFH treatments. Three studies by Wei et al (2018), Wei et al. (2019), and Wason et al. (2021) used containers that had steam release valves during the RFH treatment. Tong et al. (2022) used a vacuum-sealed bag to hold the black pepper. We could not identify whether the treated black pepper was in a sealed, vented, or open container for all other studies. We analyzed RFH methods using subgroup analysis and meta–regression according to the following parameters: matrix, power, ventilation, and water activity. The results of the meta-analysis are presented in Tables 4.31. to 4.34 while meta-regression results are reported in Table 4.35.

When categorizing RFH–treatments by matrix, experiments have an overall effect size of 0.6 (0.37 to 0.86), with whole black pepper having a mean effect of 0.3 (0 to 0.67) and ground black pepper having a mean effect of 0.9 (0.80 to 1.01). The τ^2 is 0.11 (0.03 to 0.58) in all experiments, while the τ^2 for whole and ground are 0.11 and 0, respectively. I^2 values for all experiments are 82.2% (70.1% to 89.4%), which was close to that of whole black pepper (81.2%) but much higher than ground black pepper (0.0%). It should be noted that ground black pepper consisted of five experiments, four of which came from a single study (Jeong & Kang, 2014). The matrix appears to have a strong influence on log D–values, as indicated by the test result of 9.99 ($p = 0.0016$). Overall, log D–values seem to be smaller for whole black pepper than ground black pepper.

When categorizing methods by the amount of power used, subgroup differences were even more substantial ($p < 0.0001$). Methods using 6 kW had the smallest effect size of -0.3 (-0.9;0.28), followed by 0.5 (0.26; 0.71) effect size for experiments using 9 kW and 0.9 (0.81; 1.01) for 12 kW methods. Overall, log D–values had an increasing tendency as the power increased. I^2 values were 0% for 6 and 12 kW, but were 83.0% (61.3%; 92.5%) for the 9 kW studies. The 9-kW study had the highest τ^2 as well (0.04), and the overall τ^2 was 0.11 (0.03;0.58). These data points suggest that determining a precise and accurate I^2 is difficult; however, within each group, variability between studies appears to be low. Overall, the inactivation methods exhibit a clear trend, where higher power is correlated with lower log D–values.

Since only three studies explicitly reported ventilation methods, we could only separate methods based on the following techniques: controlled ventilation and not reported. Since water has high heat conductivity and pressurized steam can contribute to microbial inactivation, we decided to compare studies where steam release was controlled through a valve with those where it was not reported.

Overall, subgroups showed significant differences ($p = 0.0014$) with high heterogeneity among methods, with no reports on ventilation ($I^2 = 84.4\%$). Studies with controlled ventilation had a mean effect size of -0.31 (-0.90 to 0.28), whereas studies with no comments on ventilation had a mean effect size of 9.7 (0.51 to 0.89). Variability in studies with controlled steam ventilation was minimal ($\tau^2 = 0$) while variability was somewhat larger in the case of studies not reported ($\tau^2 = 0.059$). Studies in the not-reported section employed multiple different designs, utilizing sample containers such as jars, beakers, or vacuum-sealed pouches, with no report on ventilation. This may explain a significant fraction of heterogeneity among studies, as evident in our meta–regression analysis below.

Water activity has a direct relationship with moisture content as seen in a moisture-sorption isotherm. Water, being an excellent heat conductor, a strong solvent for ions, and a polar molecule, is directly affected by RFH treatments. Therefore, we decided to evaluate subgroups based on the water activity of the black pepper before treatment.

Overall, we found that the subgroup difference test yielded a of 3.79 ($p = 0.051$). The mean effect sizes were 0.5 (0.12 to 0.87) for medium a_w (0.54–0.66) and 0.88 (0.77 to 0.99) for high a_w (0.76–0.87). For these studies, water activity ranged from 0.54 to 0.87. At first glance, it appears that lower water activities correspond to more significant reductions in cell count. One possible cause for the more intense reduction at lower water activities may be the higher solute concentration in *Salmonella* cells, which may be polar or ionic substances. However, his narrow range and limited number of experiments ($k = 10$) mean more research is needed.

With only 12 studies, we were limited to conducting our meta–regression analysis by using one predictor at a time. Overall, ventilation appeared to have the strongest predictive power, with an R^2* explaining 84.07% of the heterogeneity, as indicated by I^2 (62.66%). Power had a near-identical predictive power, while using the matrix as a moderator variable yielded an I^2 of 72.82% and an R^2* of 72.29%. Overall, results indicate that ventilation, matrix, and power are all suitable moderator variables ($p < 0.001$). Interestingly, conducting a meta–regression using water activity as a covariate provided little explanation ($R^2 = 0.00\%$, $I^2 = 92.38\%$, $p = 0.274$). Graphical representations can be seen in the bubble plots below (Figures 4.28, 4.29, and 4.30).

Running the rank correlation test yielded a bias estimate of 2.00 and a z-statistic of 0.14 ($p = 0.891$), indicating little evidence of publication bias (Table 4.36).

Meta-Analysis for Steam Treatment

Throughout our systematic review, we identified 11 studies that employed various forms of steam-based inactivation to eliminate *Salmonella* from black pepper. However, we were only able to use seven experiments since the first measurement in the survival curve was below the limit of detection. We analyzed the steam inactivation methods by categorizing them according to their temperature (low (88–100°C), medium (120–140°C), and high (160–180°C) respectively), water activity (0.3 and 0.65), and method (Superheated steam, vacuum steam). Superheated steam is a variation of steam–treatment whereby heat is added to steam beyond its saturation point. This provides the steam with a higher enthalpy, which speeds up heat transfer. Moreover, superheated steam does not condense if the temperature is higher than the saturation temperature at a given pressure (Alfy et al., 2016). Vacuum steam pasteurization is another method that utilizes shorter runtimes and lower temperatures to prevent the loss of volatile compounds, thereby preserving quality. In this case, steam condenses on the surface of peppercorns and penetrates their crevices, where it transfers the heat needed to inactivate *Salmonella* (Newkirk et al., 2018). The results of the meta-analysis on steam treatment log D–values can be seen in Tables 4.37 and 4.38.

Temperature had a significant effect on subgroups ($p < 0.0001$). However, precise log D–values were difficult to determine because of the rapid inactivation caused by steam pasteurization techniques. The I^2 of all studies was 87.5% (73.3 to 94.2%) with a τ^2 of 0.19 (0.05 to 1.72). Overall, higher temperatures tend to correspond to lower log D–values, with a mean estimate of -1.16 (-1.37 to -0.95) in the case of low heat. Medium heat resulted in log D–values ranging from -1.29 to -1.51, while high temperature experiments yielded log D–values of -2.14 and -2.22.

Considering water activity as a subgroup did not provide as substantial a difference as temperature. Additionally, the subgroups by water activity and method were identical, since

Newkirk et al. (2018) used low (0.33) water activity peppercorns and vacuum steam pasteurization while Ban et al used medium (0.63) a_w with superheated steam pasteurization (Ban et al., 2018; Newkirk et al., 2018). Therefore, we cannot determine the cause of variability. The studies had an I^2 of 87.5% (73.3 to 94.2%), and a tau² of 0.19 (0.05 to 1.92). Superheated Steam treatments with medium a_w had a high I^2 (>90%), whereas the low a_w vacuum steam treatment experiments had no measurable heterogeneity. The mean effect size was -1.6 (-2.2 to 1.09) for superheated steam/medium a_w and -1.1 (-1.36 to 0.74) for low a_w /vacuum steam treatments.

Meta-Analysis of Miscellaneous Treatments

While the aforementioned methods, as well as non-treated and storage after ClO_2 fumigation experiments, had multiple studies examining similar interventions, several methods had only one comparable inactivation study. These methods included ethylene oxide (EtO) fumigation, Acetic Acid fumigation, Heat and humidity treatment, phosphine fumigation, and UV/heat treatments. We considered comparing these methods to others as impractical, so we categorized them as miscellaneous (MISC) methods. A subgroup analysis based on method was conducted for MISC methods with repeated experiments (Table 4.39). Individual meta-analyses were reported on all techniques that were repeated at least once (Tables 4.40 to 4.44).

EtO fumigation is highly effective at inactivating *Salmonella* but is considered to be a controversial antimicrobial; while used in the United States, Canada, and India, this treatment has been banned from use in the European Union due to its mutagenic and carcinogenic properties (Dudkiewicz et al., 2022). EtO inactivates *Salmonella* by adding alkyl groups to proteins and nucleic acids, causing denaturation, and is highly reactive with water (Huang et al., 2012; Wei et al., 2021). Multiple studies found that temperature and relative humidity were critical parameters for inactivating *Salmonella* with EtO However, our subgroup analysis found no significant

difference (p = 0.9925) between the different RH (%) subgroups with mean effect sizes ranging from 0.24 (-0.49 to 0.98) to 0.30 (-0.33 to 0.94). However, the for subgroup differences among temperature-based subgroups (46°C, 53°C, and 60°C) had a p-value of 0.043. The study itself reported that a five-log reduction was only possible with 20 minutes of the maximum temperature (60°C) and RH (50%) (Wei et al., 2021).

Park et al (2019) explored how combining UV-irradiation with two levels of heat treatment (45°C and 60°C) can have a combined effect on inactivation. Higher temperatures combined with UV had a more substantial effect of 0.32 (-0.04 to 0.69) when compared to Lower temperatures- a log D–value of 0.68 (0.43 to 0.93). The two experiments had an I^2 of 60% and a τ^2 of 0.038 (Park et al., 2019).

Phosphine is one of the most popular fumigants in the world. It is highly toxic but does not leave residues within the product. Thus far, we have only found one study that explored how phosphine affects *Salmonella* in black pepper. We divided the experiments by Castro et al. (2011) into two subgroups based on water activity (0.92 and 0.97).

Overall, the test between the subgroups resulted in a p-value of 0.475. The I^2 of experiments on black pepper with an a_w of 0.97 was 20.7%, while no heterogeneity was found for experiments with an a_w of 0.92. Overall, all experiments had a negligible (0.0009) τ^2 and an I^2 of 0%. It should be noted that the a_w values were very close to one another, which may contribute to the lack of difference between the subgroups.

The moisture content of bacteria is most related to the relative humidity of their environment. High humidity can increase their moisture content and make them more susceptible to heat-based inactivation. Yang et al. (2020) conducted a study that combined a 15-minute dry heating phase with humidity increases to 80%, 70%, or 60%. Since there was no reduction before

the humidity treatment, we calculated the log D–values based on the initial humidity increase (Yang et al., 2022).

Our findings clearly show that higher humidity levels came with faster reductions in colony-forming units. Overall, the mean log D–values were -0.23 (-0.39 to -0.07), 0.09 (-0.28 to 0.46), and 0.55 (0.15 to 0.95) for 80%, 70%, and 60% humidity, respectively. All treatments had an I^2 of 85.5% and a τ^2 of 0.132 (Yang et al., 2022).

To find a new alternative for spice disinfection, Nei et al. (2017) tested acetic acid at concentrations of 0.3, 0.6, and 4.7 mmol/L. The results of 0.3 and 0.6 were close with respective log D–values of 1.62 (1.02 to 2.23) and 1.6 (1.06 to 2.14). Concentrations of 4.7 mmol/L had a log D–value of 1.18. Overall, no heterogeneity was found among the results.

Six experiments lacked a dimension of comparison. These methods were based on fumigation (O3, H2O2), irradiation (Near Infrared (NIR), UV with NIR, UV with NIR and TiO2 exposure (TiO2/UVNIR, and simultaneous UV-A and C treatment. The log D–values of these methods ranged from -0.42 in the case of H2O2 fumigation to 2.00. Their primary model statistics are presented in Table 4.45. Many of these methods are novel and, to the best of our knowledge, have not been previously reported. Therefore, more research is needed to verify the efficacy and predicted outcome of using these methods.

Meta-Analysis of Experiments with No Treatment, Survival after ClO_2 Fumigation

Among all the reviewed studies, we identified 28 experiments in which the researchers provided no treatment for the inoculated black pepper samples. Effects for these studies varied greatly, with Song (2023) measuring a 1 log reduction in one day and Xie et al. (2022) measuring up to a 0.18 log increase throughout the experiment (Song, 2023; Xie et al., 2022). Both the sample matrix and water activity were key determinants in *Salmonella* survival.

When comparing all experiments, NT studies had a mean effect of 1.2 (0.85 to 1.52), with high a_w (0.92–9.97) studies having a mean effect of 0.2 (0.14 to 0.27), compared to low a_w (0.30) studies, which had a mean effect of 1.8 (1.06 to 2.46). This indicates that *Salmonella* tends to die more quickly in high a_w environments, particularly in black pepper. When evaluating the matrix, only one study (Song 2023) used whole black pepper for the evaluation. However, their experiments yielded a markedly smaller effect of 0.3 (-0.3 to 0.83) compared to the mean for ground black pepper studies: 1.5 (1.11 to 1.97). The findings in our subgroup analysis align well with our response surface analysis results, suggesting that both water activity and sample matrix can influence how *Salmonella spp.* survive. The meta-analysis on D-90 values of NT studies can be seen in Tables 4.46 and 4.47.When evaluating the subgroups, both categorizations showed high heterogeneity (I^2 = 98.5%, τ^2 = 1.23), and subgroup differences as measured by the test showed p-values below 0.001.

Conducting a meta–regression (Table 4.48) shows us that using both a_w and sample matrix as a predictor explains 52% of heterogeneity that is not due to sampling error (I^2 = 92.5%). The QM statistic has a p-value below 0.0001, indicating that both moderator variables are significant. This is again in line with our findings from the response surface analysis.

Not all survival studies relied on no treatments. Wei et al. (2019) and Golden et al. (2019) both investigated the behavior of *Salmonella* after ClO_2 fumigation. We referred to these methods as ClO_2 (storage), and their results were provided in the same units as NT studies (D–90). To be able to analyze these results correctly, we chose the final CFU count of the ClO_2 treatment as our initial CFU count for D90 determination (Golden et al., 2019; Wei et al., 2023). These methods can be grouped according to the parameters of their respective ClO_2 treatments: relative humidity and concentration. The results of the meta-analysis on ClO_2 (storage) studies can be seen in Tables

4.49 and 4.50. In the case of concentration, the effect on survival ($p = 0.027$) was more prominent than that of RH ($p = 0.313$). This suggests that while concentration may not be the primary determinant of ClO_2 inactivation, it plays a significantly stronger role in cell survival after inactivation. The I^2 for medium (5–5.14 mg/L) and high (10–12.86 mg/L) concentration subgroups were 72.7% (8.1% to 91.9%) and 54.6% (0.0% to 88.9%), respectively. The τ^2 for medium concentration was 0.04, while the τ^2 was 0.2 for studies that used higher concentrations, suggesting greater variance between studies. The treatments with 80% RH showed no heterogeneity, whereas experiments using 41.5% RH resulted in a τ^2 of 0.35 and an I^2 of 88.4% (72.9% to 95.1%). Since these experiments came from only two separate studies using 41.5% and 80% RH, the number of studies performed, and variations thereof (such as concentration), can be assumed to contribute to this disparity between heterogeneity statistics.

Table 4.1: Data Extraction by Treatment

Inactivation Treatment	Data Extracted for All	Covariates used for Secondary Modeling and Subgroup Analysis
Heat	Authors, Log Reduction (log CFU), Matrix (ground or whole), Method, Treatment Time (min), and Temperature(°C)	Temperature, Matrix, a_w
UV		Intensity, Matrix
Plasma		Gas
Indirect Plasma		N/A
ClO_2		RH, Concentration
Gamma Irradiation		N/A
Steam		Water Activity
Electron Beam		N/A
Gamma Irradiation		N/A
Radiofrequency		Matrix
ClO_2/Storage		RH, Concentration
No Treatment		Matrix, a_w
Miscellaneous		Method, RH, Matrix

Table 4.2: Primary Model Data for Heat Inactivation with Key Predictors

Author	Method	Matrix	D-value	SE	Temp (°C)	a_w	RMSE	AIC	Effect Type
Song 2023	Heat	Whole	54.804	6.977	80	NA	0.347	31.129	D–value (min)
Gautam et al 2020	Heat	Ground	5.734	0.117	65	0.75	0.376	32.094	D–value (min)
Gautam et al 2020	Heat	Ground	3.263	1.493	70	0.75	0.232	26.33	D–value (min)
Gautam et al 2020	Heat	Ground	1.684	0.647	75	0.75	0.157	21.65	D–value (min)
Wason et al 2022	Heat	Ground	3.232	0.252	65	0.7	0.417	33.346	D–value (min)
Wason et al 2022	Heat	Ground	3.84	0.875	70	0.7	0.253	27.347	D–value (min)
Wason et al 2022	Heat	Ground	1.429	0.211	75	0.7	0.129	-9.012	D–value (min)
Park et al 2019	Heat	Ground	4.533	1.516	45	0.65	0.625	9.731	D–value (min)
Park et al 2019	Heat	Ground	1.584	1.516	60	0.65	0.611	9.352	D–value (min)
Wei et al 2021b	Heat	Ground	6.032	5.49	65	0.65	0.287	-2.724	D–value (min)
Wei et al 2021b	Heat	Ground	3.142	1.961	70	0.65	0.23	-6.299	D–value (min)
Wei et al 2021b	Heat	Ground	1.604	0.621	75	0.65	0.211	25.177	D–value (min)
Wason et al 2022	Heat	Ground	2.999	3.087	70	0.55	0.575	37.201	D–value (min)
Wason et al 2022	Heat	Ground	2.268	0.852	75	0.55	0.361	31.624	D–value (min)
Wason et al 2022	Heat	Ground	1.259	0.561	80	0.55	0.399	11.234	D–value (min)
Gautam et al 2020	Heat	Ground	6.015	0.152	75	0.54	0.392	32.617	D–value (min)
Gautam et al 2020	Heat	Ground	2.618	1.18	80	0.54	0.385	32.389	D–value (min)
Gautam et al 2020	Heat	Ground	0.853	0.459	85	0.54	0.246	27.01	D–value (min)
Wei et al 2021b	Heat	Ground	35.31	0.771	65	0.45	0.418	3.271	D–value (min)
Wei et al 2021b	Heat	Ground	15.721	0.837	70	0.45	0.327	-0.654	D–value (min)
Wei et al 2021b	Heat	Ground	5.948	0.445	75	0.45	0.279	-3.163	D–value (min)
Ahmad et al 2022	Heat	Ground	20	0.5	65	0.45	NA	NA	D–value (min)
Ahmad et al 2022	Heat	Ground	8.6	0.2	70	0.45	NA	NA	D–value (min)
Ahmad et al 2022	Heat	Ground	3.3	0.1	75	0.45	NA	NA	D–value (min)
Ahmad et al 2022	Heat	Ground	21.5	0.4	65	0.45	NA	NA	D–value (min)
Ahmad et al 2022	Heat	Ground	9.8	0.1	70	0.45	NA	NA	D–value (min)

Author	Method	Matrix	D-value	SE	Temp (°C)	a_w	RMSE	AIC	Effect Type
Ahmad et al 2022	Heat	Ground	3.4	0.1	75	0.45	NA	NA	D–value (min)
Wason et al 2022	Heat	Ground	18.086	0.832	70	0.4	0.597	37.641	D–value (min)
Wason et al 2022	Heat	Ground	8.446	3.495	75	0.4	0.303	29.494	D–value (min)
Wason 2024a	Heat	Ground	4.612	0.17	80	0.4	0.299	29.357	D–value (min)
Wason 2024a	Heat	Ground	3.035	0.711	80	0.4	0.255	27.438	D–value (min)
Wason et al 2022	Heat	Ground	3.225	0.35	80	0.4	0.373	31.995	D–value (min)
Gautam et al 2020	Heat	Ground	9.273	2.796	75	0.33	0.143	20.461	D–value (min)
Gautam et al 2020	Heat	Ground	5.127	1.016	80	0.33	0.136	19.877	D–value (min)
Gautam et al 2020	Heat	Ground	1.751	0.297	85	0.33	0.189	23.845	D–value (min)
Xie et al 2022	Heat	Ground	34.06	0.271	70	0.3	0.202	NA	D–value (min)
Wei et al 2021b	Heat	Ground	42.718	3.283	75	0.25	0.311	-20.789	D–value (min)
Wei et al 2021b	Heat	Ground	16.528	0.292	80	0.25	0.323	30.268	D–value (min)

Table 4.3: Primary Model Data for ClO_2–Inactivation with Key Predictors

Author	Method	Matrix	D-value	SE	RH (%)	Concentration (mg/L)	RMSE	AIC
Wei et al 2021c	ClO_2	Whole	77.890	16.257	60	5	0.161	-1.506
Wei et al 2021c	ClO_2	Whole	97.553	23.418	70	5	0.385	10.718
Wei et al 2021c	ClO_2	Whole	87.040	18.274	80	5	0.528	15.152
Wei et al 2021c	ClO_2	Whole	137.727	21.415	60	10	0.177	-0.190
Wei et al 2021c	ClO_2	Whole	40.643	9.108	70	10	0.331	8.595
Wei et al 2021c	ClO_2	Whole	64.163	7.459	80	10	0.520	2.481
Wei et al 2021c	ClO_2	Whole	101.755	19.293	60	15	0.292	6.825
Wei et al 2021c	ClO_2	Whole	88.808	22.043	70	15	0.530	36.228
Wei et al 2021c	ClO_2	Whole	43.619	9.465	80	15	0.667	38.989
Wei et al 2023	ClO_2	Whole	44.118	0.000	80	10	0.000	NA
Chai et al 2022	ClO_2	Whole	151.103	13.790	70	10	0.281	-29.460
Golden et al 2019	ClO_2	Whole	450.603	189.740	41.5	10	0.718	24.127
Golden et al 2019	ClO_2	Whole	450.102	198.171	41.5	10	0.751	24.584

4.4: Primary Model Data for No–treatment Experiments with Key Predictors

Author	Method	Matrix	D-value	SE	a_w	RMSE	AIC	Effect Type
Song 2023	NT	Whole	0.022	0.005	NA	0.445	34.123	D90 (day)
Golden et al 2019	NT	Whole	9.065	0.412	NA	0.056	-16.291	D90 (day)
Golden et al 2019	NT	Whole	6.901	1.549	NA	0.056	-16.291	D90 (day)
Song 2023	NT	Whole	0.023	0.006	NA	0.443	34.078	D90 (day)
Song 2023	NT	Whole	0.023	0.006	NA	0.443	34.078	D90 (day)
Castro et al 2011	NT	Whole	1.818	0.477	0.97	0.196	NA	D90 (day)
Castro et al 2011	NT	Whole	1.587	0.053	0.92	0.021	NA	D90 (day)
Castro et al 2011	NT	Whole	2.517	0.962	0.67	0.459	NA	D90 (day)
Castro et al 2011	NT	Whole	9.969	3.184	0.67	0.606	9.567	D90 (day)
Keller et al 2013	NT	Ground	14.485	1.183	0.55	0.565	-5.249	D90 (day)
Keller et al 2013	NT	Ground	3.263	0.230	0.55	0.418	-8.691	D90 (day)
Keller et al 2013	NT	Ground	5.482	1.128	0.55	0.727	-6.028	D90 (day)
Keller et al 2013	NT	Ground	15.460	3.146	0.55	0.336	-39.001	D90 (day)
Xie et al 2022	NT	Ground	44.785	21.903	0.5	0.083	-30.093	D90 (day)
Xie et al 2022	NT	Ground	16.734	5.854	0.5	0.159	-19.704	D90 (day)
Xie et al 2022	NT	Ground	106.371	6.268	0.5	0.226	-28.856	D90 (day)
Xie et al 2022	NT	Ground	114.078	8.351	0.5	0.248	-20.158	D90 (day)
Sun et al 2014	NT	Whole	6.888	6.827	0.47	1.121	NA	D90 (day)
Wason et al 2022	NT	Ground	10.864	4.226	0.4	0.215	-23.347	D90 (day)
Xie et al 2022	NT	Ground	168.476	369.927	0.3	0.099	-27.264	D90 (day)
Xie et al 2022	NT	Ground	1.49E+08	4.63E+14	0.3	0.282	-10.583	D90 (day)
Xie et al 2022	NT	Ground	323.367	36.441	0.3	0.134	-32.363	D90 (day)
Xie et al 2022	NT	Ground	439.549	95.310	0.3	0.190	-25.412	D90 (day)
Bowman et al 2015	NT	Whole	21.870	7.052	0.3	0.165	22.205	D90 (day)
Bowman et al 2015	NT	Whole	74.241	95.912	0.3	0.437	5.634	D90 (day)

Bowman et al 2015	NT	Whole	31.280	13.346	0.3	0.177	23.090	D90 (day)
Bowman et al 2015	NT	Whole	4.427	0.762	0.3	0.434	33.834	D90 (day)
Wei et al 2019	NT	Whole	1.906	0.241	0.66	0.11	-25.378	D90 (day)

Table 4.5: Primary Model Data for Miscellaneous Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	RMSE	AIC	Effect Type
Kim et al 2023	NIR	Whole	10.006	0.513	0.067	11.377	D–value (min)
Kim et al 2023	UV-NIR	Whole	2.76	0.147	0.352	3.035	D–value (min)
Park et al 2019	UV-Heat	Ground	4.781	0.617	0.592	4.921	D–value (min)
Park et al 2019	UV-Heat	Ground	2.099	0.39	0.879	12.028	D–value (min)
Wei et al 2021a	EtO	Whole	1.392	0.523	0.427	12.162	D–value (min)
Wei et al 2021a	EtO	Whole	0.7	0.056	0.182	0.206	D–value (min)
Wei et al 2021a	EtO	Whole	1.842	0.97	1.124	25.714	D–value (min)
Wei et al 2021a	EtO	Whole	0.891	0.189	0.377	10.409	D–value (min)
Wei et al 2021a	EtO	Whole	11.432	5.607	1.069	25.011	D–value (min)
Wei et al 2021a	EtO	Whole	1.675	0.894	1.039	24.619	D–value (min)
Wei et al 2021a	EtO	Whole	12.799	6.515	0.99	23.947	D–value (min)
Wei et al 2021a	EtO	Whole	2.489	0.602	0.756	20.162	D–value (min)
Wei et al 2021a	EtO	Whole	0.327	0	0	NA	D–value (min)
Nei et al 2017	Acetic Acid	NA	42.062	12.932	1.144	28.787	D–value (min)
Nei et al 2017	Acetic Acid	NA	39.535	10.862	1.087	28.282	D–value (min)
Nei et al 2017	Acetic Acid	NA	15	0	0	NA	D–value (min)
Park et al 2020	TiO2/UV-AC	Ground	109.399	24.612	0.369	-6.232	D–value (min)
Park et al 2020	UV-AC	Ground	72.834	12.026	0.248	-5.05	D–value (min)
Rane et al 2020	O3	Whole	400	0	0	NA	D–value (min)
Yang et al 2020	Heat/Humidity	Whole	0.589	0.047	0.302	NA	D–value (min)
Yang et al 2020	Heat/Humidity	Whole	1.221	0.231	0.579	NA	D–value (min)
Yang et al 2020	Heat/Humidity	Whole	3.574	0.732	0.586	NA	D–value (min)
Song and Kang	H2O2	Whole	0.3787	0.114	NA	NA	D–value (min)

Author	Method	Matrix	D-value	SE	RMSE	AIC	Effect Type
Castro et al 2011	PH3	Whole	1319.89	201.108	0.372	NA	D–value (min)
Castro et al 2011	PH3	Whole	1932.886	180.427	0.155	NA	D–value (min)
Castro et al 2011	PH3	Whole	813.559	366.258	1.127	NA	D–value (min)
Castro et al 2011	PH3	Whole	1231.822	181.151	0.384	NA	D–value (min)

Table 4.6: Primary Model Data for Acetic Acid Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	RMSE	AIC	Effect Type
Nei et al 2017	Acetic Acid	NA	42.062	12.932	1.144	28.787	D–value (min)
Nei et al 2017	Acetic Acid	NA	39.535	10.862	1.087	28.282	D–value (min)
Nei et al 2017	Acetic Acid	NA	15	0	0	NA	D–value (min)

Table 4.7: Primary Model Data for EtO Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	Temperature (°C)	RH	RMSE	AIC	Effect Type
Wei et al 2021a	EtO	Whole	1.392	0.523	46	30	0.427	12.162	D–value (min)
Wei et al 2021a	EtO	Whole	0.7	0.056	46	40	0.182	0.206	D–value (min)
Wei et al 2021a	EtO	Whole	1.842	0.97	46	50	1.124	25.714	D–value (min)
Wei et al 2021a	EtO	Whole	0.891	0.189	53	30	0.377	10.409	D–value (min)
Wei et al 2021a	EtO	Whole	11.432	5.607	53	40	1.069	25.011	D–value (min)
Wei et al 2021a	EtO	Whole	1.675	0.894	53	50	1.039	24.619	D–value (min)
Wei et al 2021a	EtO	Whole	12.799	6.515	60	30	0.99	23.947	D–value (min)
Wei et al 2021a	EtO	Whole	2.489	0.602	60	40	0.756	20.162	D–value (min)
Wei et al 2021a	EtO	Whole	0.327	0	60	50	0	NA	D–value (min)

Table 4.8: Primary Model Data for Heat with Humidity Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	Temperature (°C)	RH	RMSE	AIC	Effect Type
Yang et al 2020	Heat/Humidity	Whole	0.589	0.047	80	80	0.302	NA	D–value (min)
Yang et al 2020	Heat/Humidity	Whole	1.221	0.231	80	70	0.579	NA	D–value (min)
Yang et al 2020	Heat/Humidity	Whole	3.574	0.732	80	60	0.586	NA	D–value (min)

Table 4.9: Primary Model Data for PH3 Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	a_w	RMSE	Effect Type
Castro et al 2011	PH3	Whole	1319.89	201.108	0.92	0.372	D–value (min)
Castro et al 2011	PH3	Whole	1932.886	180.427	0.97	0.155	D–value (min)
Castro et al 2011	PH3	Whole	813.559	366.258	0.92	1.127	D–value (min)
Castro et al 2011	PH3	Whole	1231.822	181.151	0.97	0.384	D–value (min)

Table 4.10: Primary Model Data for Steam Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	Temp (°C)	a_w	Method Variation	RMSE	AIC	Effect Type
Ban et al 2018	Steam	Whole	0.056	0.008	100	0.62	Superheated	0.483	35.101	D–value (min)
Ban et al 2018	Steam	Whole	0.053	NA	120	0.62	Superheated	0.5831	17.770	D–value (min)
Ban et al 2018	Steam	Whole	0.031	0.008	140	0.62	Superheated	0.911	22.771	D–value (min)
Ban et al 2018	Steam	Whole	0.007	0.001	160	0.62	Superheated	0.488	NA	D–value (min)
Ban et al 2018	Steam	Whole	0.006	NA	180	0.62	Superheated	1.215	NA	D–value (min)
Newkirk et al 2018	Steam	Whole	0.077	0.018	88	0.33	Vacuum	0.649	10.326	D–value (min)
Newkirk et al 2018	Steam	Whole	0.101	0.022	88	0.33	Vacuum	0.812	13.909	D–value (min)

Table 4.11: Primary Model Data for Radiofrequency Heat Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	a_w	Power (kW)	Ventilation	RMSE	AIC	Effect Type
Wason et al 2024b	RFH	Whole	0.318	1.841	0.66	6	Controlled	2.087	29.423	D–value (min)
Wei et al 2018	RFH	Whole	0.496	0.15	0.6	6	Controlled	1.723	32.889	D–value (min)
Wei et al 2019	RFH	Whole	0.413	1.54703	0.66	6	Controlled	0.110	-25.378	D–value (min)
Jeong and Kang 2014	RFH	Ground	11.022	1.434	0.54	12	Not Reported	0.795	3.185	D–value (min)
Jeong and Kang 2014	RFH	Ground	6.335	0.713	0.76	12	Not Reported	0.648	10.353	D–value (min)
Jeong and Kang 2014	RFH	Ground	7.426	0.674	0.84	12	Not Reported	0.549	3.260	D–value (min)
Jeong and Kang 2014	RFH	Ground	9.004	0.874	0.87	12	Not Reported	0.647	-0.095	D–value (min)
Kim et al 2012	RFH	Whole	0.299	0.2186	NA	9	Not Reported	0.302	6.230	D–value (min)
Kim et al 2012	RFH	Ground	0.206	0.3142	NA	9	Not Reported	0.434	10.590	D–value (min)
Tong et al 2022	RFH	Whole	4.897	0.173	0.65	9	Closed	0.076	-15.379	D–value (min)
Tong et al 2022	RFH	Whole	3.316	0.214	0.65	9	Closed	0.097	NA	D–value (min)
Tong et al 2022	RFH	Whole	1.997	0.169	0.65	9	Closed	0.190	NA	D–value (min)

Table 4.12: Primary Model Data for Direct Plasma Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	Gas	RMSE	AIC	Effect Type
Hertwig et al 2015	Plasma	Whole	1.420	0.250	Modified (Argon)	0.250	-5.170	D–value (min)
Song 2023	Plasma	Whole	41.220	3.630	Modified (Argon)	0.050	8.610	D–value (min)
Sun et al 2014	Plasma	Whole	18.220	1.620	Modified (Argon)	0.250	13.760	D–value (min)
Sun et al 2014	Plasma	Whole	14.340	0.900	Air	0.230	26.090	D–value (min)
Sun et al 2014	Plasma	Whole	12.720	1.850	Air	0.510	NA	D–value (min)

Table 4.13: Primary Model Data for Indirect Plasma Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	Gas	Power (kW)	RMSE	AIC	Effect Type
Garcia Casado et al 2024	Indirect Plasma	Whole	13.941	0.962	Modified (Argon)	0.08	0.042	NA	D–value (min)
Hertwig et al 2015	Indirect Plasma	Whole	5.233	1.990	Air	1.2	0.967	16.704	D–value (min)

Table 4.14: Primary Model Data for Gamma Irradiation Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	RMSE	AIC	Effect Type
Song et al 2014	Gamma	Whole	3.158	0.795	0.478	34.994	D–value (min)
Song et al 2014	Gamma	Ground	9.829	1.828	0.372	31.983	D–value (min)
Arias Rios et al 2019	Gamma	Whole	0.204	0.041	0.305	7.438	D–value (min)
Doca et al 2021	Gamma	Whole	2.151	0.737	0.741	NA	D–value (min)

Table 4.15: Primary Model Data for UV Irradiation Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	Intensity (mW/cm2)	RMSE	AIC	Effect Type
Gabriel et al 2020	UV	Whole	5.946	0.523	1.7	0.192	-20.575	D–value (min)
Park et al 2019	UV	Ground	2.088	0.206	2320	0.285	-8.267	D–value (min)
Park et al 2020	UV	Ground	72.834	12.026	1110	0.248	-5.05	D–value (min)
Park et al 2020	UV	Ground	118.134	10.061	1110	0.13	-23.003	D–value (min)
Park et al 2020	UV	Ground	165.241	44.453	0.57	0.193	NA	D–value (min)
Kim et al 2023	UV	Whole	9.337	2.381	1110	0.178	-10.346	D–value (min)

Table 4.16: Primary Model Data for Electron Beam Treatments with Key Predictors

Author	Method	Matrix	D-value	SE	RMSE	AIC	Effect Type
Gaba et al 2022	Electron Beam	Whole	4.576	0.210	0.108	-7.115	D–value (min)
Murdoch et al 2024	Electron Beam	Whole	0.783	0.027	0.226	-21.971	D–value (min)

Table 4.17: Primary Model Data for ClO_2/Storage with Key Predictors

Author	Method	Matrix	D-value	SE	RH (%)	Concentration (mg/L)	RMSE
Wei et al 2023	ClO_2/Storage	Whole	6.618	1.777	80	5.00	0.127
Wei et al 2023	ClO_2/Storage	Whole	5.107	0.506	80	10.00	0.061
Golden et al 2019	ClO_2/Storage	Whole	2.5	0	41.5	2.57	0
Golden et al 2019	ClO_2/Storage	Whole	2.273	0	41.5	5.14	0
Golden et al 2019	ClO_2/Storage	Whole	3275	7777.383	41.5	12.86	0.006
Golden et al 2019	ClO_2/Storage	Whole	31.192	10.332	41.5	2.57	0.254
Golden et al 2019	ClO_2/Storage	Whole	2.632	0.069	41.5	5.14	0.005
Golden et al 2019	ClO_2/Storage	Whole	23.905	6.086	41.5	12.86	0.086

Table 4.18: Goodness of fit statistics for our secondary models

Method	AIC	RMSE	R^2	P-Value (Normality)	Predictors
Heat	-3.20	0.214	NA	0.924	Temperautre (°C), a_w
NT	39.90	0.548	0.479	0.425	a_w
ClO_2 (RH)	-4.24	0.177	0.747	0.756	RH (%)
ClO_2 (C)	13.55	0.352	0.006	0.088	Concentration (mg/L)

Table 4.19: Meta-Analysis Results for Heat Treatments by Heat Intensity

Method	Subgroup	Log D	P	k	I^2	τ^2
Heat (Temp)	All Experiments	0.9 (0.69 ; 1.02)	0	39	99.2% (99.1% ; 99.3%)	0.21 (0.12 ; 0.37)
Heat (Temp)	75–85 °C	0.8 (0.39 ; 1.21)	0	11	96.7% (95.4% ; 97.6%)	0.41 (NA ; NA)
Heat (Temp)	60–75°C	0.9 (0.71 ; 1.07)	0	26	99.5% (99.4% ; 99.5%)	0.17 (NA ; NA)
Heat (Temp)	45–60 °C	0.6 (-0.01 ; 1.23)	0.055	2	0.0% (NA ; NA)	0 (NA ; NA)

Table 4.20: Meta-Analysis Results for Heat Treatments by Matrix

Method	Subgroup	Log D	P	k	I^2	τ^2
Heat (Matrix)	All Experiments	0.9 (0.69 ; 1.02)	0	39	99.2% (99.1% ; 99.3%)	0.21 (0.12 ; 0.37)
Heat (Matrix)	Whole	1.9 (1.42 ; 2.46)	0	2	64.2% (0.0% ; 91.8%)	0.1 (NA ; NA)
Heat (Matrix)	Ground	0.8 (0.66 ; 0.96)	0	37	99.3% (99.2% ; 99.3%)	0.16 (NA ; NA)

Table 4.21: Meta-Analysis Results for Heat Treatments by Water Activity

Method	Subgroup	Log D	P	k	I^2	τ^2
Heat (a_w)	All Experiments	0.8 (0.66 ; 0.96)	0	37	99.3% (99.2% ; 99.3%)	0.16 (0.08 ; 0.26)
Heat (a_w)	0.65–0.75	0.5 (0.31 ; 0.69)	0	11	66.4% (36.5% ; 82.2%)	0.04 (NA ; NA)
Heat (a_w)	0.45–0.55	0.9 (0.66 ; 1.09)	0	15	99.2% (99.0% ; 99.3%)	0.14 (NA ; NA)
Heat (a_w)	0.25–0.40	0.9 (0.66 ; 1.23)	0	11	98.9% (98.6% ; 99.1%)	0.2 (NA ; NA)

Table 4.22: Results of Meta–Regression Analysis and Begg and Mazumdar's Rank Correlation Test for Heat–treatment experiments

Statistic	**Value**
Covariates	a_w, Temperature
τ^2	0.027 (SE = 0.011)
I^2	95.21%
H2	20.88
R^2*	83.09%
QE	561.44 ($p < 0.0001$)
QM	111.62 ($p < 0.0001$)
Rank correlation test z	1.39 ($p = 0.016$)
Bias Estimate	115.000 (SE = 82.666)

Table 4.23: *Meta-Analysis Results for* ClO_2*–Treatments by Concentration*

Method	Subgroup	Log D	P	k	I^2	τ^2
ClO_2 by RH	All Experiments	2.0 (1.85 ; 2.11)	0	12	39.6% (0.0% ; 69.4%)	0.02 (0 ; 0.2)
ClO_2 by RH	60% (RH)	2 (1.83 ; 2.24)	0	3	0.0% (0.0% ; 89.6%)	0 (NA ; NA)
ClO_2 by RH	70% (RH)	2 (1.72 ; 2.24)	0	4	50.1% (0.0% ; 83.5%)	0.04 (NA ; NA)
ClO_2 by RH	80% (RH)	1.8 (1.62 ; 1.98)	0	3	0.0% (0.0% ; 89.6%)	0 (NA ; NA)

Table 4.24: Meta-Analysis Results for ClO_2–Treatments by Concentration

Method	Subgroup	Log D	P	k	I^2	τ^2
ClO_2 (conc.)	All Experiments	2.0 (1.85 ; 2.11)	0	12	39.6% (0.0% ; 69.4%)	0.02 (0 ; 0.2)
ClO_2 (conc.)	5 mg/L	1.9 (1.69 ; 2.18)	0	3	0.0% (0.0% ; 89.6%)	0 (NA ; NA)
ClO_2 (conc.)	10 mg/L	2.1 (1.81 ; 2.31)	0	6	65.5% (17.3% ; 85.6%)	0.06 (NA ; NA)
ClO_2 (conc.)	15 mg/L	1.9 (1.63 ; 2.12)	0	3	0.0% (0.0% ; 89.6%)	0 (NA ; NA)

Table 4.25: Results of Meta–Regression Analysis for ClO_2–treatment experiments

Statistic	By Concentration	By RH
τ^2	0.020 (SE = 0.024)	0.01 (SE = 0.019)
I^2	38.50%	23.53%
H2	1.63	1.31
R^2*	0.00%	39.90%
QE	18.08 (p = 0.054)	10.84(p = 0.370)
QM	0.105 (p = 0.747	6.01 (p = 0.014)

Table 4.26: Begg and Mazumdar's Rank Correlation Test for ClO_2–treatments

Statistic	**Value for ClO_2**
Rank correlation test z	0.27 (p =0.784)
Bias Estimate	4.00 (SE = 14.583)

Table 4.27: Meta-Analysis Results for Plasma Treatments by Gas Used.

Method	Subgroup	Log D	P	k	I^2	τ^2
Plasma (Indirect)	Full Group	1.1 (0.83 ; 1.36)	0	2	17.5% (NA ; NA)	0.02 (NA ; NA)
Plasma	Full Group	0.8 (0.23 ; 1.28)	0.005	7	96.7% (95.0% ; 97.8%)	0.47 (0.18 ; 2.45)
Plasma	Has Argon	0.9 (0.45 ; 1.42)	0	4	91.0% (80.1% ; 95.9%)	0.23 (NA ; NA)
Plasma	Air Only	0.5 (-0.63 ; 1.63)	0.387	3	98.6% (97.6% ; 99.2%)	0.96 (NA ; NA)

Table 4.28: Meta-Analysis Results of UV Treatments by Intensity (mW/cm^2)

Method	Subgroup	Log D	P	k	I^2	τ^2
UV (intensity)	Full Group	1.4 (0.73 ; 1.99)	0	6	97.9% (96.9% ; 98.6%)	0.59 (0.21 ; 3.67)
UV (intensity)	0.57–1.7 mW/cm^2	1.5 (0.06 ; 2.89)	0.041	2	96.2% (89.3% ; 98.6%)	1 (NA ; NA)
UV (intensity)	1110–2320 mW/cm^2	1.3 (0.5 ; 2.12)	0.002	4	98.4% (97.5% ; 99.0%)	0.66 (NA ; NA)

Table 4.29: Meta-Analysis Results of UV Treatments by Matrix

Method	Subgroup	Log D	P	k	I^2	τ^2
UV (matrix)	Full Group	1.4 (0.73 ; 1.99)	0	6	97.9% (96.9% ; 98.6%)	0.59 (0.21 ; 3.67)
UV (matrix)	Whole	0.8 (0.63 ; 0.96)	0	2	0.0% (NA ; NA)	0 (NA ; NA)
UV (matrix)	Ground	1.6 (0.73 ; 2.48)	0	4	98.5% (97.6% ; 99.1%)	0.76 (NA ; NA)

Table 4.30: Meta-Analysis Results of Gamma and Electron Beam Irradiation Treatments

Method	Subgroup	Log D	P	k	I^2	τ^2
Gamma Irradiation	Full Group	0.3 (-0.44 ; 1.01)	0.446	4	92.3% (83.4% ; 96.4%)	0.48 (0.12 ; 6.91)
Electron Beam	Full Group	0.3 (-0.47 ; 1.03)	0.471	2	99.4% (99.0% ; 99.7%)	0.29 (NA ; NA)

Table 4.31: Meta-Analysis results of Radiofrequency Heating Treatments by Matrix

Method	Subgroup	Log D	P	k	I^2	τ^2
RFH (Matrix)	All Experiments	0.6 (0.37 ; 0.86)	0	12	82.2% (70.1% ; 89.4%)	0.11 (0.03 ; 0.58)
RFH (Matrix)	Whole	0.3 (0 ; 0.67)	0.053	7	81.2% (62.1% ; 90.7%)	0.11 (NA ; NA)
RFH (Matrix)	Ground	0.9 (0.8 ; 1.01)	0	5	0.0% (0.0% ; 79.2%)	0 (NA ; NA)

Table 4.32: Meta-Analysis for Radiofrequency Heat Treatments by Power

Method	Subgroup	Log D	P	k	I^2	τ^2
RFH (Power)	All Experiments	0.6 (0.37 ; 0.86)	0	12	82.2% (70.1% ; 89.4%)	0.11 (0.03 ; 0.58)
RFH (Power)	6kW	-0.3 (-0.9 ; 0.28)	0.31	3	0.0% (0.0% ; 89.6%)	0 (NA ; NA)
RFH (Power)	12kW	0.9 (0.81 ; 1.01)	0	4	0.0% (0.0% ; 84.7%)	0 (NA ; NA)
RFH (Power)	9kW	0.5 (0.26 ; 0.71)	0	5	83.0% (61.3% ; 92.5%)	0.04 (NA ; NA)

Table 4.33: Meta-Analysis for Radiofrequency Heat Treatments by Steam Ventilation

Method	Subgroup	Log D	P	k	I^2	τ^2
RFH (Ventilation)	All Experiments	0.6 (0.37 ; 0.86)	0	12	82.2% (70.1% ; 89.4%)	0.11 (0.03 ; 0.58)
RFH (Ventilation)	Controlled	-0.3 (-0.9 ; 0.28)	0.31	3	0.0% (0.0% ; 89.6%)	0 (NA ; NA)
RFH (Ventilation)	Not Reported	0.7 (0.51 ; 0.89)	0	9	84.4% (72.0% ; 91.3%)	0.06 (NA ; NA)

Table 4.34: Meta-Analysis for Radiofrequency Heat Treatments by Water Activity

Method	Subgroup	Log D	P	k	I^2	τ^2
RFH (a_w)	All Experiments	0.7 (0.42 ; 0.89)	0	10	84.6% (73.3% ; 91.1%)	0.1 (0.03 ; 0.46)
RFH (a_w)	0.54–0.66	0.5 (0.12 ; 0.87)	0.009	7	85.2% (71.4% ; 92.3%)	0.16 (NA ; NA)
RFH (a_w)	0.76–0.87	0.9 (0.77 ; 0.99)	0	3	0.0% (0.0% ; 89.6%)	0 (NA ; NA)

Table 4.35: Meta–Regression results for Radiofrequency Heating

Statistic	By Ventilation	By Matrix	By a_w	By Power
τ^2	0.018 (SE = 0.016	0.031 (SE = 0.024)	0.106 (SE = 0.070)	0.020 (SE = 0.017)
I^2	62.66%	72.82%	92.38%	63.83%
H2	2.68	3.68	13.13	2.76
R^2*	84.07%	72.29%	0.00%	82.12%
QE	27.67 (p = 0.0011)	35.38 (p = 0.0001)	47.20 (p < 0.0001)	29.77 (p = 0.0009)
QM	18.49 (p < 0.0001)	11.03 (p = 0.0009)	1.20 (p = 0.274)	18.72 (p < 0.0001)

Table 4.36: Begg and Mazumdar's Rank Correlation Test for Radiofrequency–treatments

Statistic	Value for ClO_2
Rank correlation test z	0.14 (p = 0.891)
Bias Estimate	2.00 (SE = 14.583)

Table 4.37: Meta-Analysis Results for Steam Treatment by Temperature

Method	Subgroup	Log D	P	k	I^2	τ^2
Steam (Temperature)	All Experiments	-1.4 (-1.84 ; -0.99)	0.00	5	87.5% (73.3% ; 94.2%)	0.19 (0.05 ; 1.72)
Steam (Temperature)	88–100°C	-1.2 (-1.37 ; -0.95)	0.00	3	0.0% (0.0% ; 89.6%)	0 (NA ; NA)
Steam (Temperature)	120–140°C	-1.5 (-2.01 ; -1)	0.00	1	NA (NA ; NA)	NA (NA ; NA)
Steam (Temperature)	160–180°C	-2.2 (-2.43 ; -1.87)	0.00	1	NA (NA ; NA)	NA (NA ; NA)

Table 4.38: Meta-Analysis results for Steam Treatments by Water Activity and Method

Method	Subgroup	Log D	P	k	I^2	τ^2
Steam (a_w /Method)	All Experiments	-1.4 (-1.84 ; -0.99)	0.00	5	87.5% (73.3% ; 94.2%)	0.19 (0.05 ; 1.72)
Steam (a_w /Method)	0.65 /Superheated	-1.6 (-2.2 ; -1.09)	0.00	3	90.2% (74.1% ; 96.3%)	0.21 (NA ; NA)
Steam (a_w /Method)	0.33/Vacuum	-1.1 (-1.36 ; -0.74)	0.00	2	0.0% (NA ; NA)	0 (NA ; NA)

Table 4.39: Meta-Analysis Results for Miscellaneous Treatments

Method	Subgroup	Log D	P	k	I^2	τ^2
EtO	All Experiments	0.2 (-0.09 ; 0.54)	0.155	8	56.8% (5.1% ; 80.4%)	0.09 (0 ; 0.75)
EtO	30% (RH)	0.3 (-0.33 ; 0.86)	0.376	3	54.6% (0.0% ; 87.0%)	0.15 (NA ; NA)
EtO	40% (RH)	0.3 (-0.33 ; 0.94)	0.345	3	80.3% (37.7% ; 93.7%)	0.24 (NA ; NA)
EtO	50% (RH)	0.2 (-0.49 ; 0.98)	0.514	2	0.0% (NA ; NA)	0 (NA ; NA)
Acetic Acid	All Experiments	1.6 (1.21 ; 2.01)	0	2	0.0% (NA ; NA)	0 (NA ; NA)
Heat/Humidity	All Experiments	0.1 (-0.34 ; 0.56)	0.64	3	85.5% (57.5% ; 95.1%)	0.13 (0.02 ; 6.1)
PH_3	All Experiments	3.2 (3.06 ; 3.34)	0	4	0.0% (0.0% ; 84.7%)	0 (0 ; 0.24)
PH_3	0.92 (a_w)	3.1 (2.82 ; 3.38)	0	2	0.0% (NA ; NA)	0 (NA ; NA)
PH_3	0.97 (a_w)	3.2 (3.04 ; 3.4)	0	2	20.7% (NA ; NA)	0 (NA ; NA)
UV/Heating	All Experiments	0.5 (0.18 ; 0.87)	0.003	2	60.0% (0.0% ; 90.6%)	0.04 (NA ; NA)

Table 4.40: Meta-analysis results for Ethylene-Oxide Treatments by Temperature and Relative Humidity

Method	Subgroup	Log D	P	k	I^2	τ^2
EtO (Temp)	46°C	-0.1 (-0.28 ; 0.02)	0.085349	3	0.0% (0.0% ; 89.6%)	0 (NA ; NA)
EtO (Temp)	53°C	0.3 (-0.35 ; 0.97)	0.355966	3	53.8% (0.0% ; 86.8%)	0.19 (NA ; NA)
EtO (Temp)	60°C	0.6 (-0.03 ; 1.25)	0.061408	2	37.2% (NA ; NA)	0.09 (NA ; NA)
EtO (RH)	All Experiments	0.2 (-0.09 ; 0.54)	0.155057	8	56.8% (5.1% ; 80.4%)	0.09 (0 ; 0.75)
EtO (RH)	30% RH	0.3 (-0.33 ; 0.86)	0.375508	3	54.6% (0.0% ; 87.0%)	0.15 (NA ; NA)
EtO (RH)	40% RH	0.3 (-0.33 ; 0.94)	0.345366	3	80.3% (37.7% ; 93.7%)	0.24 (NA ; NA)
EtO (RH)	50% RH	0.2 (-0.49 ; 0.98)	0.513504	2	0.0% (NA ; NA)	0 (NA ; NA)

Table 4.41: Meta-analysis results for UV/Heating Treatments

Method	Subgroup	Log D	P	k	I^2	τ^2
UV/Heating	All Experiments	0.5 (0.18 ; 0.87)	0.003	2	60.0% (0.0% ; 90.6%)	0.04 (NA ; NA)

Table 4.42: Meta-Analysis Results for Phosphine Fumigation Treatments

Method	Subgroup	Log D	P	k	I^2	τ^2
PH_3	All Experiments	3.2 (3.06 ; 3.34)	0	4	0.0% (0.0% ; 84.7%)	0 (0 ; 0.24)
PH_3 (a_w)	0.92	3.1 (2.82 ; 3.38)	0	2	0.0% (NA ; NA)	0 (NA ; NA)
PH_3 (a_w)	0.97	3.2 (3.04 ; 3.4)	0	2	20.7% (NA ; NA)	0 (NA ; NA)

Table 4.43: Meta-Analysis Results for Heat/Humidity Treatments

Method	Subgroup	Log D	P	k	I^2	τ^2
Heat/Humidity	All Experiments	0.1 (-0.34 ; 0.56)	0.640	3	85.5% (57.5% ; 95.1%)	0.13 (0.02 ; 6.1)

Table 4.44: Meta-analysis results for Acetic Acid Treatments

Method	Subgroup	Log D	P	k	I^2	τ^2
Acetic Acid	All Experiments	1.6 (1.21 ; 2.01)	0	2	0.0% (NA ; NA)	0 (NA ; NA)

Table 4.45: Primary Model Statistics for Other Miscellaneous Methods

Author	Method	Matrix	Log D	RMSE	AIC
Kim et al 2023	NIR	Whole	1.00	0.067	11.377
Kim et al 2023	UV/NIR	Whole	0.44	0.352	3.035
Park et al 2020	TiO_2/UV-A-C	Ground	2.04	0.369	-6.232
Park et al 2020	UV-A-C	Ground	1.86	0.248	-5.05
Rane et al 2020	O_3	Whole	2.60	0	NA
Song and Kang	H_2O_2	Whole	-0.42	NA	NA

Table 4.46: Meta-Analysis Results for Non-Treated Sample Experiments by Matrix

Method	Subgroup	Log D–90 (day)	P	k	I^2	τ^2
NT (matrix)	Full Group	0.8 (0.39 ; 1.28)	0	27	98.5% (98.2% ; 98.7%)	1.23 (0.69 ; 2.25)
NT (matrix)	Whole	0.3 (-0.31 ; 0.83)	0.375	15	96.6% (95.6% ; 97.5%)	1.12 (NA ; NA)
NT (matrix)	Ground	1.5 (1.11 ; 1.97)	0	12	97.7% (96.9% ; 98.2%)	0.49 (NA ; NA)

Table 4.47: Meta-Analysis Results for Non-Treated Sample Experiments by Water Activity

Method	Subgroup	Log D–90 (day)	P	k	I^2	τ^2
NT (a_w)	Full Group	1.2 (0.85 ; 1.52)	0	22	98.5% (98.2% ; 98.7%)	0.53 (0.25 ; 0.97)
NT (a_w)	0.92–9.97	0.2 (0.14 ; 0.27)	0	2	0.0% (NA ; NA)	0 (NA ; NA)
NT (a_w)	0.40–0.67	1.1 (0.76 ; 1.46)	0	13	97.4% (96.6% ; 98.0%)	0.33 (NA ; NA)
NT (a_w)	0.30	1.8 (1.06 ; 2.46)	0	7	93.8% (89.6% ; 96.3%)	0.63 (NA ; NA)

Table 4.48: Meta–Regression Results for No–treatment Experiments

Statistic	Value
Covariates	a_w, Matrix
τ^2	0.245 (SE = 0.105)
I^2	92.50%
H2	13.34
R^2*	53.42%
QE	387.18 ($p < 0.0001$)
QM	21.12 ($p < 0.0001$)

Table 4.49: Meta-Analysis Results for ClO_2/Storage by Concentration

Method	Subgroup	Log D–90 (day)	P	k	I^2	τ^2
ClO_2/Storage by Conc.	Full Group	0.9 (0.52 ; 1.31)	0	6	85.6% (70.7% ; 92.9%)	0.16 (0.03 ; 3.99)
ClO_2/Storage by Conc.	5–5.14 mg/L	0.5 (0.18 ; 0.88)	0.003	2	54.6% (0.0% ; 88.9%)	0.04 (NA ; NA)
ClO_2/Storage by Conc.	10–12.86 mg/L	1.1 (0.39 ; 1.71)	0.002	3	72.7% (8.1% ; 91.9%)	0.2 (NA ; NA)
ClO_2/Storage by Conc.	2.57 mg/L	1.5 (0.84 ; 2.14)	0	1	NA (NA ; NA)	NA (NA ; NA)

Table 4.50: Meta-Analysis Results for ClO_2/Storage by Relative Humidity

Method	Subgroup	Log D–90 (day)	P	k	I^2	τ^2
ClO_2/Storage by RH	Full Group	0.9 (0.52 ; 1.31)	0	6	85.6% (70.7% ; 92.9%)	0.16 (0.03 ; 3.99)
ClO_2/Storage by RH	80% (RH)	0.7 (0.54 ; 0.9)	0	2	0.0% (NA ; NA)	0 (NA ; NA)
ClO_2/Storage by RH	41.5% (RH)	1.1 (0.39 ; 1.81)	0.002	4	88.4% (72.9% ; 95.1%)	0.35 (NA ; NA)

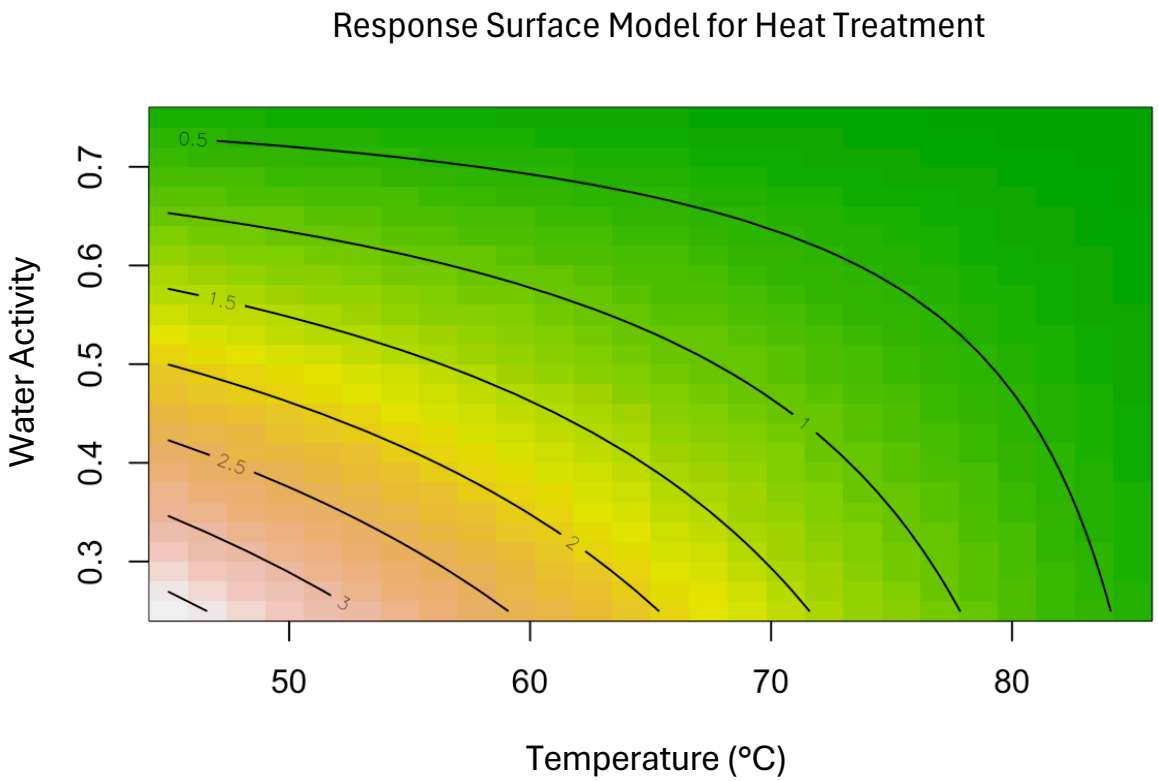


Figure 4.1: Contour plot of temperature and water activity for predicting D–value during heat treatment Deeper green colors correspond to lower log D–values while red/white colors correspond to higher log D–values.

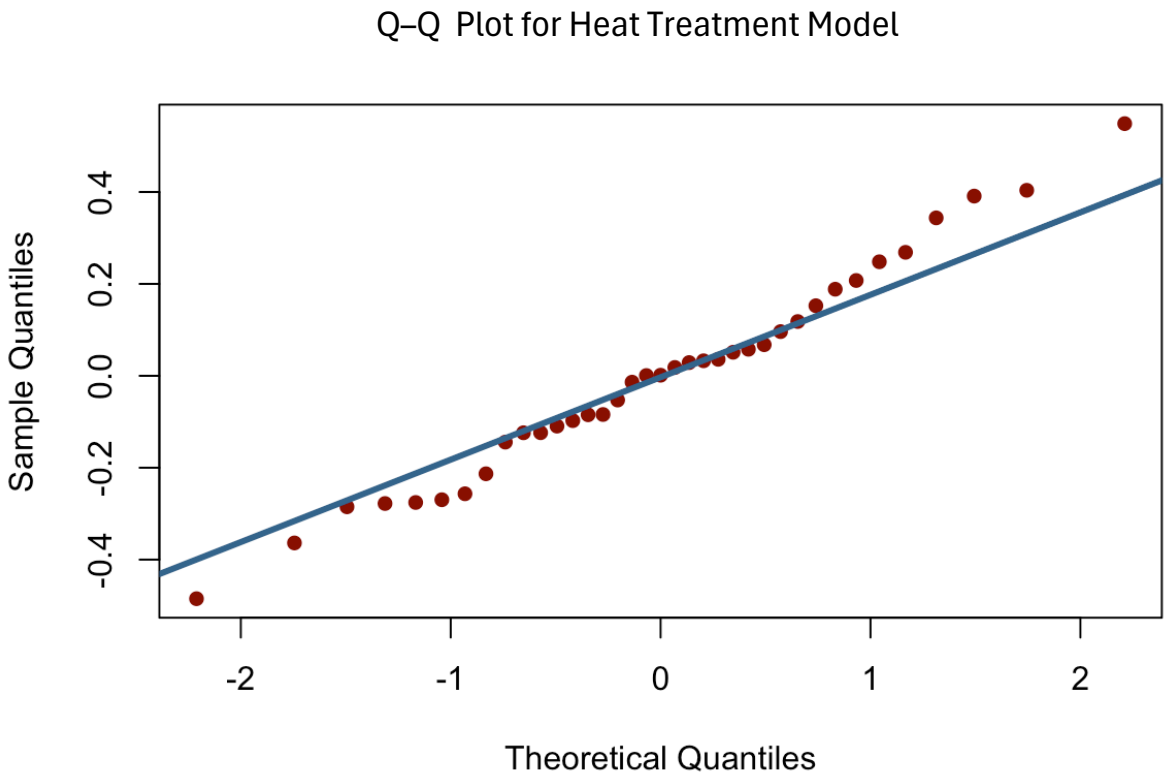


Figure 4.2: Q–Q Plot of the Heat Inactivation Model. Predictors are heat and water activity. Shapiro-Wilkes Test p-Value is 0.924.

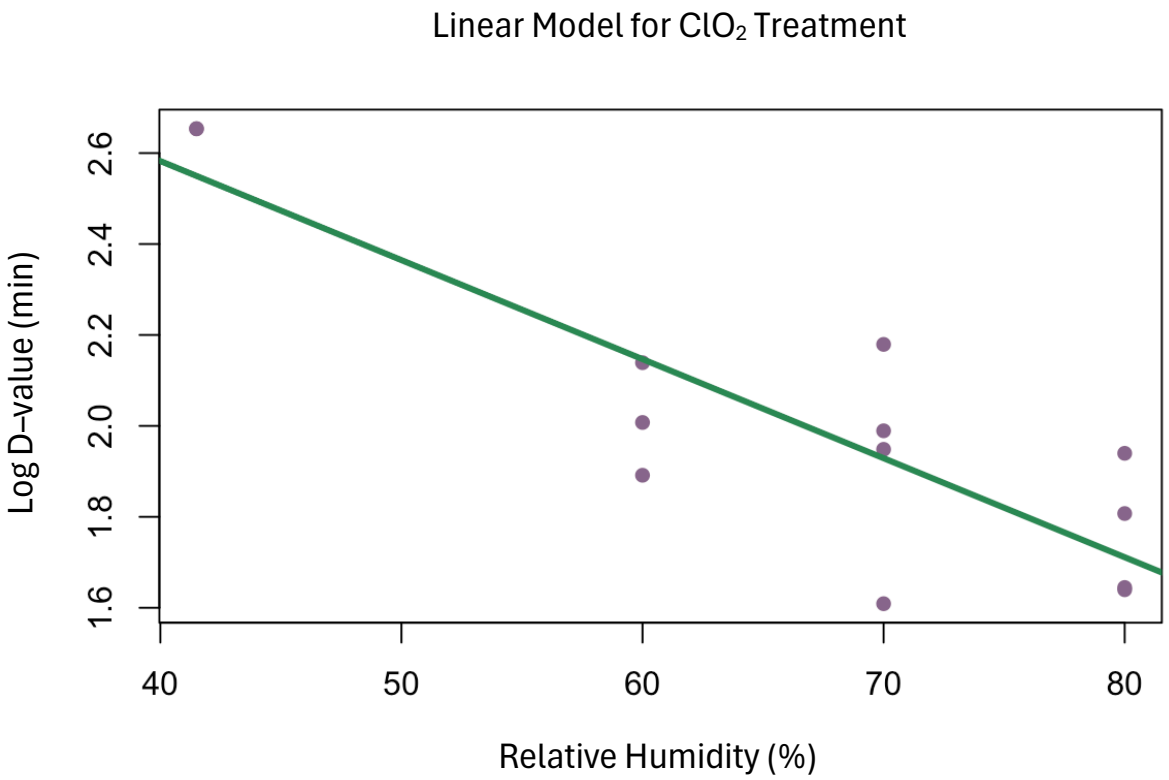


Figure 4.3: Secondary Log-Linear Model for ClO_2– Fumigation Treatment Predicting Log D–values from RH

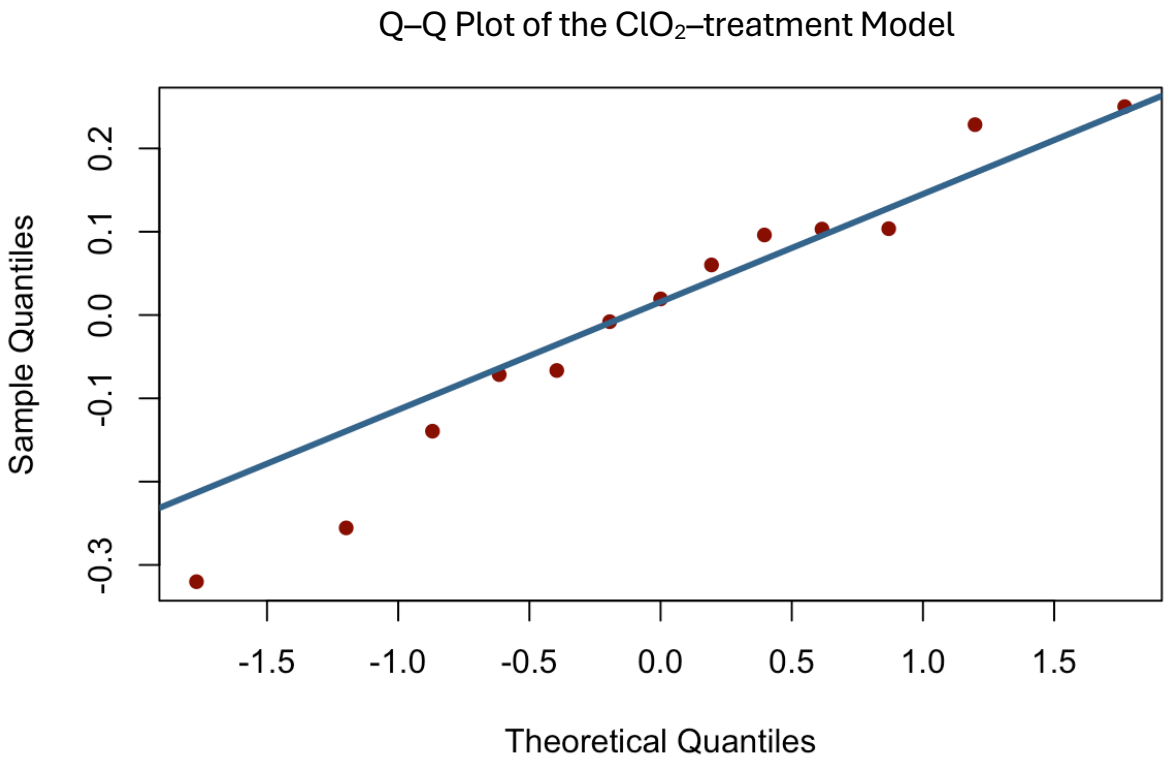


Figure 4.4: Q–Q Plot of the ClO_2–Inactivation Model. Shapiro-Wilkes Test p-Value is 0.756.

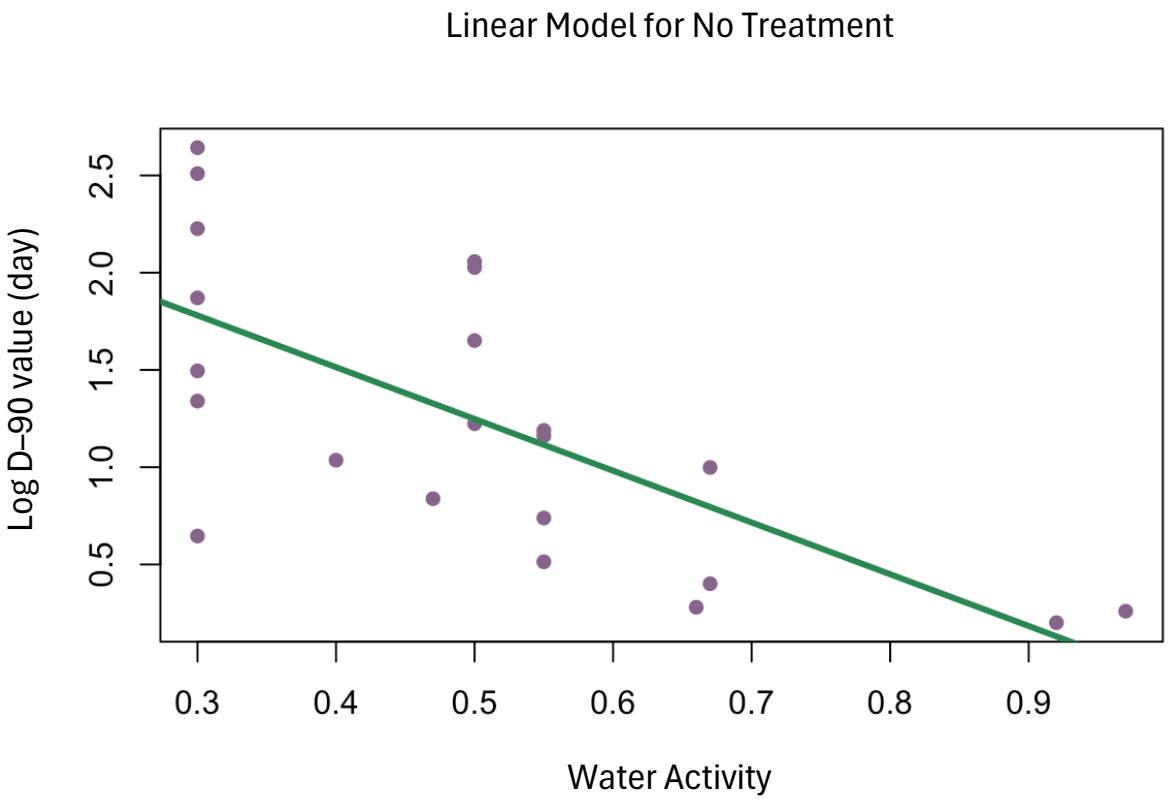


Figure 4.5: Secondary Log-Linear Model for Non-Treated Sample Experiments Predicted from a_w

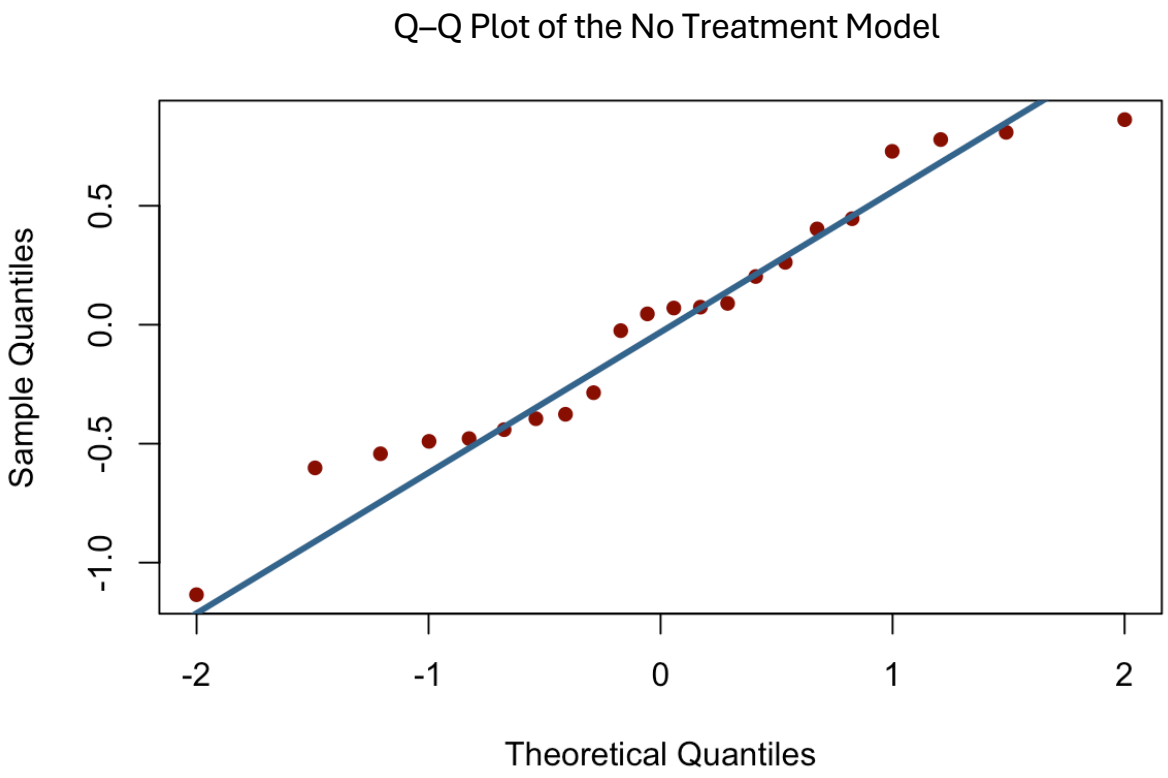


Figure 4.6: Q–Q Plot for Non-Treated Sample Model. Shapiro-Wilkes Test p-Value is 0.425

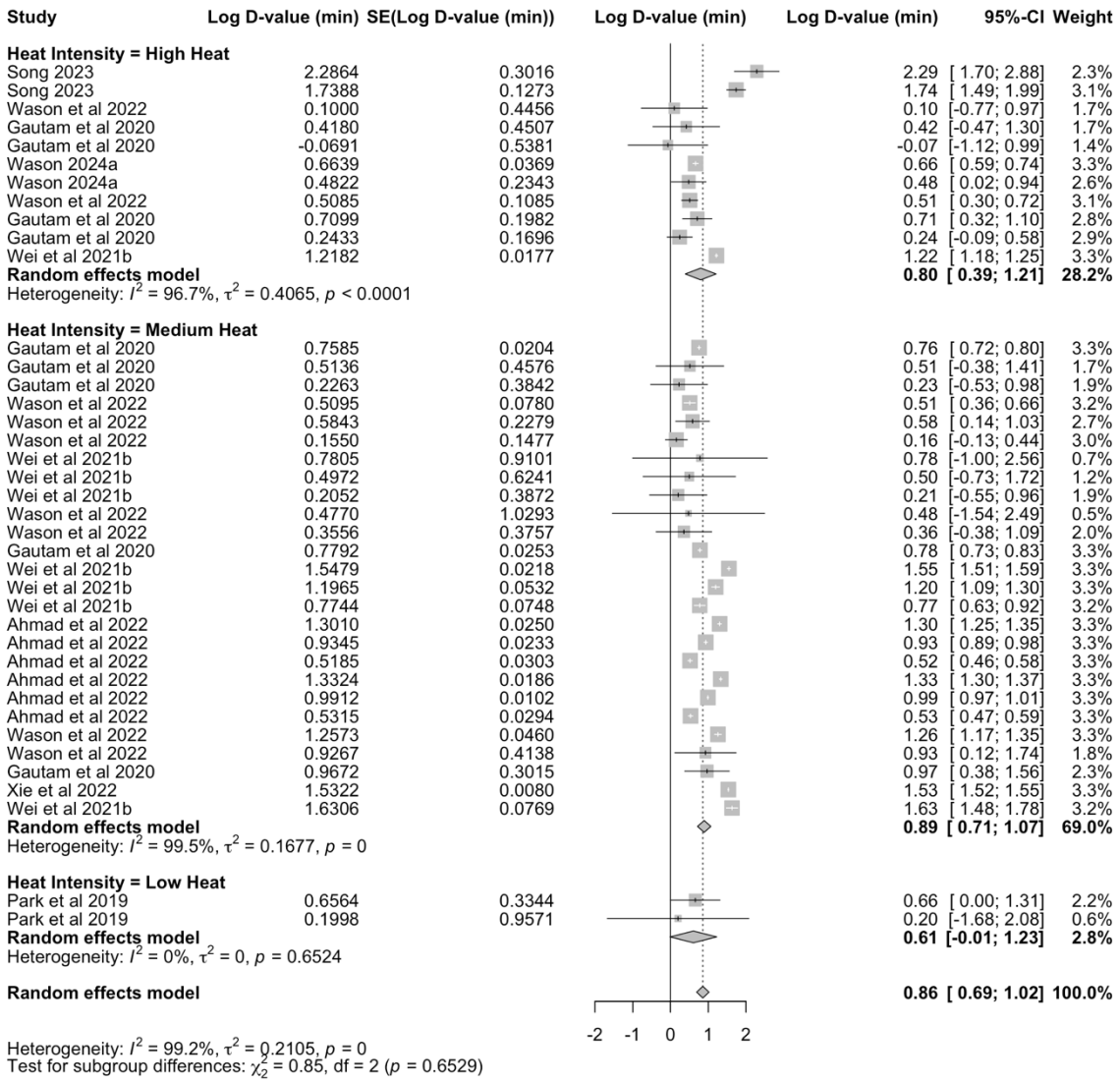


Figure 4.7: Forest Plot for Heat Treatments by Heat Intensity

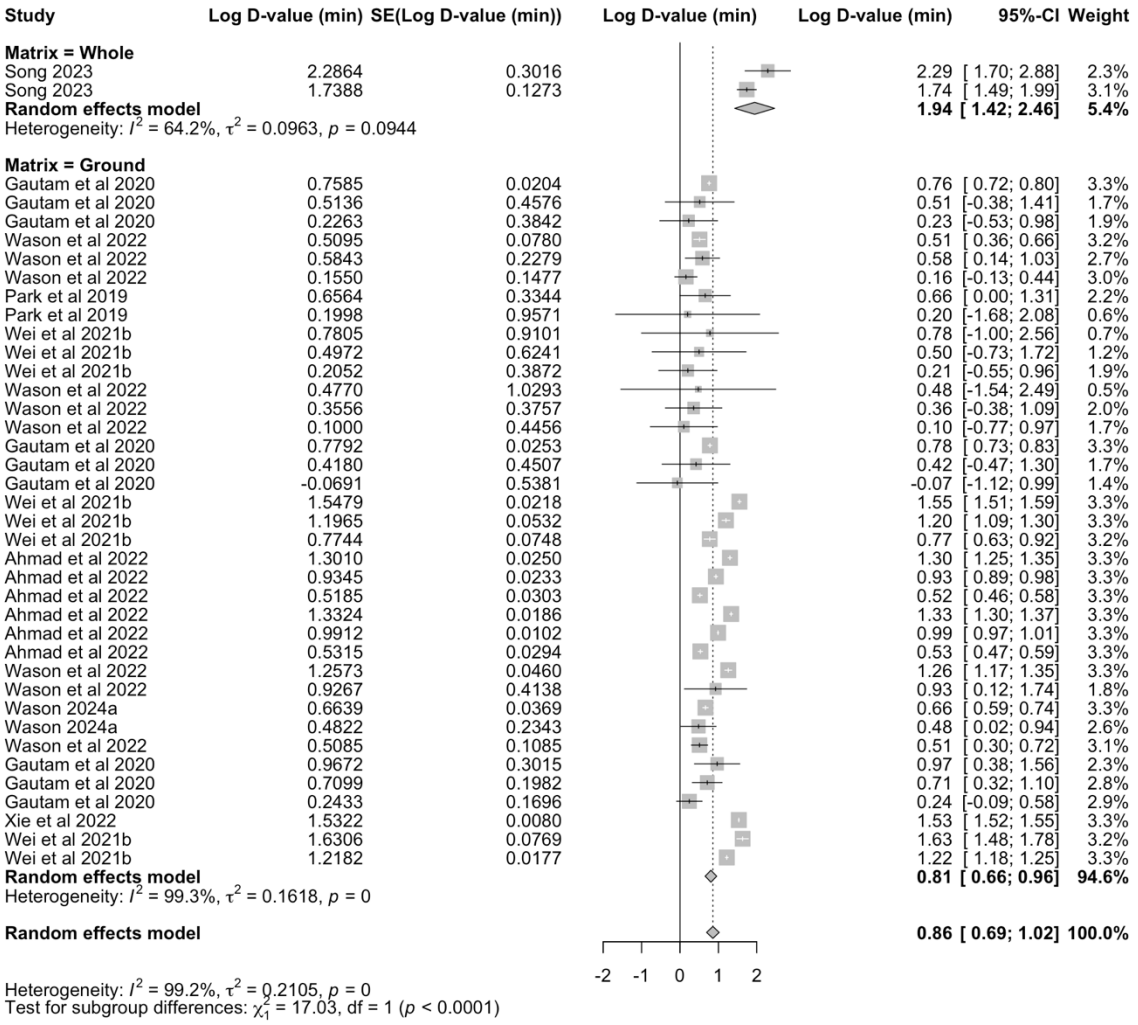


Figure 4.8: Forest Plot for Heat Treatments by Sample Matrix

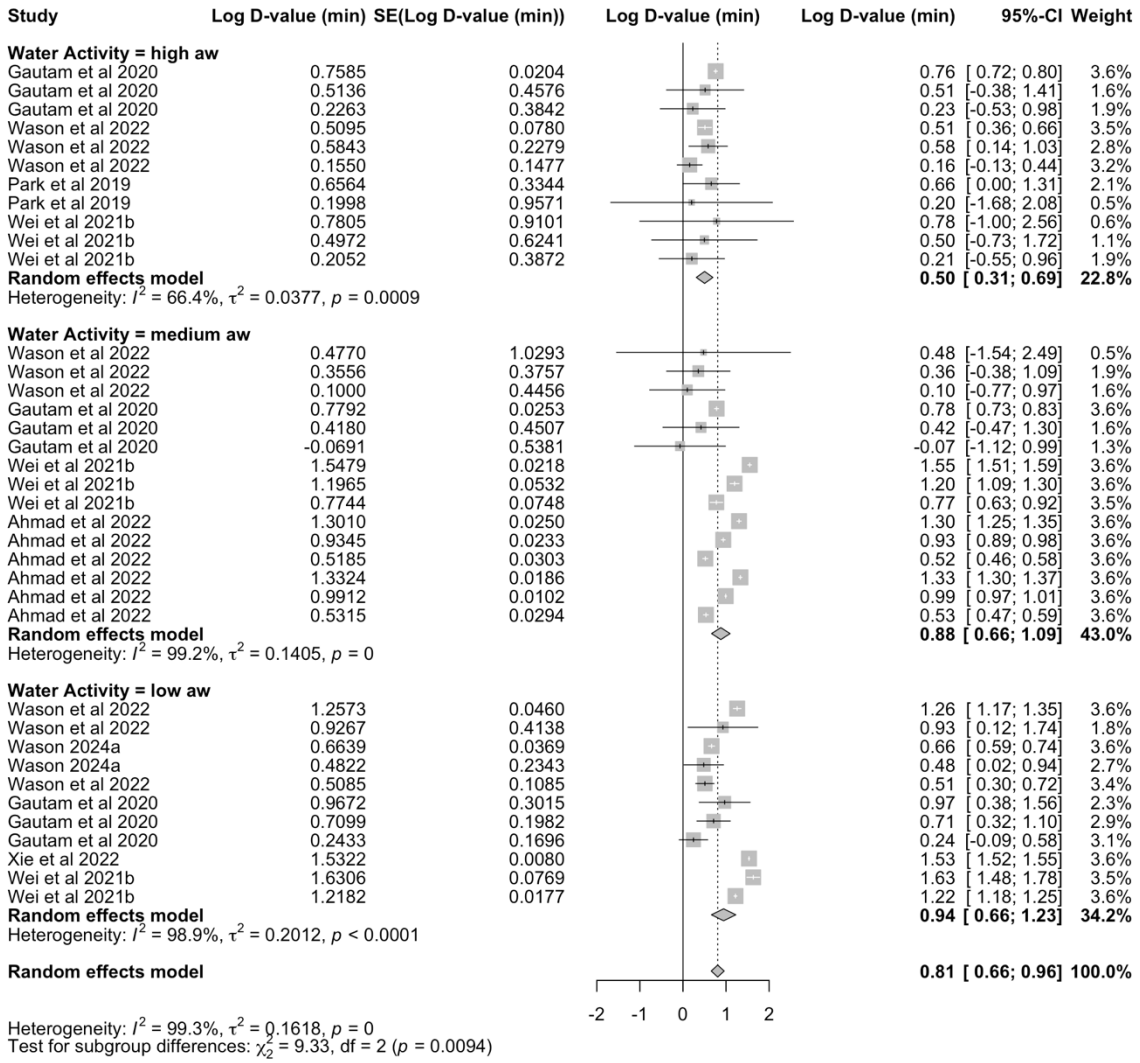


Figure 4.9: Forest Plot for Heat Treatments by Sample Water Activity

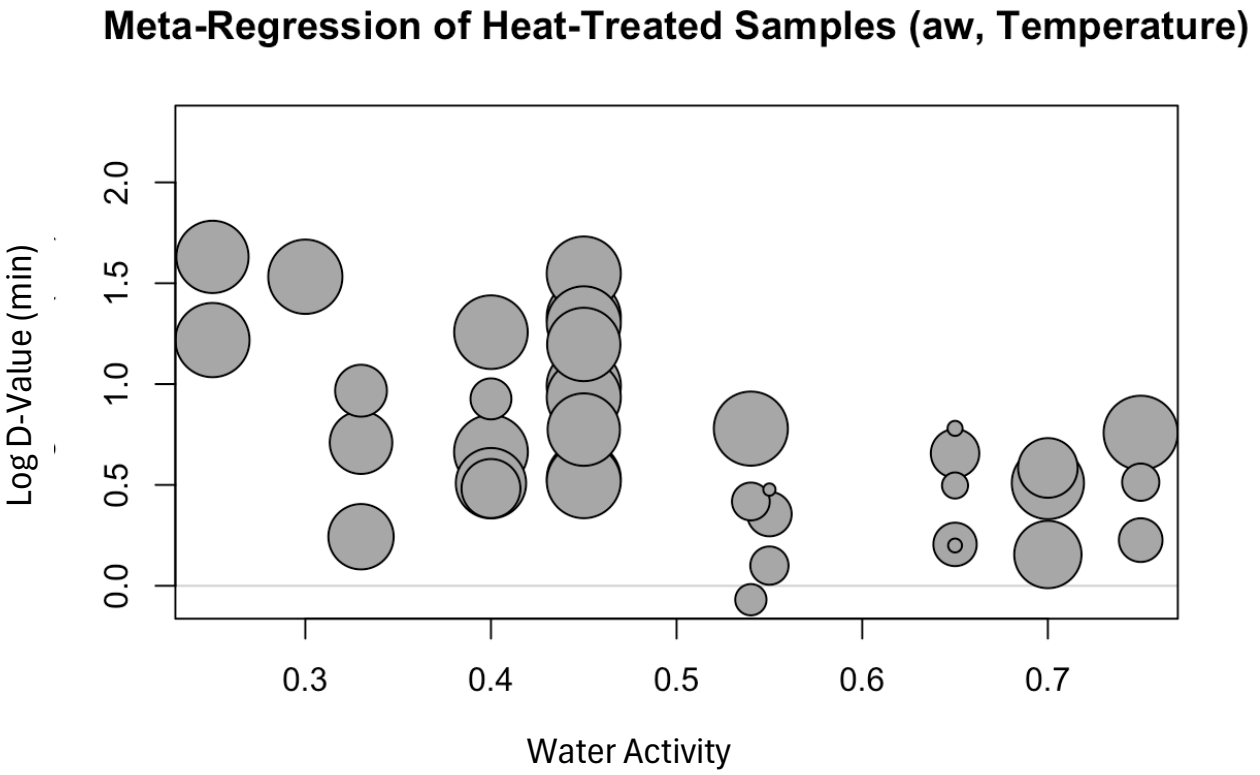


Figure 4.10: Bubble Plot of Heat Treatment Meta–Regression with a_w and Temperature as Predictors. Bubble size corresponds to temperature.

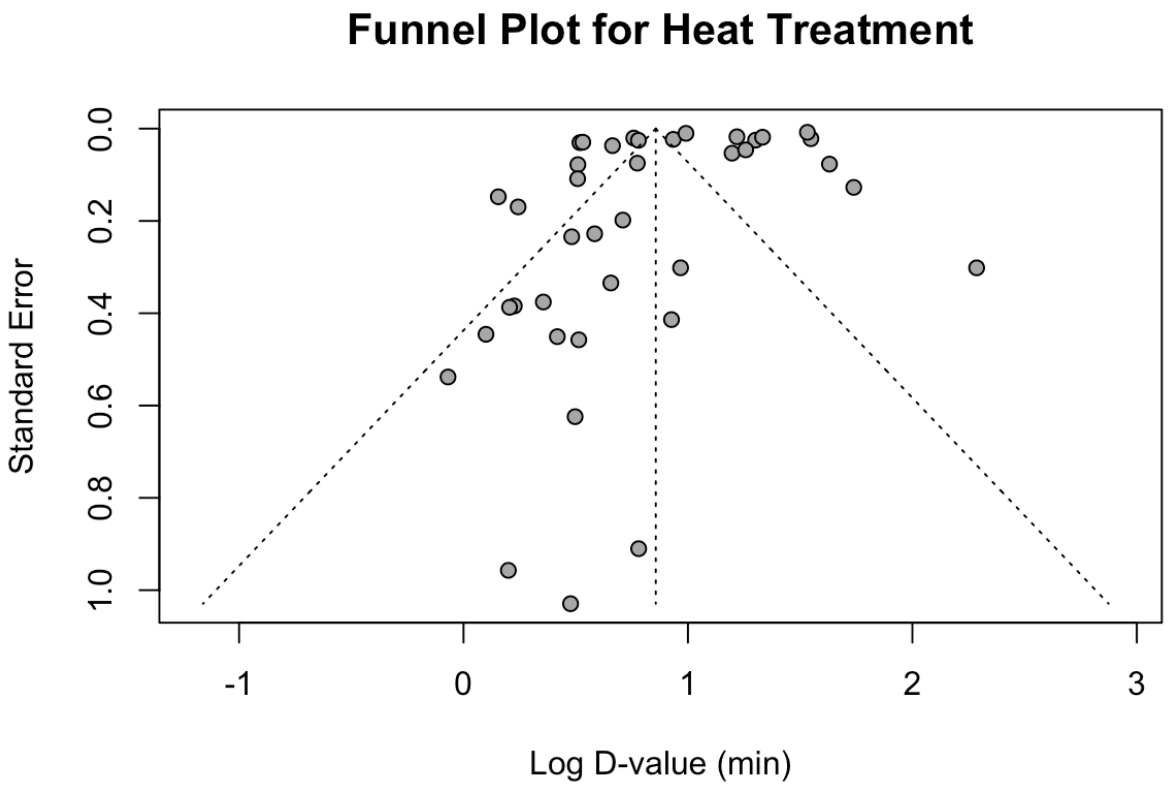


Figure 4.11: Funnel Plot for Heat Treatment Experiments

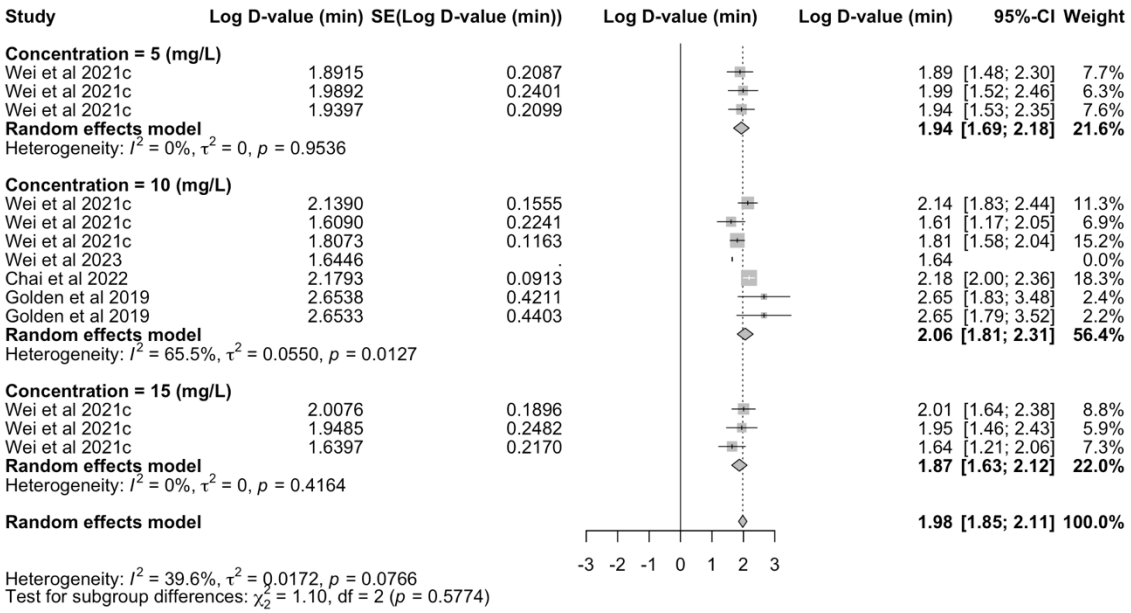


Figure 4.12: Forest Plot for Log D–value of ClO_2-treated Samples Grouped by Concentration

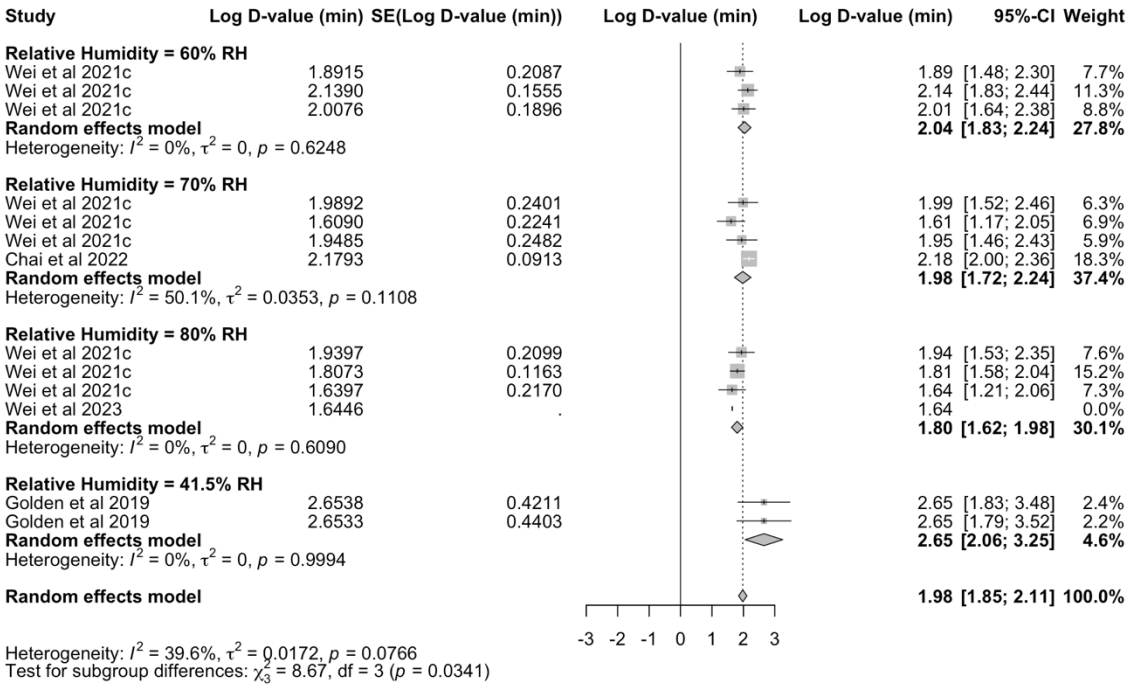


Figure 4.13: Forest Plot for Log D–value of ClO_2-treated Samples Grouped by Relative Humidity

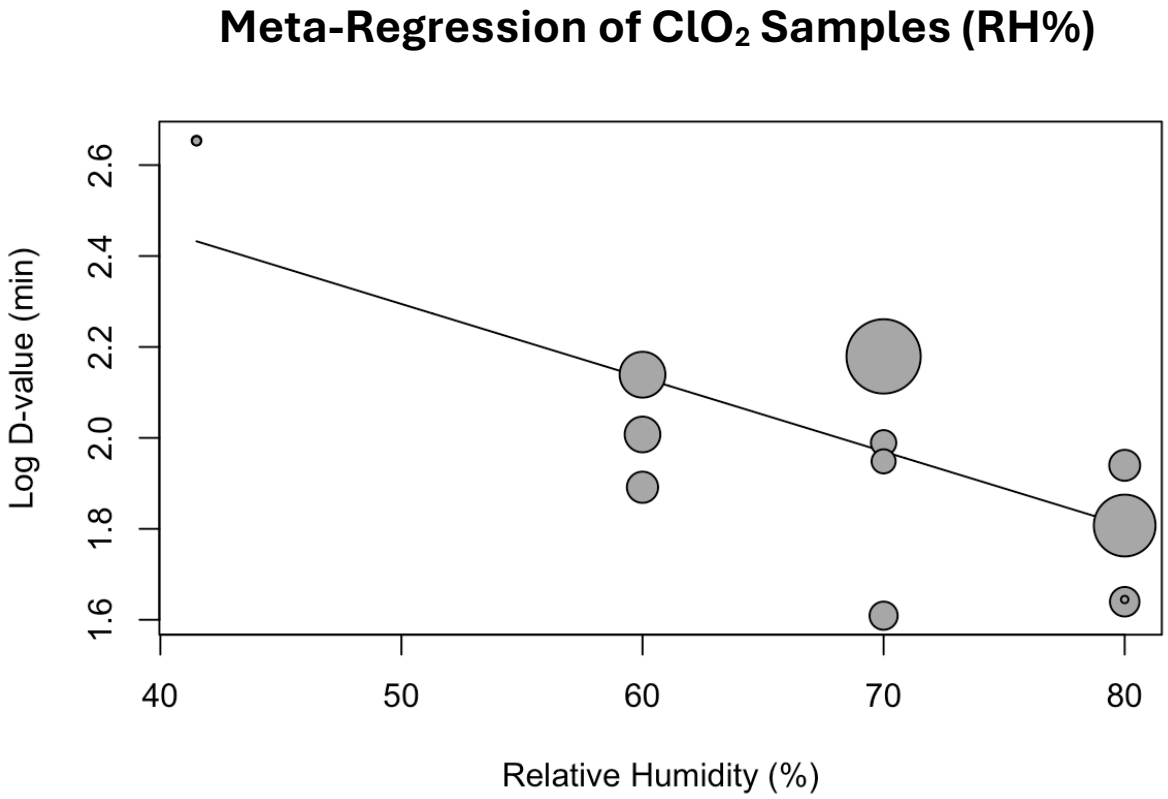


Figure 4.14: Bubble Plot of Meta–Regression for ClO_2-treated Samples, with RH as Covariate

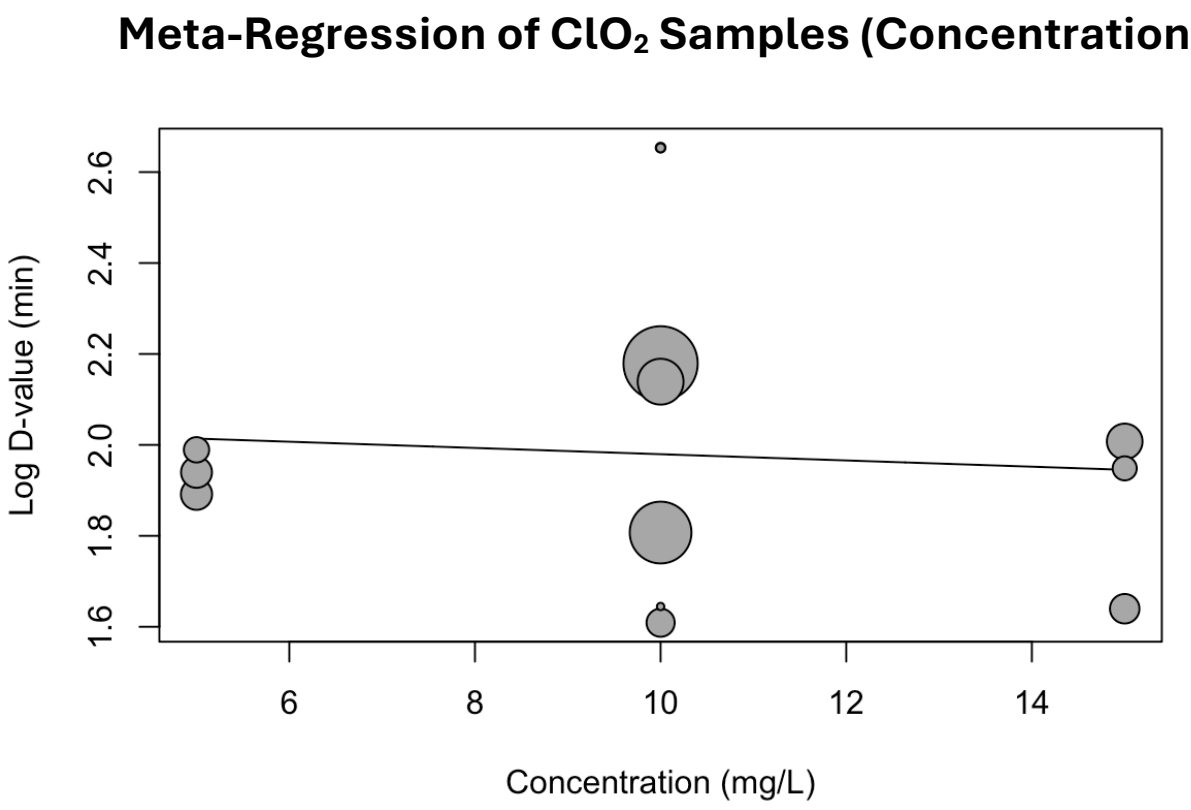


Figure 4.15: Bubble Plot of Meta–Regression for ClO_2-treated Samples, with Concentration as Covariate

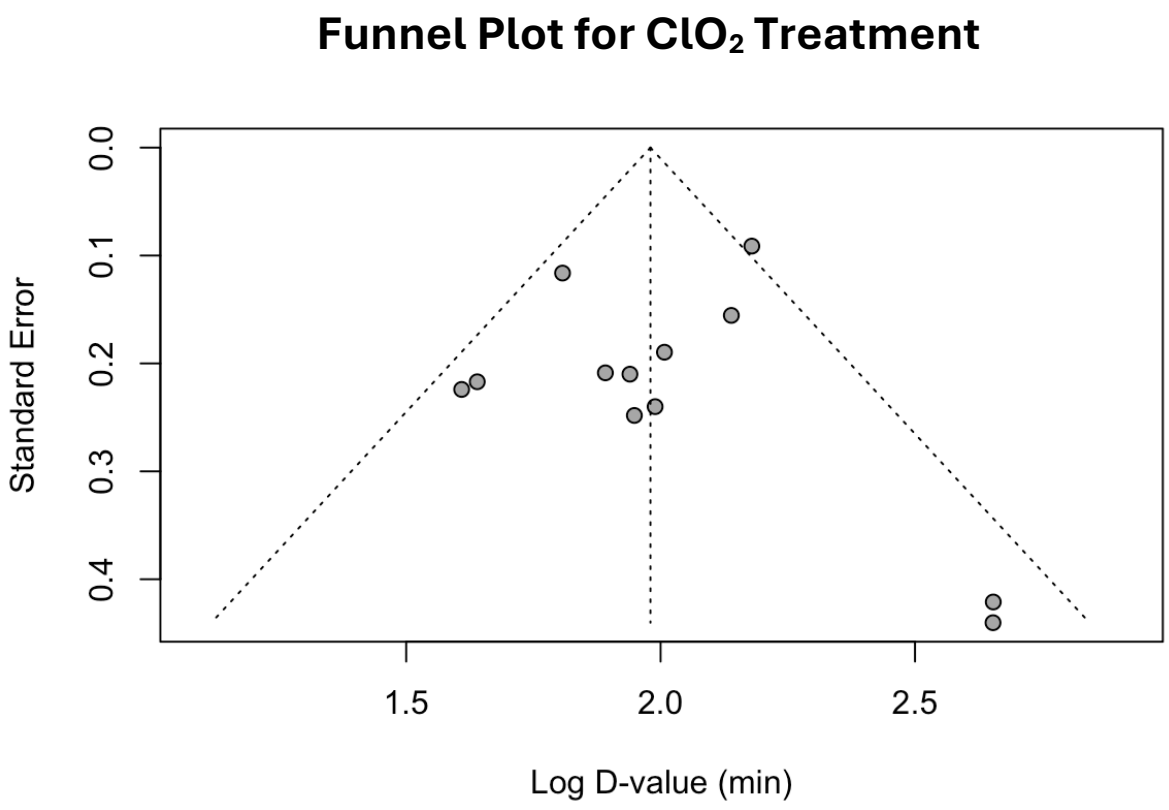


Figure 4.16: Funnel Plot for ClO_2- Treatment Experiments

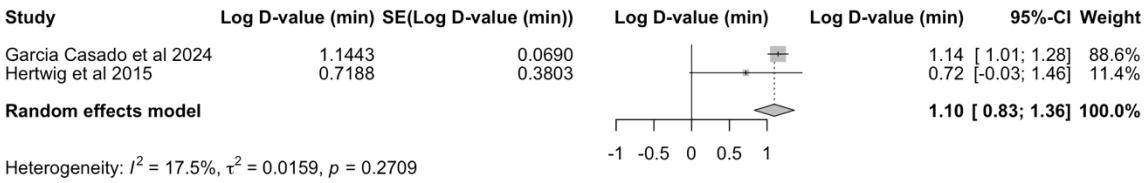


Figure 4.17: Forest Plot for Log D–value of Indirect Plasma-treated Samples Grouped by Relative Humidity

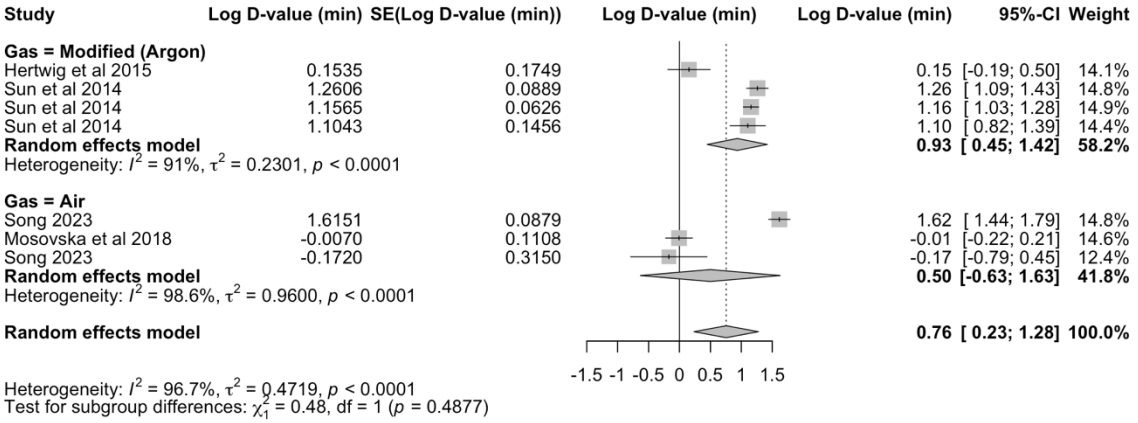


Figure 4.18: Forest Plot for Log D–value of Direct Plasma-treated Samples Grouped by Gas Used

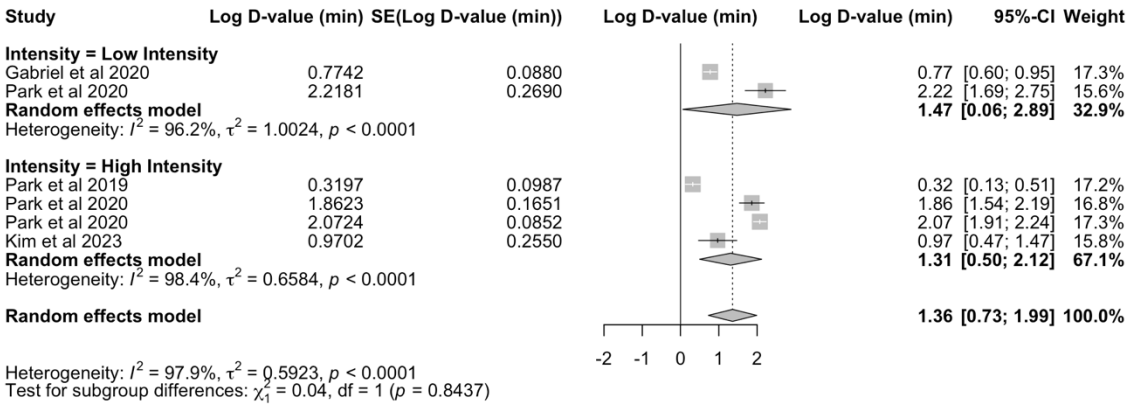


Figure 4.19: Forest Plot for Log D–value of UV-treated Samples Grouped by Intensity (mW/cm^2)

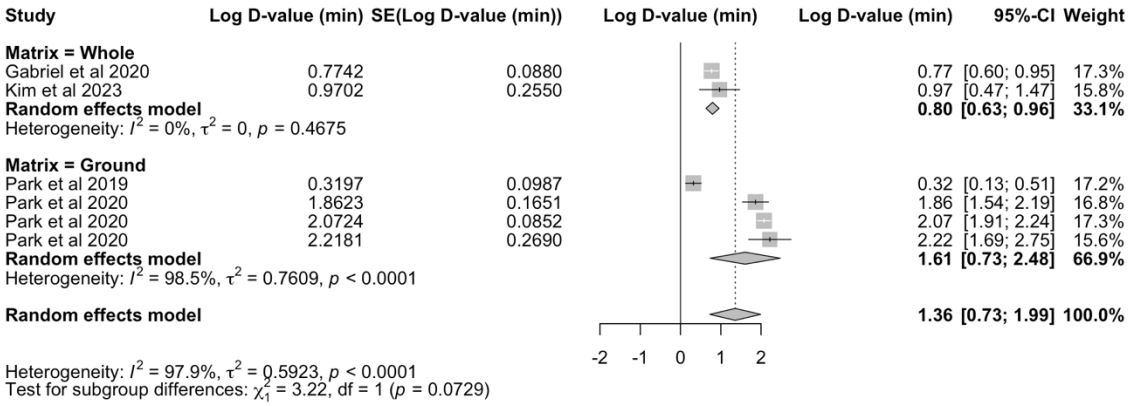


Figure 4.20: Forest Plot for Log D–value of UV-treated Samples Grouped by Matrix

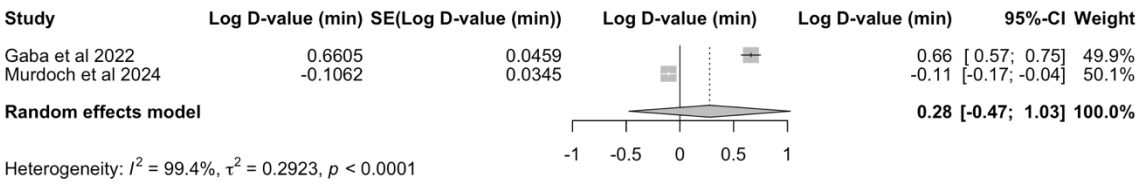


Figure 4.21: Forest Plot for Log D–value of Electron Beam Treatments

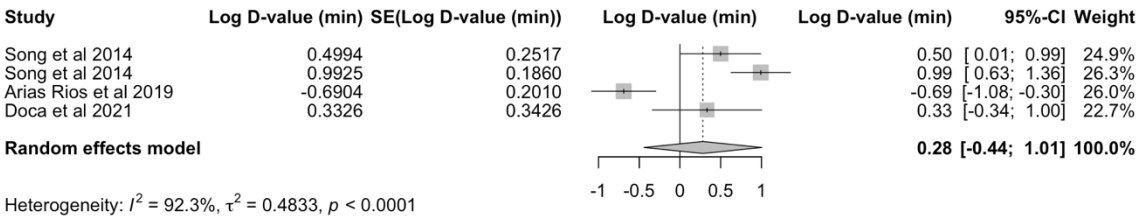


Figure 4.22: Forest Plot for Log D–value of Gamma Irradiation Treatments

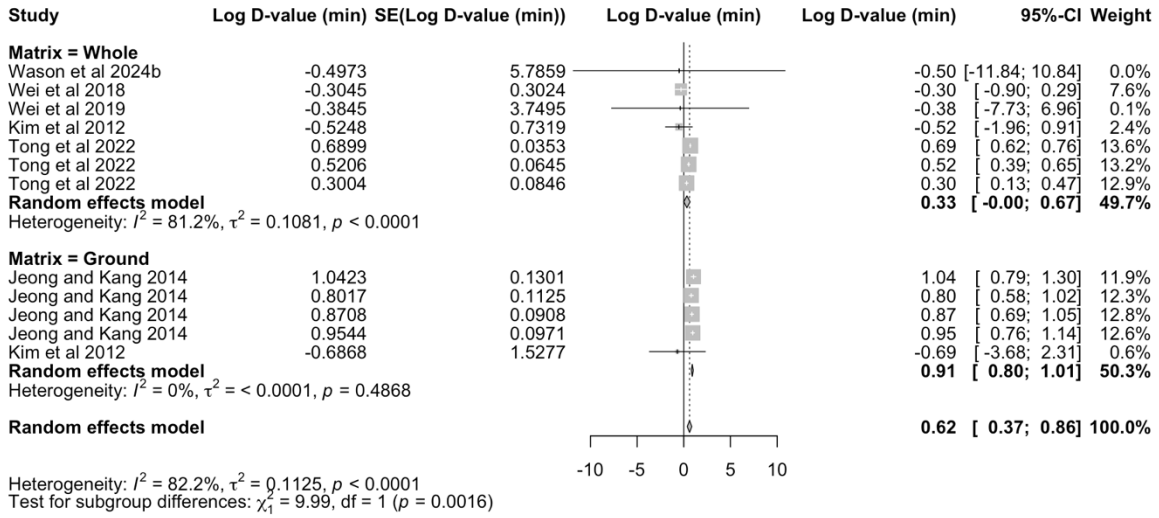


Figure 4.23: Forest Plot for Log D–value of Radiofrequency Heat Treatments by Matrix

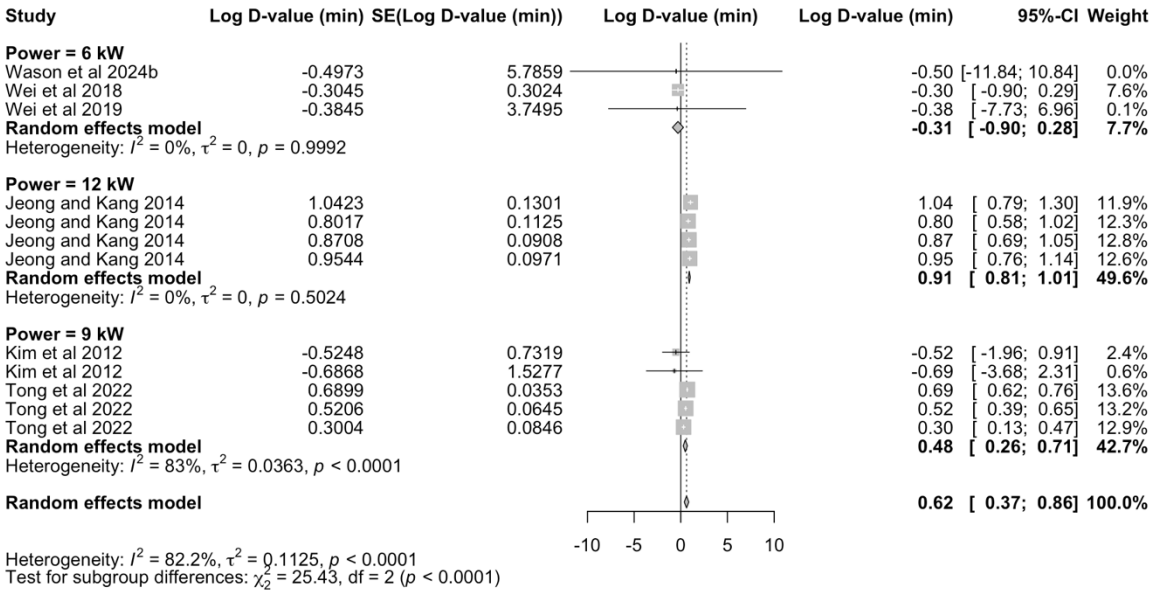


Figure 4.24: Forest Plot for Log D–value of Radiofrequency Heat Treatments by Power

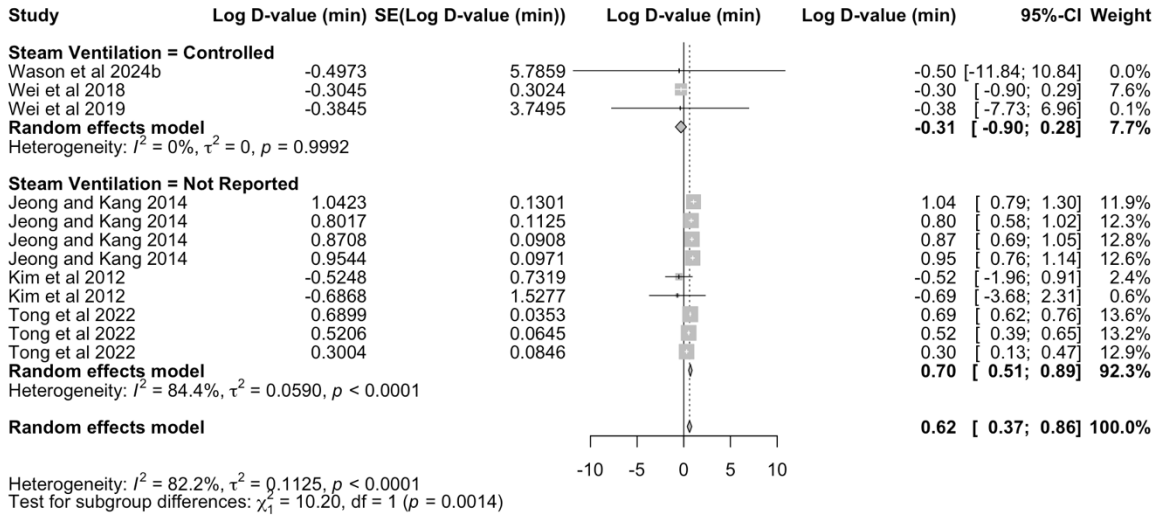


Figure 4.25: Forest Plot for Log D–value of Radiofrequency Heat Treatments by Steam Ventilation

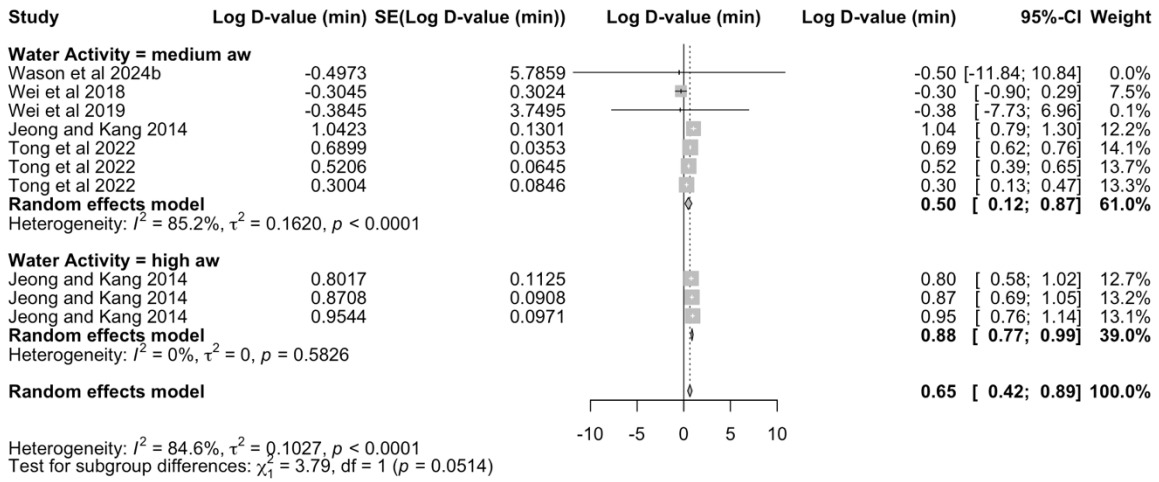


Figure 4.26: Forest Plot for Log D-value of Radiofrequency Heat Treatments by Water Activity

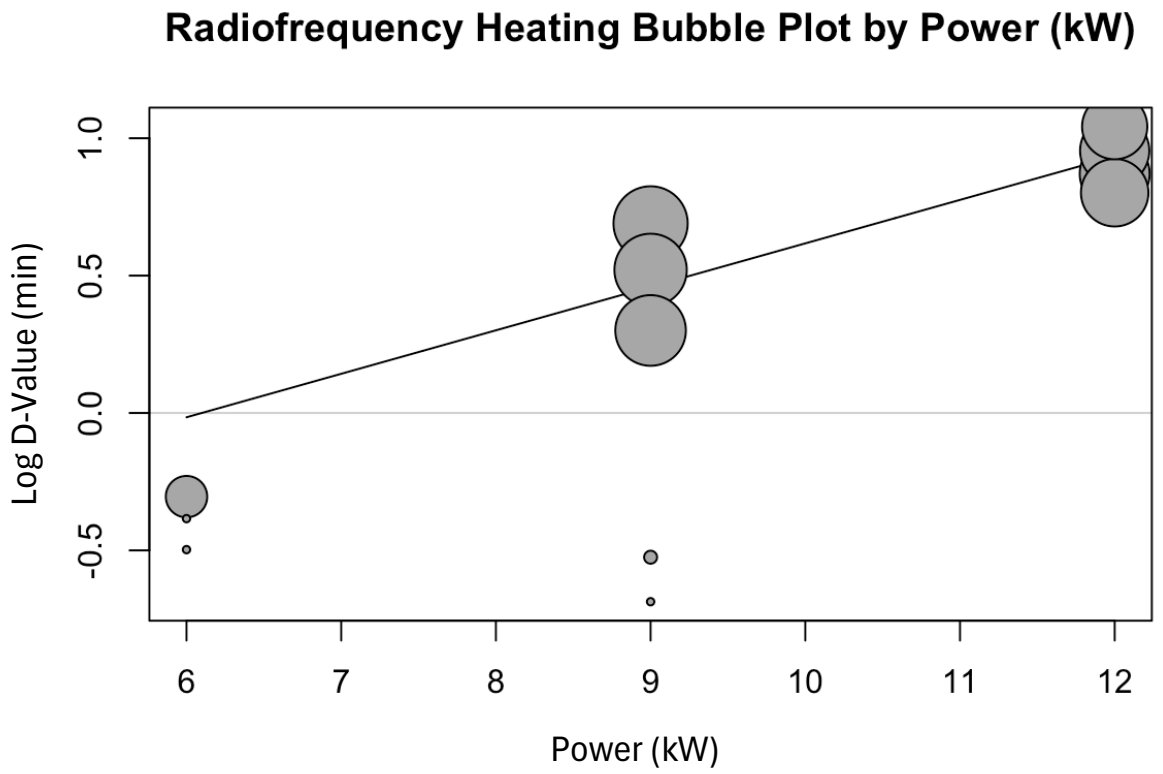


Figure 4.27: Bubble Plot of Meta–Regression for Radiofrequency-treated Samples, with Power as a Covariate

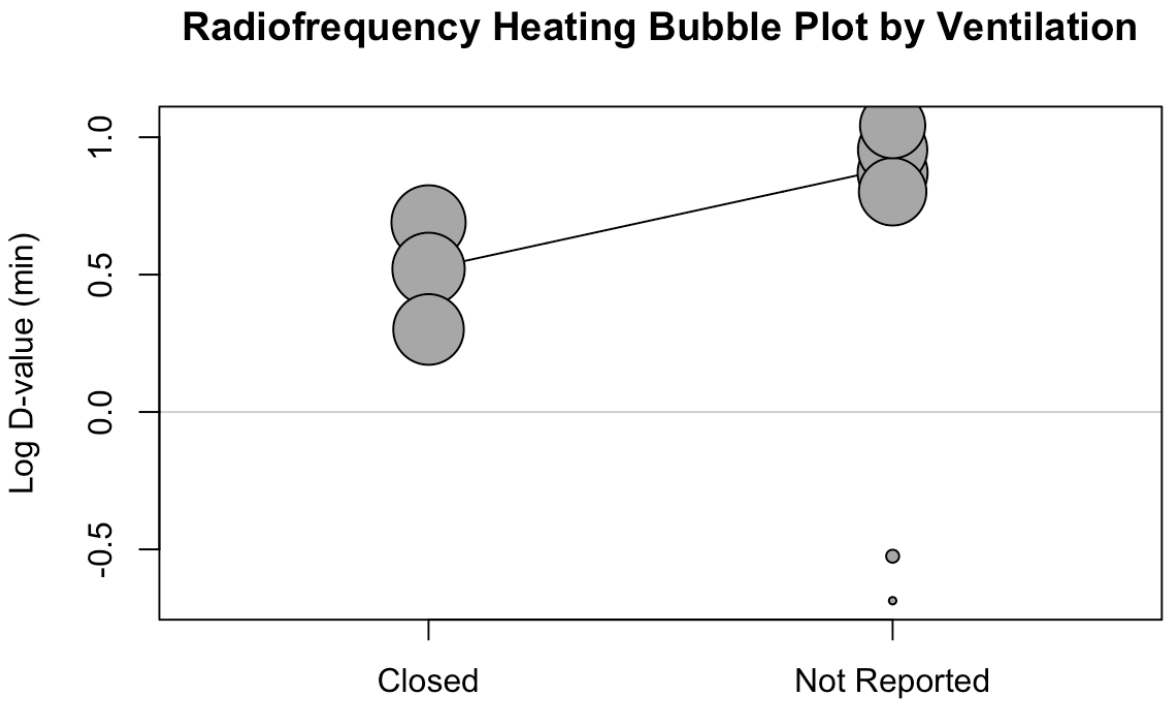


Figure 4.28: Bubble Plot of Meta–Regression for Radiofrequency-treated Samples, with Ventilation as the Covariate

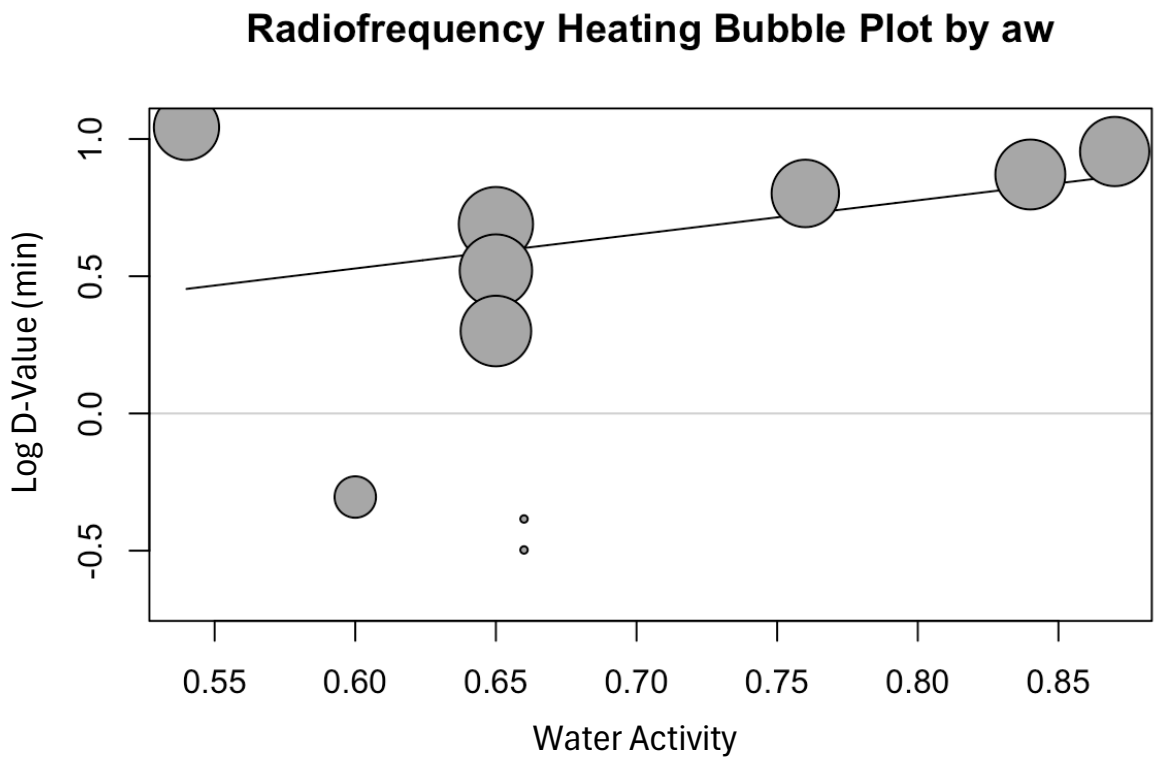


Figure 4.29: Bubble Plot of Meta–Regression for Radiofrequency-treated Samples, with Water Activity as the covariate

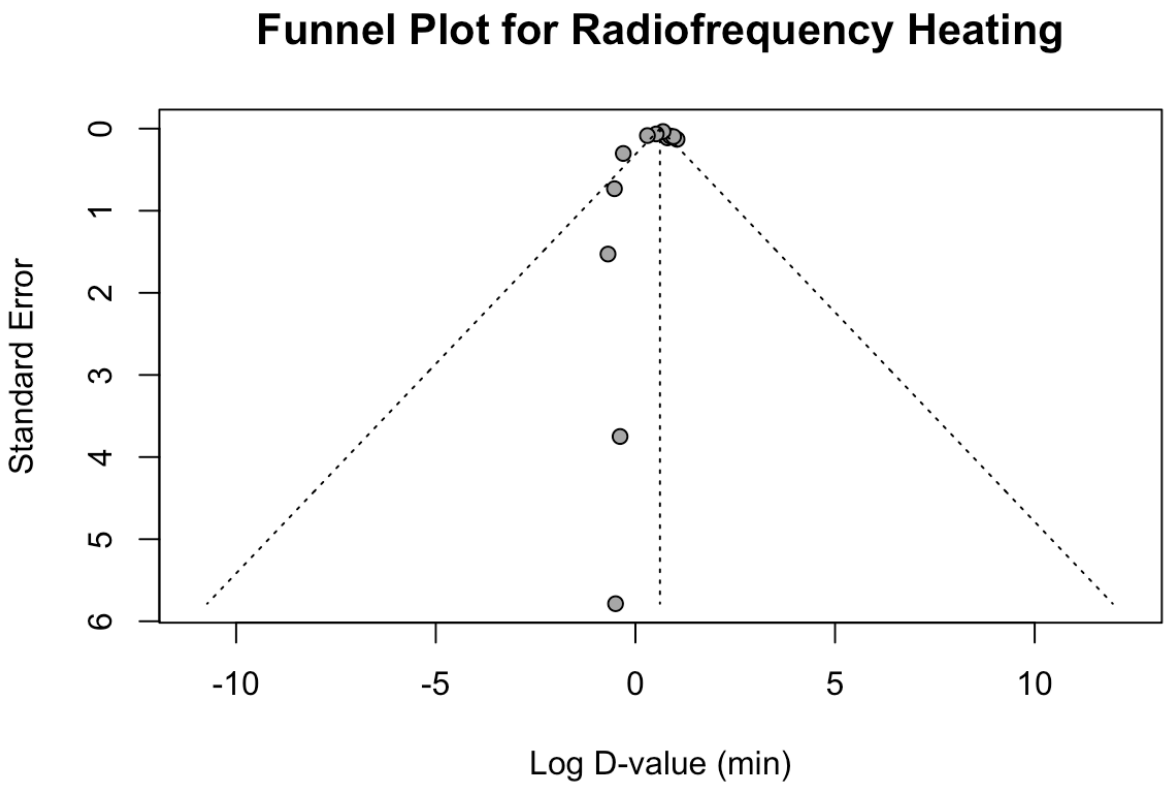


Figure 4.30: Funnel Plot for Radiofrequency Treatment Experiments

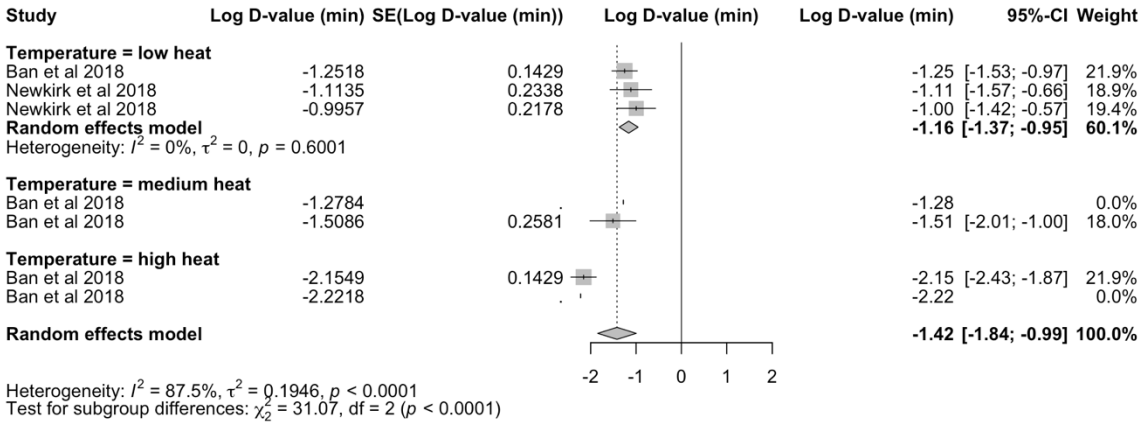
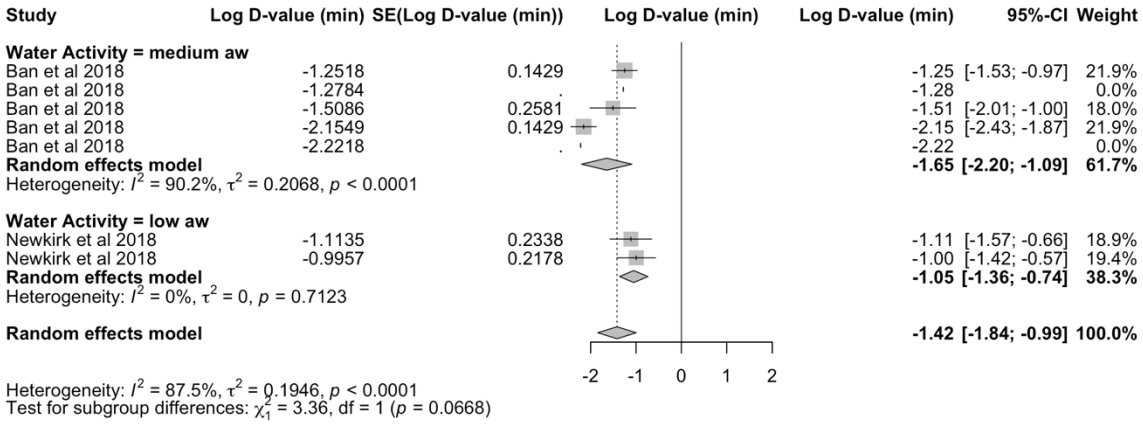


Figure 4.31: Forest Plot of Steam Treatments by Temperature

A)



B)

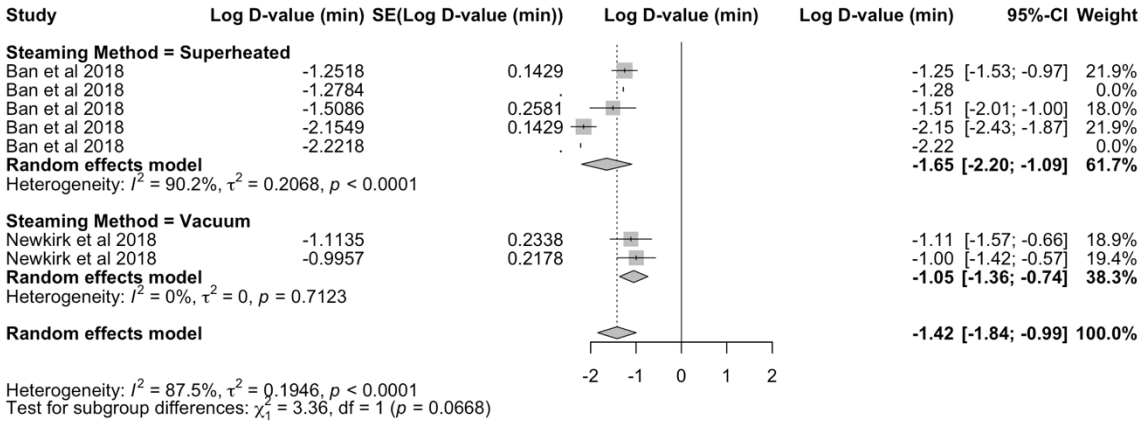


Figure 4.32: Forest plots of Steam Treatments by Water Activity (A) and Steaming Method (B). Forest Plots are Identical.

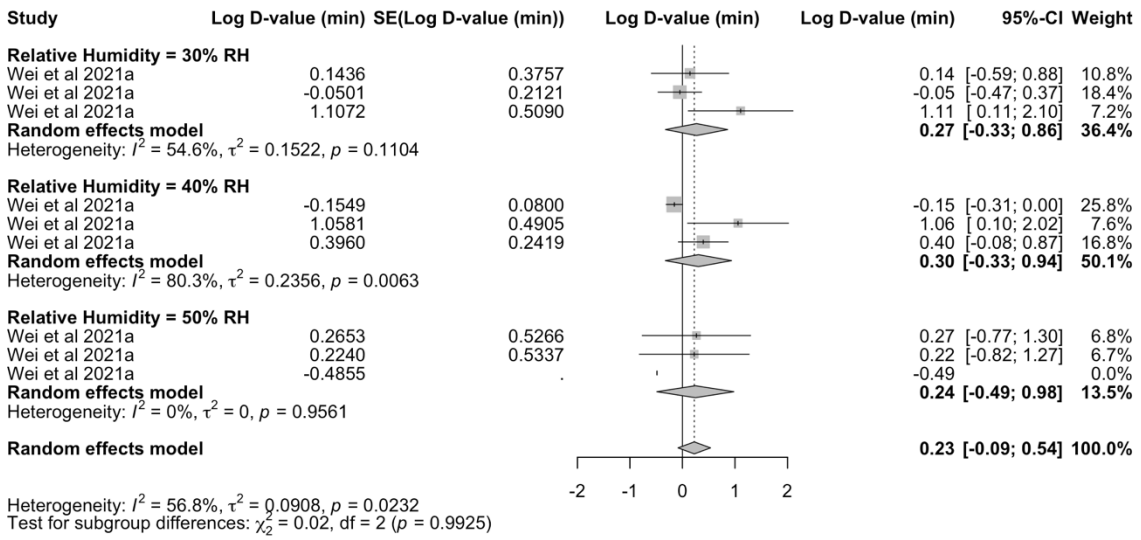


Figure 4.33: Forest Plot of Ethylene-Oxide Treatments by Relative Humidity

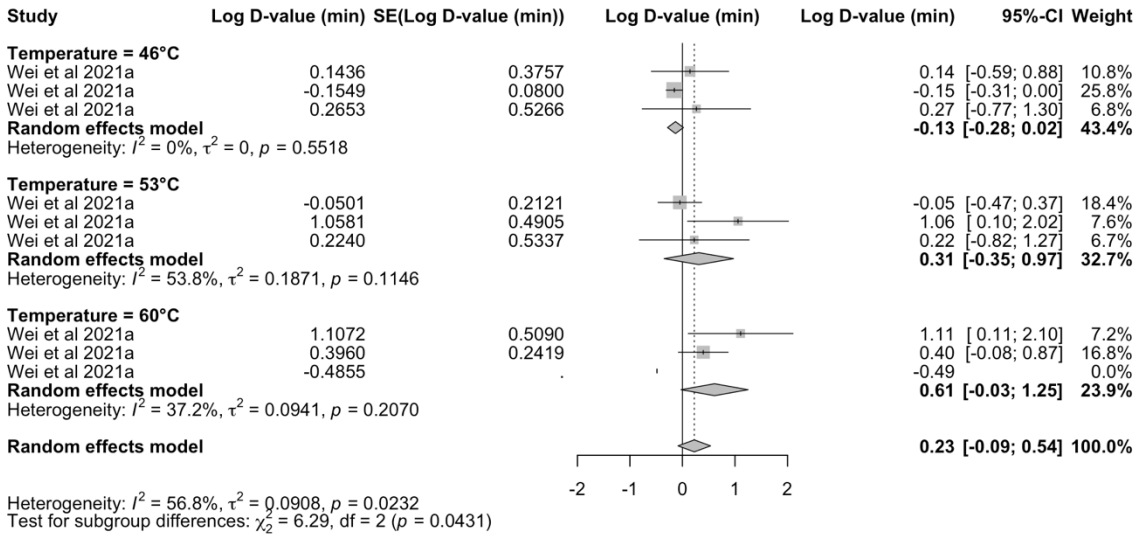


Figure 4.34: Forest Plot of Ethylene-Oxide Treatments by Temperature

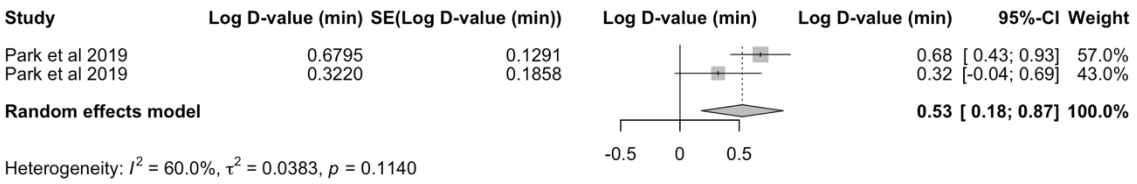


Figure 4.35: Forest Plot for UV/Heating Treatments

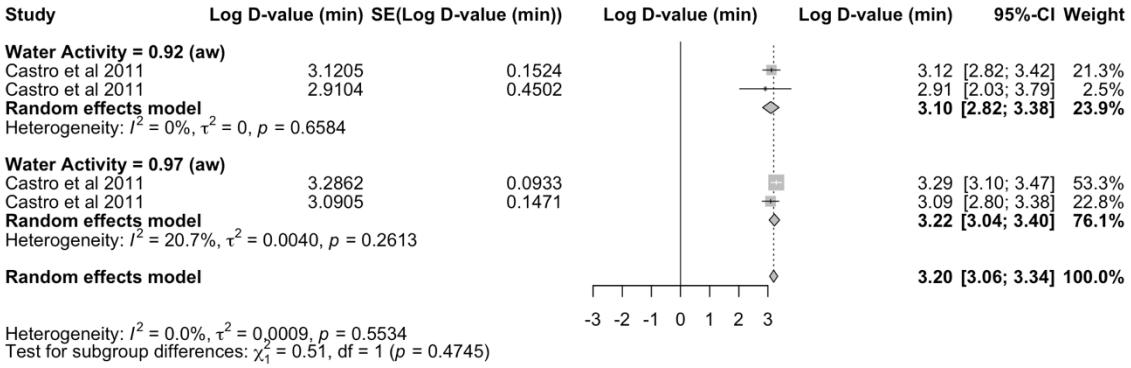


Figure 4.36: Forest Plot for Phosphine Fumigation Treatments

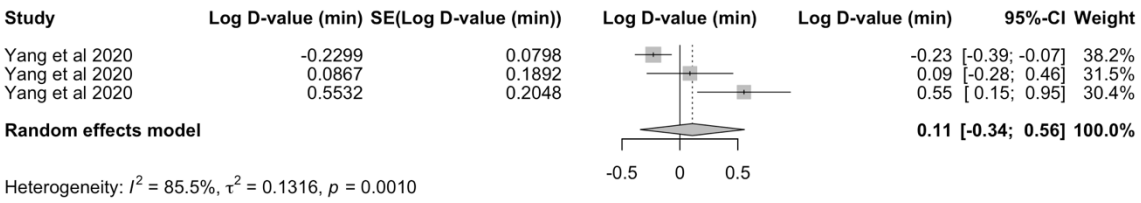


Figure 4.37: Forest Plot for Heat/Humidity Treatments

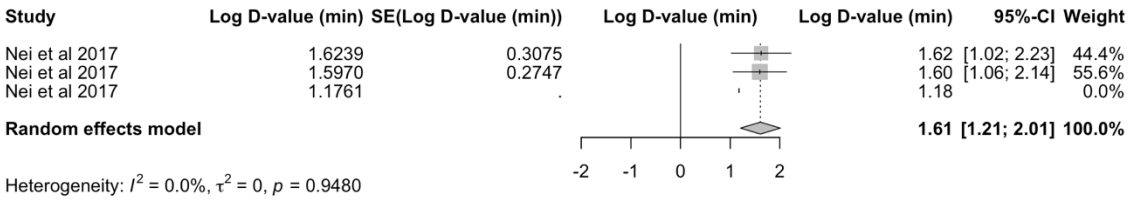


Figure 4.38: Forest Plot for Acetic Acid Treatments

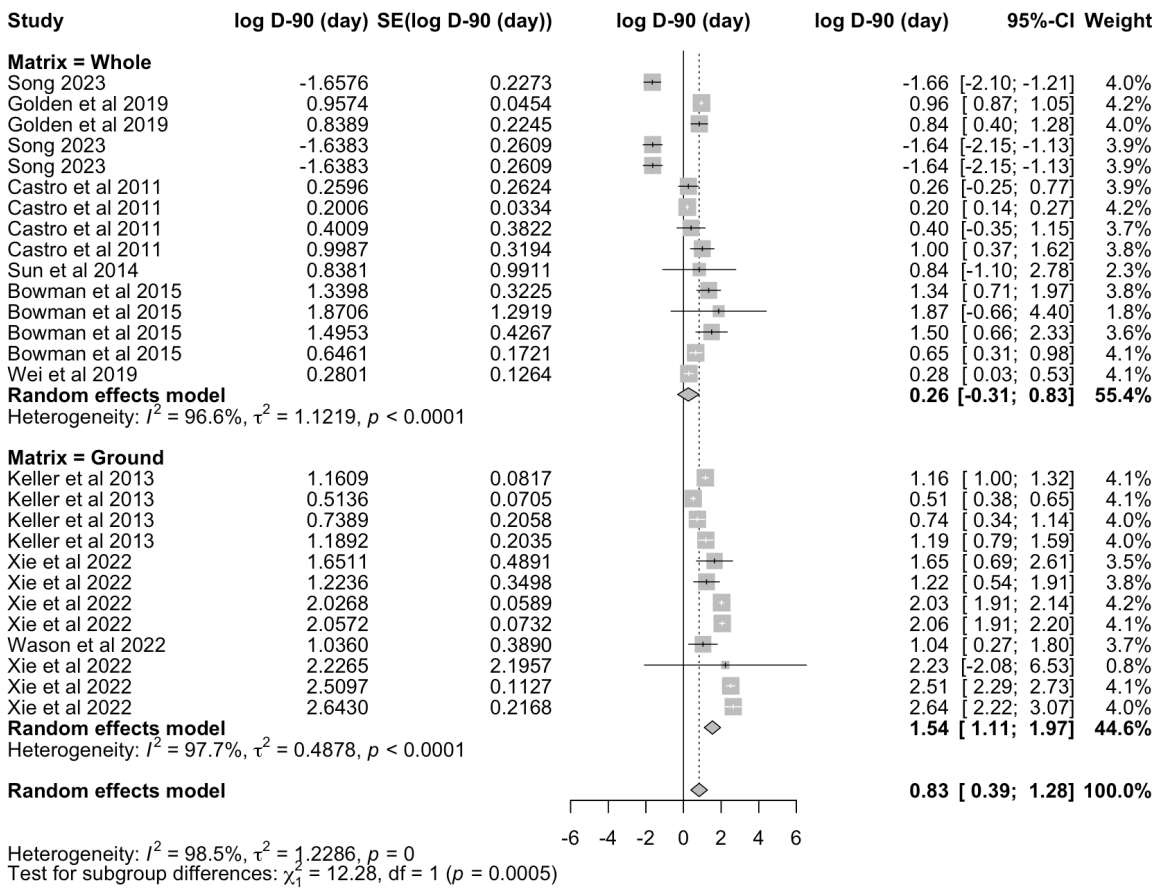


Figure 4.39: Forest Plot for Non-Treated Sample Experiments by Matrix

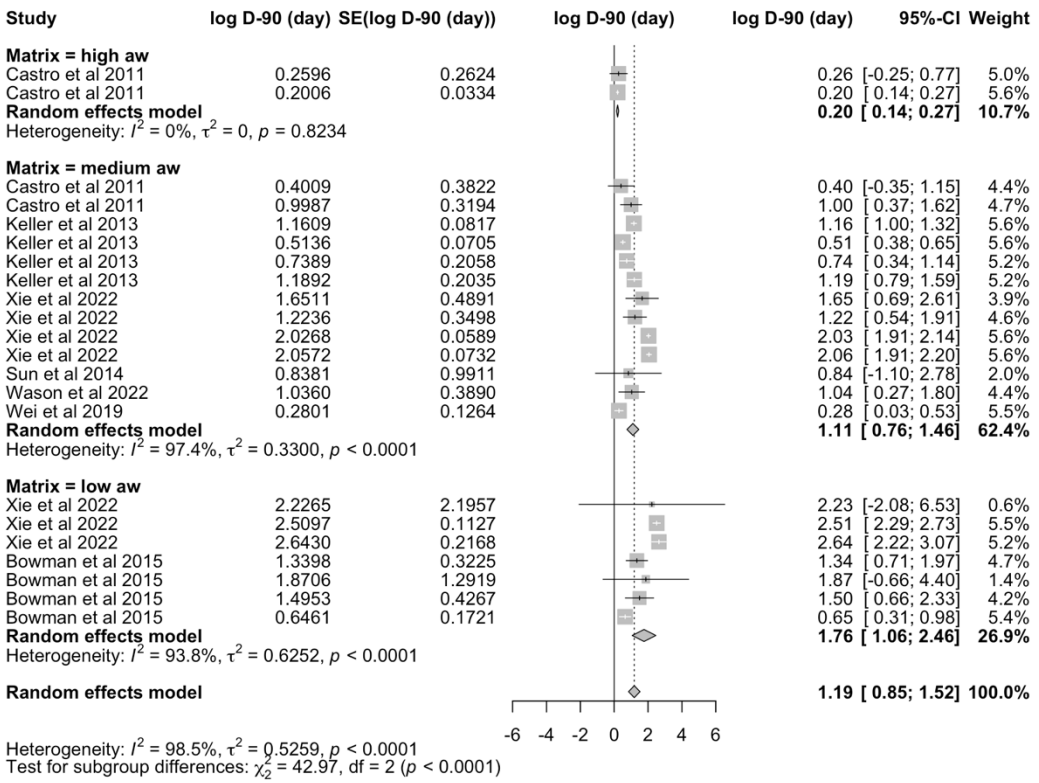


Figure 4.40: Forest Plot for Non-Treated Sample Experiments by Water Activity

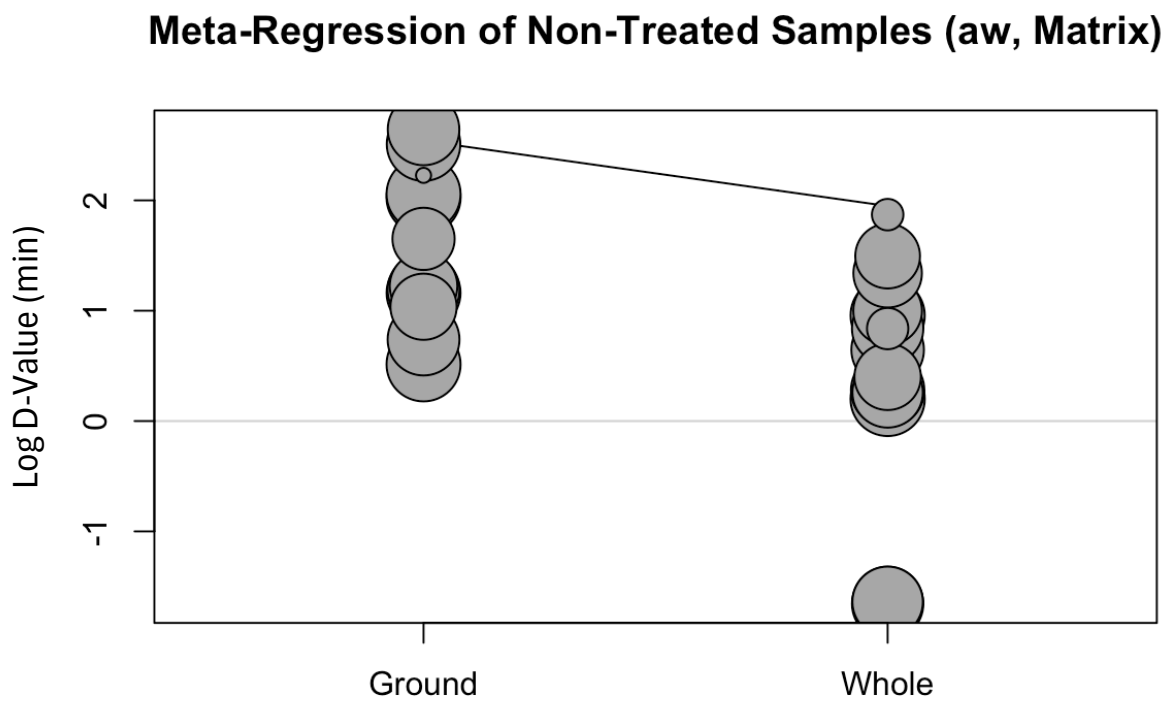


Figure 4.41: Bubble Plot for Meta–Regression for Non-Treated Samples, Covariates are Matrix and Water Activity, with bubble size corresponding to water activity.

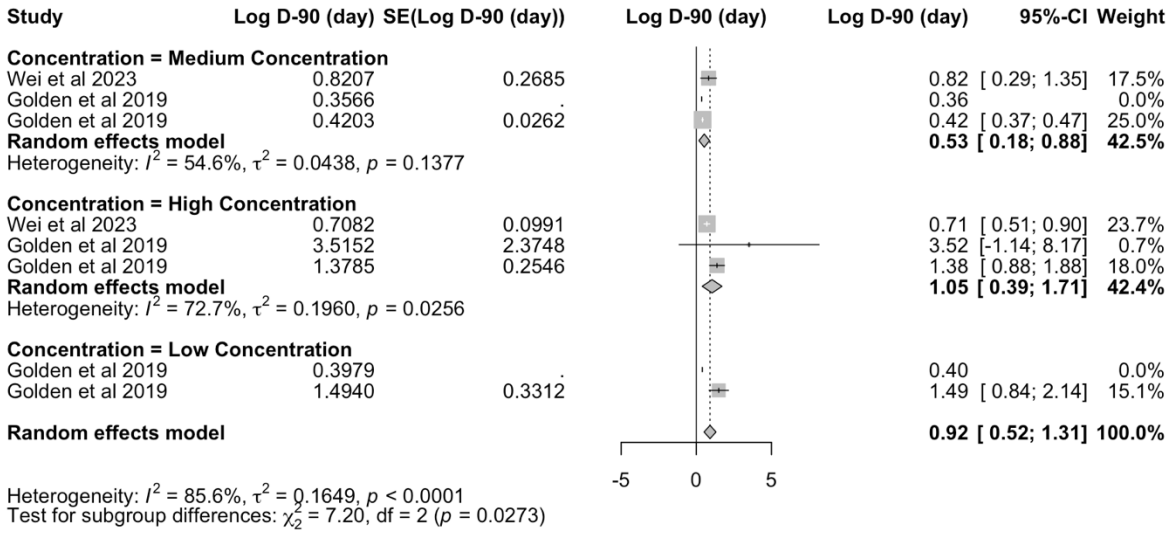


Figure 4.42: Forest Plot for ClO_2/Storage Experiments by Concentration

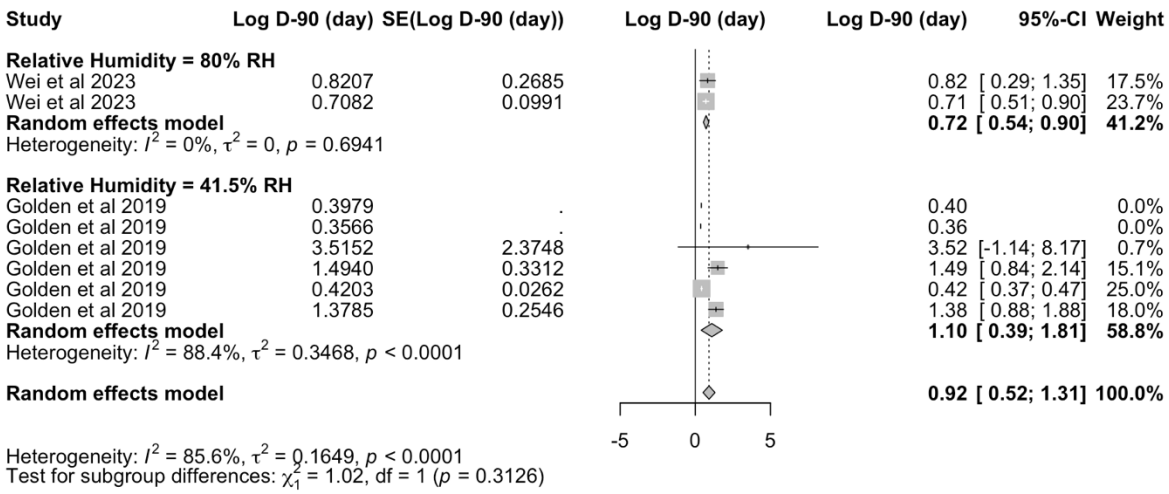


Figure 4.43: Forest Plot for ClO_2/Storage Experiments by Relative Humidity

CONCLUSIONS

This study examined available literature on different inactivation methods for *Salmonella* in black pepper. Through a systematic review and meta-analysis, we identified commonly used as well as less-conventional methods for microbial control in spices. We also identified some of the key factors contributing to the efficacy of each method and compared studies based on their log D–value and 95% confidence interval. For methods with sufficient data (i.e., putative predictors and at least 10 studies), we developed response surface and linear models, which were evaluated for goodness of fit. We found that higher water activity tends to correlate with shorter D–values, and the sample matrix strongly influences heat-based inactivation as well as survival under no treatment.

In contrast to previous reports, we found that relative humidity was the key factor in predicting D–value for ClO_2-based treatments, while concentration did not play a significant role. However, we found that concentration does play a more substantial role in determining how cells survive after treatment. We also discussed possible future studies and the limitations of this study. Overall, while over 40 studies have been conducted on eliminating *Salmonella* in black pepper, further research is needed to improve our understanding of how different factors such as matrix, water activity, and temperature — affect the performance of these methods. Further research in this field could help organizations and individuals within the global food safety community develop comprehensive predictive models that enhance food safety. With black pepper being the king of spices, it is essential to ensure that the public is protected from pathogens that may survive and even grow within it.

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Chapter 5- Limitations and Future Research

Many studies have been conducted on multiple inactivation methods for *Salmonella* in black pepper. However, significant knowledge gaps remain that could be critical when developing a food safety system. The black pepper matrix was one of the most critical factors in any method where sufficient data was provided. Some methods, such as steam, ClO_2, and Gamma Irradiation, were only tested on one matrix of black pepper. In the case of heat inactivation and no treatment, whole black peppercorns allowed *Salmonella* to survive for a longer period.

In contrast, radiofrequency treatments provided a more suitable matrix for *Salmonella* survival when ground black pepper was used. Future studies should focus on evaluating the behavior of *Salmonella* within ground and whole black pepper. Understanding whether whole black pepper kernels can protect *Salmonella* is essential when considering the parameters of a chosen inactivation method. The relationship between heating method (e.g., direct, indirect, volumetric) and the sample matrix may also shed light on factors that should be considered when combining heat treatments with either ground black pepper or whole peppercorns.

Additionally, testing other methods, such as steam treatments, irradiation-based treatments, and ClO_2–fumigation on whole and ground black pepper could yield important information when applying these techniques. Our results also suggest that water activity plays a key role in the efficacy of each treatment. Lower water activity tends to correspond with better survival. While this may be attributed to cross-protection from an unfavorable environment, further research is needed to determine how methods such as ClO_2–fumigation and UV-irradiation are affected by water activity. For instance, it is reasonable to consider whether ClO_2, a highly

soluble gas in water, would be more effective on a sample with higher water activity, and whether this would impact residue concentration post–treatment. Moreover, past research on ClO_2-based treatments has identified temperature, relative humidity, concentration, and time as the most critical factors (Han et al., 2001). However, we found that concentration had little to no independent effect on immediate inactivation (though it did seem to have a more substantial effect on survival after treatment). Moreover, all experiments were conducted at or near room temperature, so the effect of temperature could not be studied. Future studies may investigate how higher temperatures impact ClO_2–treatments on *Salmonella* in black pepper.

Furthermore, one challenge in our analysis was that many methods lacked sufficient replications for meta–regression analysis and testing for publication bias. To properly validate these methods, further studies are needed to establish relationships between covariates and their effects. While subgroup analysis may have identified some critical variables, their effect on the response variable (D–value) in comparison to other variables that may or may not have been reported leaves much room for further study. Furthermore, predictive modeling techniques require at least 10 studies, following the "one in ten rule," to create a model with sufficient statistical power. For more accurate models, more than 20 or 30 models would be needed for reliable prediction.

Additionally, our predictive model for NT experiments yielded an R^2 of 0.479, an RMSE of 0.548, and an AIC of 39.90. We believe this is due to substantial variability between studies. Identifying potential confounding variables within these studies would be crucial to improving the goodness-of-fit.

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