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Thermal and Nonthermal Inactivation of Foodborne Pathogens on Low-Moisture Foods: A Systematic Review

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Abstract

Low-moisture foods (LMF) are generally regarded as safe because microbes typically do not survive in water activity less than 0.85. Previous studies have shown that pathogens are capable of persisting in dry conditions for an extended period and may acquire resistance to subsequent killing steps. As a result, there are major foodborne outbreaks and product recalls associated with LMF causing hospitalizations and even death. Given that the persistence of the foodborne pathogens in LMF is not widely understood. The objective of this review is to provide the current knowledge of thermal and nonthermal treatments of foodborne pathogens in LMF and discuss the effectiveness of thermal and nonthermal treatments for LMF processing. A total of 137 articles were used after inclusion and exclusion criteria were applied. Many parameters affect the effectiveness of the treatments such as water activity, temperature, time, and product formulation. Predictive models for instance Bigelow, Weibull, and Kopelman model can be used to design the inactivation steps in LMF. Different treatments are needed to inactivate different LMF products thus food manufacturers should implement the most effective way to kill the pathogen according to the food produced.

Keywords: *Low-moisture food, Thermal processing, Nonthermal processing, Predictive model, Foodborne pathogens*

1.INTRODUCTION

Low-moisture foods (LMF) are food that contains water activity (aw) less than 0.85 (Codex, 2018). Some examples of low-moisture foods are cereals, chocolate, cocoa powder, dried fruits and vegetables, egg powder, fermented dry sausage, flour, meal and grits, herbs, spices and condiments, honey, hydrolyzed vegetable protein powder, meat powders, dried meat, milk powder, pasta, peanut butter, peanuts, tree nuts, powdered infant formula (PIF), grains and seeds such as sesame, melon, pumpkin, linseed (Beuchat et alet al., 2013). These past years many foodborne outbreaks have been related to low-moisture foods, and caused severe illnesses and deaths (Alshammari et al., 2020; Beuchat et al., 2013; Mondal et al., 2014; Shah et al., 2017; Van Doren et al., 2013). In low water activity and dry food processing and preparation conditions, some of these foodborne pathogens can survive for months, even years. They are higher resistant to heat and other treatments than high water activity products/conditions (Beuchat et al., 2013). The main foodborne pathogens that cause outbreaks in low-moisture foods such as flour, cake mix, cereals, ginger powder, and herbal teas around the globe are Listeria monocytogenes (Taylor et al., 2018), Escherichia coli (CDC, 2019; CDC, 2021), and *Salmonella* (CDC, 2018; Falkenstein, 2017; Keller et al.,

2015). While most strains of *E. coli* are harmless, others can cause illness and some E. coli strains can cause diarrhea, while others can cause urinary tract infections, respiratory illness, *pneumonia*, and other illnesses (CDC, 2022). *Listeria* can cause fever and diarrhea in the same way that other foodborne germs do, but this type of *Listeria* infection is uncommon (CDC, 2022). The symptoms of invasive listeriosis, which means the bacteria has spread beyond the gut, vary depending on whether the person is pregnant (CDC, 2022). There are few ways to inactivate foodborne pathogens in foods for example thermal and nonthermal treatments.

Thermal processing is the combination of temperature and time needed to reduce the microbial load in the food. There are a few examples of thermal processing technologies for example extrusion, radiofrequency, and steam (Wason et al., 2021a). Thermal processing is also one of the most effective ways and is commonly used to kill harmful germs in food (Pan et al., 2017; Silva and Gibbs, 2012a). Proper time and temperature exposure can eliminate most of the microbial load present in the food. Heat is used to kill pathogens as well as develop the flavor, aroma, texture, and color of a cooked dish (Silva and Gibbs, 2012). However, pathogens are difficult to eliminate from foods with low water activity using techniques like mild heat treatment, which works well for foods with high water activity which is concerning since some of the foodborne pathogens such as *Salmonella* and *Escherichia coli* O157:H7 only need a few cells to survive and cause further outbreaks (Beuchat et al., 2013). In a dehydrated condition, the metabolism of the pathogens is greatly reduced thus there is no growth, but the vegetative cells and spores may remain viable for several months or even years (Beuchat et al., 2013). Thermal processing also causes some undesirable changes and may form a by-product that will negatively affect the flavor, texture, and nutritional value of the final product (Pan et al., 2017). This may adversely affect the sales of the product since people are becoming conscious about the nutritional value present in their food and thus prefer more natural and less processed food.

Nonthermal methods are gaseous technologies that are being used to eliminate the microbial load present in food (Wason et al., 2021a). Nonthermal technologies are based on an electromagnetic field which includes pulsed electric fields, high voltage arc discharge, pulsed light, ionizing radiation, microwave and cold plasma (Pan et al., 2017). Nonthermal inactivation is significant to reduce the negative impact on the food and increase the shelf life of the products. The benefit of using the nonthermal method is the ability of the gaseous to diffuse through the air spaces and pores thus allowing the gaseous to eliminate the microbial load even with irregularly shaped food (Wason et al., 2021a). Furthermore, combining these approaches with thermal treatment can lower the treatment temperature while still achieving large microbe reductions in food (Pan et al., 2017). Some of the nonthermal processing is not applicable for low-moisture food for example chlorine dioxide (ClO_2), hydrogen peroxide ($H2O_2$), and high-pressure processing (HPP) (Wason et al., 2021).

LMF's are commonly assumed to be safe food from microorganisms, however, there are many recorded outbreaks of LMF particularly caused by *Salmonella*. Mountain Mel's is recalling herbal teas, including those that are intended for babies and toddlers, due to a risk of *Salmonella* infection of ingredients (Food and Drug Administration, 2019). *Salmonella* can survive for a long time, and because low-moisture foods are long-lasting and have a long shelf life, *Salmonella* can harm customers for years by causing subsequent infections. Thus, more studies about the inactivation of foodborne pathogens in low-moisture need to be

conducted to improve food safety around the globe. The expected outcome of this review is to cover the effective ways to reduce or kill the foodborne pathogens that survive in LMF. The objective of this review is to provide the current thermal and nonthermal treatments of foodborne pathogens in LMF and discuss the effectiveness of thermal and nonthermal treatments for LMF processing.

2. MATERIALS AND METHOD

2.1. Literature Search and Search Strategy

A literature search was conducted through Scopus and Science Direct databases using keywords searches for low-moisture foods, thermal inactivation, and non-thermal inactivation. The keywords used for the Scopus search engine were "low-moisture foods" and ("thermal inactivation" or "non-thermal inactivation") while for Science Direct search engines were "low-moisture foods" AND ("thermal inactivation" OR "non-thermal inactivation"). No restrictions were placed on the subject area of the searches. Restrictions are only placed on the date published, document type and language. The date of publications was restricted between 2000 to the present; the last search was conducted on 1st September 2021. This review only included the research articles document type that has been written in English. After removing duplicates and reviewing papers, the remaining articles were screened based on inclusion and exclusion criteria (Table 1), by three researchers independently to minimize bias.

Table 1. Inclusion and Exclusion Criteria

Inclusion criteria	Exclusion criteria
Study on thermal inactivation or nonthermal inactivation on LMF Tested on foodborne pathogens. Surrogate study of the foodborne pathogen	Review paper Research articles on animal feed, antimicrobial agent of the LMF, only storage or survival of microbes, surveys, RSM without microbes, and studies on inoculation only or sanitizing solution.

2.2. Inclusion and Exclusion Criteria

Acceptability criteria are research articles that evaluate the effectiveness of thermal inactivation or nonthermal inactivation step for LMF. All articles in the study focused on LMF as the primary outcome. Data compilation from 137 original articles provided adequate discussion to meet the objectives of this review. As primary data needed were not always available from review articles, they were excluded from the article selection. Studies that focused on the efficacy of antimicrobial agents and optimization of processing parameters in LMF were also excluded as these studies are more relevant to food product development.

Studies that focused only on the pathogen behavior under multiple stresses including dry environment, were included although without the present of LMF, as these studies would provide an in-depth explanation of the mode of pathogen inactivation when subjected to newly developed kill step protocol. Studies that utilized surrogate microorganisms are included because selecting appropriate surrogate microorganisms for the intended pathogen is critical for process validation as the use of pathogenic microorganisms is not recommended in food processing operations. The summary of the article selection process is illustrated in Figure 1.

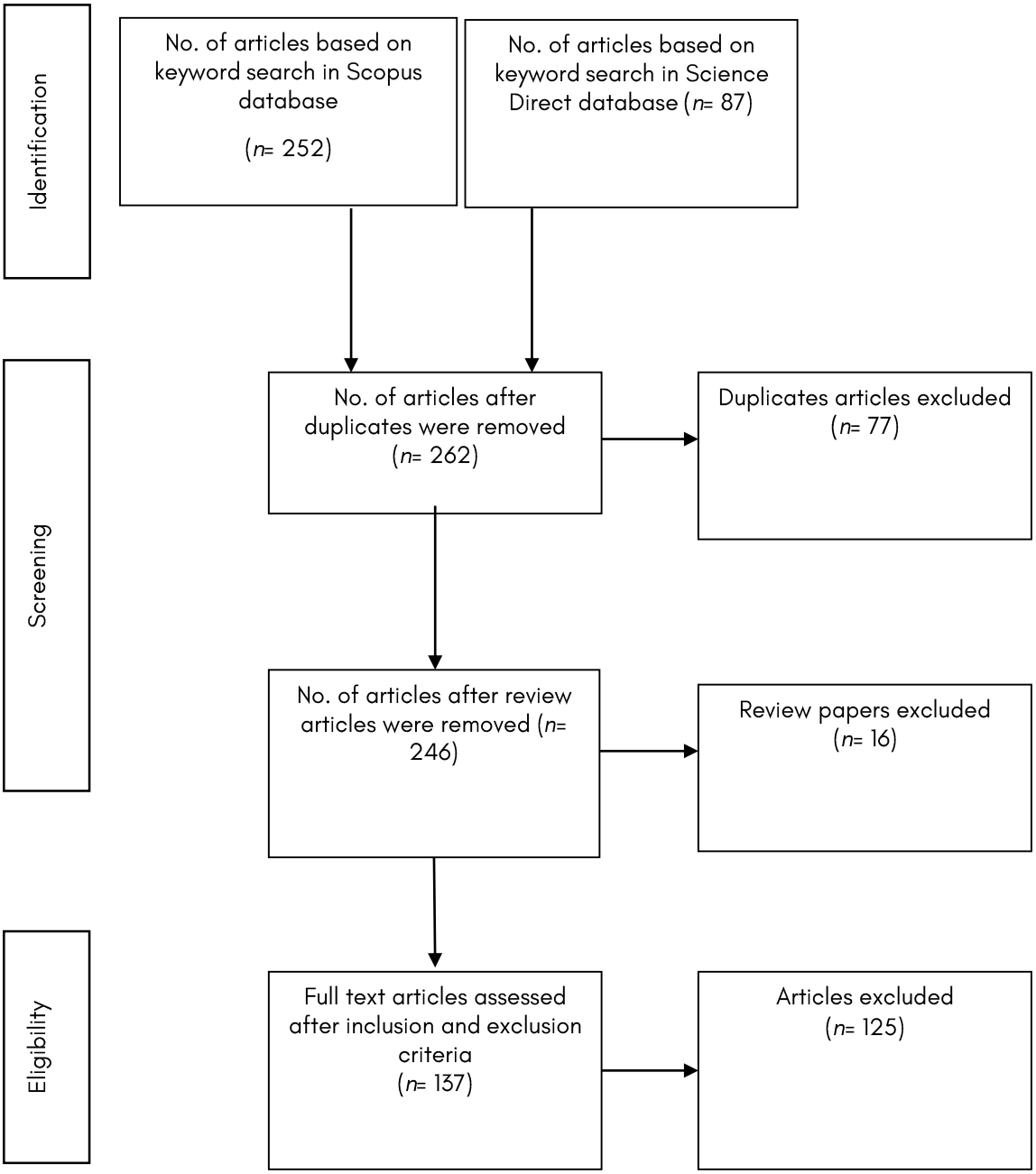

Fig 1. Flowchart of the article selection process

3. RESULTS AND DISCUSSION

The food industry has a vital task to mitigate or control the inactivation of foodborne pathogens in low-moisture foods. Preventative control may include additional treatments to ensure the safety and verification of these treatment methods. Currently, there is a lack of tools and methods for process validation in LMF processing. The treatment and product factors selected are some of the key parameters that determine the validation of the pasteurization process. The development of verification protocols for LMF processing technologies also requires the identification of appropriate surrogate microorganisms.

Overall, 137 articles were selected from a total of 262 articles derived from Scopus and Science Direct ranging from 2000-2021. Information

regarding the target microorganisms, food samples, processing parameters, log reduction or/and D- and Z-values, inactivation models used to obtain the D- and z-values, and the summary of significant findings were reported in Table 2 and Table 3, for thermal treatment and nonthermal treatment, respectively.

3.1. Thermal inactivation

C Thermal inactivation is widely used to reduce microbial loads in LMFs all over the world. While thermal treatments can eliminate or reduce microbial load, choosing the best processing method to maintain product quality is considered validation (Wason et al., 2021). Most of the industrial-scale technologies are used to eliminate or reduce the microbial loads in food processing. Thermal processing is the common technology applied and approved by regulatory and international agencies, to inactivate spoilage and pathogenic bacteria, while also providing food with adequate stability during storage. The intensity of the treatments will determine the stability of the product during storage conditions for the suggested time frame (Bermúdez-Aguirre and Corradini, 2012). The microbial cell's DNA, RNA, ribosome, cell envelope, and proteins can all be destroyed by thermal inactivation. By using infrared treatment, the magnitude of microbial damage is as follows: protein, RNA, cell wall, and lastly DNA. The magnitude of microbial damage after infrared treatment is as follows: protein is followed by RNA, then the cell wall, and finally DNA (Rifna et al., 2019). However, mild, and high heat treatment that works well for foods with high water activity might not eliminate all foodborne pathogen in LMFs. Pathogens such as *Salmonella* and *Escherichia coli* O157:H7 only need a few cells to survive and cause further outbreak. The vegetative cells and spores may remain viable for several months or even years (Beuchat et al., 2013). The review also states that the heat is transferred differently in conventional thermal treatment, processing times are reduced, and heat-food contact is reduced thus significantly reducing the negative effect of heat on food characteristics. In this section, different thermals treatments and their' efficacies are reviewed to reduce the microbial loads in low-moisture foods

3.2. Liquid-heated bath

The water bath, ethylene glycol bath, and oil bath were used as liquid medium in thermal inactivation of foodborne pathogens and surrogates such as Salmonella, Listeria monocytogenes, and Enterococcus faecium. Types of bath liquids impact thermal efficiency. An oil bath was used when the inactivation temperature was above 100℃. According to the results, the most studied low-moisture foods are powder milk, flour, and nuts (such as almonds and pecans) with a temperature range of between 60 to 90℃. The common primary models used are the Weibull model and the Bigelow model. Water bath thermal treatment shows a significant reduction of foodborne pathogens in LMF (Sekhon et al., 2021). Few factors affect the effectiveness of water baths in reducing the microbial load for instance fat content, moisture content, inoculation method, temperature, time and water activity (Daryaei et al., 2020; Limcharoenchat et al., 2018; Quinn et al., 2021). Lower moisture content and water activity may cause a lower microbial reduction, thus higher temperature and longer time exposure are needed to achieve the same log reduction as high moisture content and water activity food (Daryaei et al., 2020). High carbohydrates content in food may result in higher thermal resistances in storage and heat treatments (He et al., 2011). The findings also suggested that contamination events during prefabrication may be more concerning in process validation (Limcharoenchat et al., 2018). Additional tests are being carried out to quantify *Salmonella* thermal resistance in various product structures at different aw levels, to model *Salmonella* behavior in a variety of LMF. Future research on secondary inactivation models to apply for process validation, must account for dynamic moisture during processing.

For example, a study conducted by Quinn et al., (2021) shows that *Salmonella* spp. in powder infant formula had the highest thermotolerance among Listeria monocytogenes, *Salmonella* spp., and E. faecium in peanut butter, powder infant formula and wheat flour. *Salmonella* was also tested in dry and dehydrated non-fat dry milk and whole milk powder using a water bath (80 to 90℃), resulting low inactivation rate (Sekhon et al., 2021). Additionally, the sorption state LMF products should be considered during the prediction of bacterial inactivation kinetics, developing models and validation processes (Garces-vega et al., 2019).

The ethylene glycol bath is a promising thermal treatment that can reduce the microbial load up to 5 log reduction in LMF. Some studies have been conducted to test microbial inactivation using ethylene glycol baths in cocoa powder, non-fat dairy milk, and wheat flour. Some of the pathogens studied are Listeria monocytogenes, *Salmonella* spp., and Enterococcus faecium with a temperature range from 70 to 80℃. Water activity is a significant factor in reducing *Salmonella* loads in cocoa powder (Tsai, et al., 2019a). The log reduction of Listeria monocytogenes was more than 4 log CFU/g when tested on the same food sample which cocoa powder with the same parameters. (Tsai et al., 2019b). Listeria monocytogenes were reduced up to 5 log reduction when tested in non-fat dry milk under the parameters of water activity is 0.30, the temperature of 80℃ and 60 min of treatment time (Ballom et al., 2020). However, the most significant impact of milk powder pasteurization is browning, which is caused by the formation of melanoidins in the final stage of the Maillard reaction which happens during heating if the heat is very high (Wei et al., 2020a).

The most studied food samples in an oil bath are wheat flour and some other food such as confectionary, seasoning, chicken meat powder, peanut oil, and almonds. The most studied foodborne pathogens are *Salmonella* spp. and Enterococcus faecium. The oil bath treatment is more effective compared to hot water treatment for *Salmonella* Enteritidis PT 30 in almonds due to higher inactivation rate at the same temperature (Mohammad et al., 2020). Other than that, a study on confectionary, seasoning, and chicken meat powder was conducted and highlighted that 5-log reduction of *Salmonella*, Listeria monocytogenes, and Enterococcus faecium can be inactivated with the temperature of 111.2, 105.3 or 111.8°C respectively with the time ranging from 1.5 to 2 min (Rachon et al., 2016).

A correlation between temperature and water activity based on the fat and protein content indicates that these parameters must be considered in predicting the thermal inactivation in foods (Yuqiao et al., 2018). Moreover, a study of Enterococcus faecium in peanut oil resulting in less than 1 log reduction concluded that oil acts as a barrier to moisture diffusion is another mechanism that will cause oil's protective effect on bacteria from thermal inactivation (Amninder et al., 2021).

3.3. Steaming and vacuum steam pasteurization

Steaming is one of the methods studied that effectively reduces the microbial load in black peppercorns, almonds and pistachios and maintains the product quality and the temperature used is between 70 – 200℃ (Ban and Kang, 2016; Zhou et al., 2019). The steam process directly raises the temperature of the food, the mechanism for microbial inactivation is like that of the thermal process. The structure of proteins, nucleic acids, and lipids is harmed by these high temperatures and causes protein and nucleic acid denaturation, which disrupts cell metabolism. Lipids melt within the cell membrane to maintain cellular content, resulting in cell lysis and microorganism inactivation (Wason et al., 2021). However, there is a limitation in superheated steaming which is ineffective towards high moisture content food (Ban et al., 2018).

Ban and Kang (2016) tested *E. coli*, *Salmonella*, and Listeria spp. in almonds and pistachios at the temperature of 100 - 200℃ and the treatment

time is between 1- 30s. The log reduction is 3.0 – 6.2 log CFU/g for *E. coli*, 2.7 – 6.5 log CFU/g for *Salmonella* spp. and 2.7 – 5.7 log CFU/g for Listeria spp. and D100℃ = 5.28 – 9.88, D100℃ = 4.87 – 11.15, D100℃ = 6.68 – 11.12, respectively. The finding from this study is that superheated steaming is an effective method for inactivation foodborne pathogens in almonds and pistachios while maintaining the quality of the products. More than 5 log reduction was able to achieve targeting E. faecium in peanut butter with temperature used 125 - 250℃ and water activity 0.19 – 0.80 (Park et al., 2021). The D values and Z value recorded are D125℃ = 129.70 - 6.33s, D175℃ = 32.41 - 3.38s, D225℃= 24.62 - 1.93s, D250℃= 18.49 - 3.22s and Z value = 194.66℃ (Park et al., 2021). The highlight of this study is the inactivation results are apply to environmental surfaces for effective inactivation of the pathogens. *Salmonella* spp. was tested in black peppercorns, pecans, and almonds by using superheated steaming with temperatures ranging from 100 – 180℃ and the log reduction recorded was more than 6 log CFU/g. The D100℃ = 4.65 – 9.2 while the Z–value = 47.06 – 146.26℃ (Ban et al., 2018).

Other than that, vacuum steam pasteurization is a heat treatment process that kills pathogenic microorganisms in specific foods and beverages. Most studies using pasteurization are flaxseed, quinoa, sunflower kernels, black peppercorns, macadamia, raisins, and many more using the Weibull model and Geeraerd-tail model. The temperature range used is between 60 - 105℃. Short-time exposure of vacuum steam pasteurization in spices can effectively reduce the microbial load by up to 5 log reduction (Newkirk et al., 2018; Shah et al., 2017). Vacuum steam pasteurization has proven to effectively reduce the total aerobes, yeast, and moulds, and the chemical properties are not significantly affected by this treatment (Malekmohammadi et al., 2020; Shah et al., 2018). It is a method for improving the safety of dried fruits and nuts in a short time, depending on the temperature exposed (Acuff et al., 2020).

Salmonella Enteritidis PT30, *Enterococcus faecium* NRRL B-2354 ATCC 8459 and *Escherichia coli* O157:H7 are tested in flaxseed, quinoa, sunflower kernels, and black peppercorns with the temperature of 75 – 105℃ within the time of 0.5 to 5.0 mins able to reduce ~7 to 8 log CFU/g of the microorganisms. The significant findings from this study are that the pathogens were reduced by more than 5 logs in whole flaxseed, sunflower kernels, and peppercorns at 75°C and milled flaxseed and quinoa at 85°C (Shah et al., 2017).

A study conducted by Acuff et al. (2020) by targeting *Salmonella*, *E. coli*, *Listeria monocytogenes*, and *Pediococcus acidilactici* in apricot, macadamia and raisins, by using vacuum steam pasteurization with the temperature of 62 - 82℃ and 0 -5 mins able to achieve more than 5 log reduction with the D value recorded D72℃ = 0.8 – 7.5, D72℃ = 0.8 – 5.4, D72℃ = 0.7 – 7.3, and D72℃ = 1.1 – 10.3, respectively. The findings from the study are that depending on the temperature, low-temperature, vacuum-assisted steam pasteurization provides a strategy for improving the safety of dried fruits and nuts in relatively short time periods. *Salmonella* was tested in flaxseed with the processing parameters of 71℃ and 0.5 water activity and the D value recorded is D71°C = 1.0 to 1.5 with the significant finding that the time stored before the heat treatment would have a small impact on the time required to inactivate *Salmonella* in flaxseeds (Malekmohammadi et al.,2020). *Salmonella* and *E. faecium* were tested in whole peppercorns and cumin seed with the temperature of 177℃, the log reduction recorded are 1.92 – 1.93 and 1.64 – 2.3 log CFU/ respectively (Newkirk et al., 2018). The findings from this article are that vacuum assisted steam pasteurization of spices was effective in reducing *Salmonella* and *Enterococcus faecium* may be used as a surrogate for inactivation on whole peppercorns and cumin seeds (Newkirk et al., 2018). The conclusions and recommendations for thermal pasteurization can be extended to non-thermal pasteurization processes, as it is necessary to determine microbial

D- and z-values, or non-thermal resistance parameters of other models, concerning the new technologies (Silva and Gibbs, 2012). Future studies need to be conducted with different parameters to confirm the effectiveness of vacuum steam pasteurization.

3.4. Roasting

Roasting usually been done for LMF such as cocoa beans, sunflower seeds, etc. between the temperature range of 90 - 150℃. *Salmonella* is proven to be inactivated over 5 log reduction using roasting using the right exposure and time (Yan et al., 2021). *Salmonella* that survives during the heat treatment may survive in room temperature storage (Zhang et al., 2017). Heat resistance data obtained under specific experimental conditions, on the other hand, cannot be used to validate thermal processes by cocoa and chocolate manufacturers. A study conducted by Yan et al., (2021) found that the log reduction is 2.8 – more than 5 log CFU/g for *Salmonella Oranienburg* in cocoa beans with the treatment temperature (100 - 150°C) and time (2- 100 mins). The D-value recorded are D100°C: 33.34, D110°C: 18.7, D115°C: 12.92, D120°C: 10.50, D130°C: 4.20, D140°C: 1.9 with the Z-value: 32.0°C; the study indicates that 10 mins of roasting at 150℃ able to reduce 5 log reduction of *Salmonella*. Similarly, a few strains of *Salmonella* (*Salmonella Typhimurium*, *Salmonella Newport*, *Salmonella Enteritidis* and *Salmonella Tennessee*) were tested in sunflower seeds and the log reduction is more than 4 log CFU/g (Kottapalli et al., 2020). The temperature used is 107.2 - -135℃ with the treatment time of 5 -45 mins and it claims that after roasting the sunflower seeds for 45 minutes at 135℃ have saleable water activities, resulting in a.7-log reduction in *Salmonella* (Kottapalli et al., 2020). Zhang et al., (2017) demonstrated that more than 4 log CFU/g of *Salmonella* can be reduce in tahini after the storage time of 119 days with the temperature of 95 – 130℃ and time between 0 – 90 mins. The D values recorded are D90°C = 24.7 and D130°C = 15.0; this study mentioned that there are no changes in *Salmonella* population after 119 days of storage. These findings highlight the critical importance of aw during the roasting step, and *Salmonella* that survives roasting is likely to survive the tahini's in RT.

A risk assessment analysis is required to determine the level of safety for *Salmonella* contamination in this type of product and, as a result, the parameters of its thermal processes. Environmental conditions such as temperature and humidity, which have been linked to *Salmonella* outbreaks linked to almond consumption or the presence of *Salmonella* in almonds, require further research.

3.5. TDT Sandwich

The TDT Sandwich was created as an open-source, free alternative that uses dry heat. The system can heat samples to 140°C and keep them within ±0.2°C of the target temperature (Lau and Subbiah, 2020). Most studies conducted the TDT sandwich with the temperature range of 60 to 95℃ with food samples such as whole black peppercorns, wheat flour, almond meal, skim milk powder, desiccated shredded coconut and some types of sugar. The log reduction of foodborne pathogens ranges from 0.2 to more than 8 log CFU/g. The almond flour would need to be fully exposed to 80°C for 120 minutes to achieve a 6-log reduction of *Salmonella Enteritidis* PT30 (Xu et al., 2019). Mild thermal treatments can be used to control *Salmonella* in cinnamon powder, as well as possibly other antimicrobial-containing spices or herbs, for better product quality retention (Xie et al., 2021). Water activity had a strong influence on the D-values of *Salmonella* and *Enterococcus faecium* in ground black pepper. Lower water activity increased the thermal resistance of *Salmonella* and *Enterococcus faecium* in ground black pepper (Wei et al., 2021e).

Recently, *Salmonella* spp. in whole black peppercorns was tested using TDT cells and the log reduction achieved was 0.41 – 2.19 log CFU/g and the D75℃ = 106 – 198 mins with the temperature

used of 60 -85℃. The study highlighted that modifying the food-water activity may be an effective way to achieve the required level of pathogen activation in a relatively short period of time at low treatment temperatures (Gautam et al., 2020). Similar to the study conducted by Jin et al., (2020) more than 5 log of *Salmonella* spp. in soy protein powder was reduced by using temperature ranging from 60 - 95℃. The study concluded that *Salmonella* thermal resistance is affected by the temperature and the water activity of the food. Recently, *Salmonella* spp., Listeria spp. and Enterococcus faecium were tested in desiccated shredded coconut and the log reduction recorded is more than 5 log CFU/g with the processing temperature of 75 - 90℃ with the water activity between 0.25 – 0.45. The D-value for *Salmonella* spp. is D80℃ = 38.7 – 53.2, Listeria spp. is 14.2 – 40.2 and Enterococcus faecium NRRL B-2354 is D80℃ = 49.6 – 85.5 and the significant finding is Listeria monocytogenes has less thermal resistance than *Salmonella*. The study focuses on thermal inactivation strategies for controlling *Salmonella* and *Listeria* monocytogenes during the desiccated shredded coconut post-drying process (Dhowlaghar et al., 2021). Similarly, *E. coli* ATCC 25922 in the almond powder were reduced up 2.55 log CFU/g and the D75℃ = 12.6 – 20.5 when treated with 75℃ and the highlight of this study is that the effects of storage environment on reducing bacterial populations and D75℃ of *E. coli* became more noticeable as storage temperature increased Cheng and Wang, 2018). More than 6 log CFU/g and more than 7 log CFU/g of *Salmonella* spp. and *Enterococcus faecium*, respectively, were able to be reduced in egg powder (Pérez-Reyes et al., 2021). The temperature used are between 20 - 80℃, time ranging from 0 – 150 mins with water activity from 0.3 – 0.74. The D-value recorded for *Salmonella* is D80℃ = 5.1 – 25.9 and *Enterococcus faecium* is D80℃ = 10.4 – 43.8 and concluded the D value had a linear relationship with water activity and the composition differences (Pérez-Reyes et al., 2021).

3.5. Desiccation

Desiccation known as drying or dehydration has been used for a long time to preserve fruits such as prunes, and raisins. Most studies use desiccation for food samples for instance flour, almond meal, and peanuts. A study was conducted on wheat flour to test the survival of *Salmonella Enteritidis* PT30 under different water activities (0.3 and 0.6) and the result shows that the responses are negligible (Smith and Marks, 2015). Biofilm-producing strain of *Salmonella* was tested on wheat flour using desiccation and the finding are the thermal resistance of *Salmonella* in LMF is influenced by the strain that produces biofilm (Villa-Rojas et al., 2017b). A study was conducted to test the thermal resistance of *E. coli* O121 in wheat flour with a temperature of 70 - 80℃ and found that *E. coli* O121 is the least thermally resistant compared to *Salmonella Enteritidis* PT30 when tested under the same condition. The log reduction ranged from 0.64 - 6.95 log CFU/g and the D80℃ = 4.58 min while the Z- value = 14.57℃ (Suehr et al., 2019). Enterococcus faecium was tested in peanut oil using desiccation with the water activity around 0.33 to 0.93 and the log reduction was a range between 0 to 5 log CFU/g. The significant findings of this study are that the key factor of thermal resistance of bacteria in oil is the equilibrium of water activity and oil in the system (Yang et al., 2020).

A study conducted by Villa-Rojas et al., (2017b), found that 0.59 – 1.49 log CFU/g of *Salmonella* spp. were able to reduce in wheat flour by using desiccation. The study justified that the thermal resistance of *Salmonella* in LMF was influenced by the preformed biofilm and the D80℃ is 3.1 – 21.7. Similarly, 2.5 – 4.3 log CFU/g of *Salmonella Enteritidis* PT30 in wheat flour were able to reduce with the D80℃ ranging from 1.33 – 7.32 mins (Smith and Marks, 2015b). The study concluded that when applying thermal resistance data to industrial pasteurization validations, the response period to new water activity is negligible (Smith and Marks, 2015). Then again, 0.64 – 6.95 log CFU/g of

E. coli O121 in wheat flour were able to reduce with the temperature used of 70 - 80℃. The D value recorded are D70℃ = 18.16, D75℃ = 6.47, D80℃ = 4.58 with the Z- value = 14.57℃and the study highlighted that *E. coli* O121 was found to be least thermally resistant than *Salmonella Enteritidis* PT30 when assessed under the same environments and using the same methodology (Suehr et al., 2019).

3.6. Drying

Drying is one of the techniques to draw the water of the food thus reducing the water activity in it. Most studies conducted this thermal treatment with temperatures between 60 to 100℃. The effectiveness of drying was tested on *Salmonella* spp. in almonds and the log reduction ranged from 0.17 to more than 6.54 log CFU/g. The study concluded that the combination of dry heat and vacuum packaging can significantly inactivate *Salmonella* without affecting the color of the products (Song and Kang, 2021). *Enterococcus faecium* and *Salmonella* in dried basil leaves were tested using a dry heating method and the D-values recorded were 6.53 to 14.07 min and 3.30 to 9.14 min, respectively. The study found that Enterococcus faecium is a suitable surrogate for *Salmonella* to perform validation of the thermal process (Verma, et al., 2021a). *Salmonella* and Cronobacter sakazakii in milk powder were tested with the temperature range of 90 to 100℃ resulting in the log reduction of up to 1.12 log CFU/g. The percentage of uncultivable cells is strongly related to the loss of respiratory activity and weakly with the membrane permeability (Lang et al., 2018). Lastly, *Enterococcus faecium* in almonds is studied using a drying column resulting in less than 1 log reduction thus the study highlights that the harvested almonds need to be sorted and dehulled before drying to increase the efficiency and moisture uniformity (Chen et al., 2021a).

3.7. Extrusion

Extrusion cooking is a time-honored method that has been widely used in the food industry for decades (Wason et al., 2021). Because of the high temperature, pressure, and shear applied to the product, the extrusion process reduces the microbial load on food commodities. Extrusion processing was assumed to eliminate biological hazards from food due to the use of high temperatures and high moisture, even though the final product contains low-moisture. However, the food industries are required to validate their process as a killing step such that it will effectively control the identified hazard. In that case, twin-screw extrusion can be used as an effective process intervention step for the inactivation of *Salmonella* spp. in whole-grain oat flour (Verma and Subbiah, 2019). Whole-grain oat flour with an initial fat content of 5–15% and moisture content of 14–26% was subjected to treatment. About 0.0 to 9.0 log reduction was achieved at a temperature of 55–85°C, and a screw speed of 75–225 rpm.

3.8. Extrusion

Thermal treatments play a major role in the inactivation of pathogenic bacteria in low-moisture foods. But nowadays non-thermal inactivation methods are gaining importance as potential alternatives to thermal operations in food processing. Non-thermal methods allow the processing of foods in such a way as to preserve flavors, essential nutrients, and vitamins. In addition, *Salmonella* exhibits increasing thermal resistance at decreasing aw during heat treatment (Wei et al., 2018). So, this major challenge is expected to be overcome with non-thermal inactivation such as radiofrequency, irradiation, extrusion and many more.

Types of inactivation in high -requency heating the type of heat transfer is radiation, where the energy delivered is directly absorbed by the microbial DNA and essential proteins, resulting in physical changes in the microbial cell structure and function.

Therefore, it can be concluded that the effect of radio-frequency treatment on reducing microbial logs in food depends on the species of the target organisms and their cell wall structure them, the RF frequency used, and the uniformity of heating (Rifna et al., 2019). The mode of pulsed light inactivation was destroying cellular structure and causing the microbial DNA damage and was thought to be a key contributor to the fatal effects. Microbial destruction begins with the absorption of UV light emitted by pulsed light, followed by the formation of cross-linked pyrimidine nucleotide bases that cause mutations in the DNA (Rifna et al., 2019).

The following section discusses non-thermal methods such as radiofrequency heating, gaseous chlorine dioxide technology, pulsed light, X-ray, and high-pressure carbon dioxide for the elimination of pathogenic bacteria in various foods. Many parameters affect the effectiveness of the treatments such as water activity, temperature, time, product formulation and more. Predictive models for instance Bigelow, Weibull and Kopelman models can be used to design the inactivation steps in LMF. So those were discussed accordingly.

3.8. Radiofrequency heating

Radiofrequency (RF) heating is a novel non-thermal processing method that has gained the interest of the scientific community for the pasteurization of various food products. RF heating is a dielectric heating method operating in the range of 3 kHz–300 MHz. Friction caused by ionic conduction and dipole rotation of water molecules ended up in the formation of heat needed for the inactivation of pathogenic bacteria. Unlike irradiation and other treatment methods, RF treatment is certified organic, and natural, and can be used on an FDA-approved clean label (Lin et al., 2020).

Aspergillus flavus, which can produce aflatoxins, is a major problem in peanut production worldwide. A study by Zhang et al., (2021b) pointed out the effectiveness of radiofrequency heating in eliminating *Aspergillus flavus* in peanut kernels using the Kopelman model. Between the treatment temperature of 65-70°C, 3 to 7 log reduction of *Aspergillus flavus* was observed and the D value at 68°C with the water activity of 0.74 was 8.3 minutes. Although it is successful in inactivation, the problem observed was the non-uniformity of RF treatment causes the least Aspergillus flavus death at cold spots. And that study recommends the use of "COMSOL" software in the development of effective RF.

Similarly, a study by Zhang et al., 2020a has found the effectiveness of the radiofrequency against *Salmonella Typhimurium* in red pepper powder. In treatment under the water activity between 0.44 – 0.70, there was a 4-log reduction of *Salmonella* Typhimurium. Further, these findings confirmed the absence of *Salmonella Typhimurium* sublethal injury cells (SICs) in red pepper powder with an initial aw of 0.44 after the RF treatment. Same as for red pepper powder Jiao et al., 2019 have researched to find out the effectiveness of RF treatment in eliminating Bacillus cereus spores. B. cereus endospores are resistant to heat, radiation, disinfectants, and desiccation, and their adhesive characters facilitate their attachment to processing equipment and resistance to cleaning procedures. Jiao et al., 2019 has used the Weibull model in water activity of 0.70 at a temperature of 90°C to predict the log reduction and they reported a 4-log reduction and D value of 5.8 min and the Z value is 64.3°C. The study highlighted that the RF inactivation effects could be improved by manipulating the sample's initial water activity level. Another study has been done for *Salmonella Typhimurium* in red pepper powders using the Weibull model at a temperature of 70°C with a water activity of 0.71. It has resulted in a more than 5 log reduction (Hu et al., 2018). It has also been suggested that the increasing initial aw could first increase log reductions and then decrease the log

reductions, the optimum aw level was 0.71 for RF inactivation of *Salmonella* in red pepper powders.

In addition to that RF, treatments could be considered as an effective method to control pathogens in in-shell walnuts as well. There was a 4-log reduction of *Staphylococcus aureus* ATCC 25923 in in-shell walnuts with a 15.01% moisture (Weight basis) after RF treatment (Zhang et al., 2019). The effectiveness of radiofrequency heating against *Aspergillus parasiticus* in corn grains was studied by Zhang et al., 2017. About 5 to 6 log reduction was achieved at the temperature of 70°C in moisture content of 15.0% (Weight basis). RF treatments can provide an effective and rapid heating method to control *Aspergillus parasiticus* and maintain acceptable corn quality.

In general, RF heating is a propitious non-thermal technique rather than conventional techniques to inactivate foodborne pathogens but there is an urgent need to find out the appropriate surrogate for the target pathogen and it is needed to be validated for different food matrices. Therefore, the identification of a surrogate for different food products is crucial to help the food industry conduct in-plant validation studies. In this perspective, Ballom et al., 2021 studied the suitability of *Enterococcus faecium* NRRL B2354 and *Listeria innocua* as surrogates for *Salmonella* and *Listeria* monocytogenes respectively using the Bigelow model in cocoa powder. About 4.6 log reduction was observed at the temperature of 75°C in water activity of 0.45. D value at 90°C was observed as 2.5 minutes for *Salmonella* and 2.3 minutes for *Enterococcus faecium*. *Enterococcus faecium* and *L. innocua* were appropriate surrogate strains for controlling *Salmonella* and *L. monocytogenes*, respectively, during RF processing of cocoa powder. Verma et al. (2021b) explored the inactivation of *Salmonella* and *Enterococcus faecium* NRRL B-2354 in dried basil leaves. At 100°C of cold spot temperature, a 4.8 log reduction was observed in *Salmonella* and in the case of *Enterococcus faecium* about 2.7 log reduction. So, Enterococcus faecium was validated as a suitable surrogate for *Salmonella* in dried basil leaves under radiofrequency treatment. Furthermore, RF processing results in rapid heating of the dried basil leaves enhancing food safety with an insignificant impact on quality.

According to Wei et al. (2019) RF heating could effectively inactivate *E. coli* O157: H7 and *Salmonella Typhimurium* ATCC 14028 without significant influence on the quality of black pepper kernels. It was reported that the *E. coli* O157: H7 reached more than 6 log reductions after 7.0 min when heating to 90°C and in contrast *Salmonella Typhimurium* ATCC 14028 reached more than 6 log CFU/g reduction after 8.0 min when reaching 100°C. RF holds great potential for the industrial pasteurization of cumin seeds as well. In continuous radiofrequency heating of cumin seeds with a belt speed of 28.2 m/h and at a cold-spot temperature of 99.6°C, *Salmonella* enterica showed more than 5.52 log reduction and *Enterococcus faecium* showed more than 6.52 log reduction (Chen et al., 2020). Another study was also done to validate the inactivation of RF in cumin seeds by Chen et al., 2019. According to them, at a water activity of 0.74, *Salmonella* achieved a 5.8 log reduction and Enterococcus faecium achieved more than 6.4 log reduction. Thus, Enterococcus faecium is a suitable surrogate of *Salmonella* in cumin seeds for RF microbial inactivation. One consideration pointed out by the above-mentioned study is batch variation requires stricter process control parameters for RF microbial inactivation.

As per the study by Ozturk et al., 2020 it is suggested that the RF heating could be used as an alternative pasteurization method for spices, and it is feasible to design RF pasteurization processes using *Enterococcus faecium* as a surrogate to validate the inactivation of *Salmonella* spp. The Weibull model was used to validate the radiofrequency heating in Paprika, White pepper and Cumin powder. About 4-5 log reduction was observed in both Enterococcus faecium and *Salmonella* spp. D-value ranges from 1.21 to 4.47

minutes for *Salmonella* and for *Enterococcus faecium* it ranges from 1.82 – 9.53 minutes. The Z-value was predicted between 3.6 – 19.1℃. Wei et al. (2018) reported that RF heating is a promising thermal inactivation treatment for *Salmonella* without significant quality deterioration, and *Enterococcus faecium* seems to be a suitable surrogate for *Salmonella* to validate the efficacy of RF heating of black peppercorns. Radiofrequency heating of black peppercorn with the water activity of 0.60 ended up in a 5.31 log reduction of *Salmonella* with 5.26 log reduction in *Enterococcus faecium*.

Wei et al. (2020b) have experimented with the effectiveness of radiofrequency heating in egg white powder. At the temperature of about 80℃ with a time range from 0 - 16 h, *Salmonella* enterica and *Enterococcus faecium* showed a log reduction from 0.58 to more than 5. This study emphasized that E. faecium is a suitable surrogate for *Salmonella*. The validated RF-assisted thermal process has the potential to be scaled up for use in the egg industry. Same as for the organic wheat flour radiofrequency heating at the water activity of 0.25 resulted in 5 log reductions of *Salmonella* and 3 log reductions of *Enterococcus faecium*. RF appears to be an acceptable method to pasteurize *Salmonella* in wheat flour, and *Enterococcus* faecium B-2354 may be an adequate surrogate for future evaluation of RF inactivation on a larger scale (Villas Rojas et al., 2017a). There was another study that explored the RF inactivation capability in wheat flour at the temperature of 85°C and the water activity of 0.45 (Liu et al., 2017). It reported that there were more than 5 log reductions in both *Salmonella Enteritidis* PT 30 and Enterococcus faecium NRRL B-2354. D value at 85°C for *Salmonella Enteritidis* PT 30 was 2.92 minutes. The Z-value of *Salmonella Enteritidis* PT 30 and Enterococcus faecium NRRL B-2354 was 12.8°C and 11.7°C respectively. Although both microorganisms yielded similar z-values, Enterococcus faecium was more heat-resistant than *Salmonella Enteritidis* and it can be selected as a surrogate for the validation of inactivation of *Salmonella Enteritidis*.

In addition to that, some studies experimented with the different combinations of treatments with RF heating. Wei et al. (2021a) have studied the possibility of hot air-assisted radio frequency processing in whole milk powder and non-fat dry milk for the inactivation of *Salmonella*. At the temperature of 95°C more than 5 log reduction was observed. This study validated a hot air-assisted RF process for the pasteurization of milk powder based on previously collected microbial inactivation kinetics data. Similarly, Xu et al., 2020b was also studied the hot-air assisted RF in wheat flour for the inactivation of Enterococcus faecium NRRL B-2354 with the water activity of 0.45 at 80 – 85°C. About 2.5 – 3.7 log reduction was observed. The Bigelow model was used for the prediction and the D value at 80°C was observed as 8.3 minutes and the z-value was 11.7°C. Overall, the study concluded that the relatively slow RF heating rate with a hot air-assisted system is helpful in improving the temperature uniformity of RF treatment to obtain a uniform treated sample. The optimum RF-assisted thermal processing conditions of 80°C for 7 hour and 90°C for 2 hours were recommended for pasteurization of soft wheat flour without any compromise in the quality and functionality (Boreddy et al., 2019).

Cheng et al., 2020 have explored the combination of controlled atmosphere and RF heating. RF heating of almond kernels under a controlled atmosphere (2% O_2, 20% CO_2, and 78% N2) with a moisture content of 8% at a temperature of 72-78°C resulted in a 4-log reduction of *E. coli* ATCC 25922. D value was estimated at 75°C for 5 to 5.5 minutes. This study highlighted that RF heating under controlled atmosphere conditions may hold potential as an effective treatment method to control *E. coli* ATCC 25922 in raw almond kernels and be possibly extended for pasteurization applications. According to Cheng and Wang 2019, modified atmosphere pre-storage assisted thermal treatments induced by RF energy may hold

potential as an effective and environmentally friendly method to control *E. coli* ATCC 25922 in almond kernels. About 4 log reduction was observed under 8.0% wet basis and at the temperature of 75°C.

Zhang et al. (2020b) have used thermostatic radiofrequency for powdered infant formula milk (PIFM) with a water activity of 0.2 – 0.4 and a temperature range of 55–70°C. It was observed a 0.16 log reduction in Cronobacter sakazakii ATCC 29544. D value at 70°C was observed as 23.3 minutes. At the same time, the Z value was 14.90°C RF heating can provide an outstandingly higher heating rate compared to traditional treatment, so it has great potential to be used to produce PIFM to achieve higher pasteurization efficiency. According to Lin et al. (2020) RF assisted traditional thermal process is more suitable for the pasteurization of PIFM than the traditional thermal process due to lower lipid oxidation and much shorter overall processing time.

RF heating and freezing treatment combination was experimented with in corn flour for the inactivation of *Salmonella Enteritidis* PT30 and *Enterococcus faecium* NRRL B-2354 (Ozturk, et al., 2019). Water activity and temperature were maintained at 0.45 and 85°C respectively. *Salmonella Enteritidis* PT30 showed a 6.59 log reduction and Enterococcus faecium showed a 4.9 log reduction. D-value at 85°C for *Salmonella Enteritidis* PT30 was reported as 2.03 minutes. *Enterococcus faecium* could be used as a surrogate for validation studies in packaged corn flour. Results also confirmed that RF heating combined with freezing storage treatment could significantly reduce the survival of both microorganisms in corn flour. In the case of wheat flour, RF treatment is efficient in inactivating target microorganisms, especially Enterococcus faecium NRRL B-2354 (Xu et al., 2018). Within the temperature range of 75°C - 85°C about 1.0 – 4.9 log reduction was observed. D value at 85°C was reported as 2.79 minutes. Z-value was 13.1°C. Freeze-dried *Enterococcus faecium* proved to be an effective *Salmonella* surrogate in LMF. Michael et al. (2014) studied radiofrequency dielectric heating on non-fat dry milk. At about a temperature of 90°C, there was a 3-log reduction in Cronobacter sakazakii and *Salmonella*. D-value for Cronobacter sakazakii at 90°C was reported as 5.57 minutes. D-value for *Salmonella* at 90°C was reported as 5.82 minutes. Z-values for Cronobacter sakazakii and *Salmonella* were 23.77 and 26.92 respectively. Radio-frequency dielectric heating can be used as a faster and more uniform heating method for non-fat dry milk to achieve target temperatures for a post-process lethality treatment of non-fat dry milk before packaging.

Because no single technology can provide an ultimate solution for the shortcomings in the inactivation mechanisms, it is decisive to search for different non-thermal technologies as alternatives. Gaseous technologies gain their importance as nonthermal methods that have been used to reduce the microbial load in foods. One of the key benefits of using gases is their ability to diffuse through the air spaces and pores. This allows the gaseous technologies to perform well with irregularly shaped food products. The following section discusses nonthermal methods such as gaseous chlorine dioxide technology, pulsed light processing, twin-screw extrusion, and high-pressure carbon dioxide technology for the elimination of pathogenic bacteria in various foods that came across during manipulated literature search.

3.9. Gaseous chlorine dioxide technology

Chlorine dioxide (ClO_2) is a strong oxidizing agent that has been used as a sanitizer both in the gaseous as well as in the aqueous form. Wei et al. (2021b) have researched by applying gaseous chlorine dioxide technology for black peppercorn and cumin seeds for the inactivation of *Salmonella* and *Enterococcus faecium* NRRL B-2354. ClO_2 gas in a 15 mg/L concentration and relative humidity of 80% at a room temperature of 25°C, could be able to achieve a more than 5 log reduction of

Salmonella for both spices. However, in the case of *Enterococcus faecium* NRRL B-2354, there was a 4.36 log reduction in black peppercorn and a 4.17 log reduction in cumin seeds. It was also estimated that the D value of *Salmonella* at 25℃ for black peppercorn was 60.3 minutes and for cumin seeds was 58.7 minutes. This study elaborated that *Enterococcus faecium* is a suitable surrogate for *Salmonella* during the ClO_2 treatment.

3.10. Pulsed light technology

Pulsed Light (PL) technology is an alternative to thermal treatment for killing pathogenic and spoilage microorganisms in foods. The key component of a Pulsed Light unit is a flash lamp filled with inert gas, such as Xenon, which emits radiation that ranges from 200 nm to 1,100 nm. The exact mechanisms by which PL causes cell death are not yet fully understood, but it is generally accepted that UV plays a critical role in microbial inactivation. Liu et al., 2021 studied the efficacy of pulsed light in the inactivation of *Salmonella* in raw almonds. One-time dipping of almonds in water for 1 min followed by a PL treatment of 500 g of almonds at an intensity of 0.75 W/cm2 for 18 min have reached more than a 5-log reduction. It is reported that PL treatment in combination with prior water dipping could be a potential pasteurization method for raw almonds. Prasad et al., 2019 have done a study on the effectiveness of high-intensity pulsed light-emitting diode treatment on pet foods for the inactivation of *Escherichia coli* and *Salmonella* enterica. At about a water activity of 0.75 with a LED treatment 365nm of *Salmonella* achieved a 0.79 log reduction and with the treatment of 395 nm LED there was a 1.76 log reduction. Dose, duration of light exposure, bacterial strain, and aw played a major role in the antibacterial efficacy of the 365 and 395 nm LEDs.

3.11. X-ray technology

Irradiation is a nonthermal technique used for the preservation of food products and is approved for the elimination of pathogenic microorganisms. Ionizing radiation is a residue-free treatment, causing no thermal damage to food products. For food irradiation, three types of radiation consisting of variable energy levels are used such as gamma rays, electron beam and X-ray generated by the X-ray machine. The use of X-rays to low-moisture foods commodities is limited as it is energy-intensive and is extremely expensive. But Zhang et al. (2021a) have researched the use of 150 KeV low-energy X-rays for the inactivation of *Salmonella Typhimurium*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Listeria* monocytogenes in cardamom. The Weibull model was used to predict the D-values. At about 350 Gy more than 2 log reduction was observed in all microbial species considered. D-value for *E. coli* O157:H7 was reported as 71.43 Gy. tR values were reported as 53.57 Gy for *Salmonella Typhimurium*, 87.74 Gy for Listeria monocytogenes and 114.64 Gy for *Salmonella aureus*. The study concluded that the 150 KeV low-energy X-ray could be applied to effectively inactivate pathogens in dry cardamom. Although the 150 KeV low-energy X-ray irradiation affected the content of PUFAs, no 2-DCBs and 2-TCBs were detected after up to 350 Gy irradiation.

3.12. High-pressure CO_2

Carbon dioxide (CO_2) is a non-toxic, inert, and economically viable gas that does not leave any toxic residues on the treated commodities. In low-moisture foods such as grains, CO_2 gas has been used to induce hypoxia, lethal to insects and moulds; but may not be enough to achieve pasteurization at shorter durations. Research on microbial inactivation of low-moisture foods using high-pressure CO_2 is limited, while fewer studies have been done so far. Chen et al., 2017, evaluated the effectiveness of high-pressure carbon dioxide in inactivating *Escherichia coli* AW1.7 in dry cells. It was reported a more than 3 log reduction at a temperature of 35°C. It was reported that the liquid and supercritical CO_2 were ineffective in reducing the cell counts of dry *E. coli* isolates, and the

effectiveness of gaseous CO_2 was related to the diffusivity of CO_2.

4. CONCLUSION

Few nonthermal treatments can reduce the microbial load in LMF and ensure food safety such as radiofrequency, high-pressure CO_2, and extrusion. Some new evolving technologies that were approved by the FDA (Food and Drug Administration) such as high-pressure processing, pulsed electric field, and ultraviolet light have many concerns the customers thus more research needs to be conducted to investigate the effectiveness of those treatments and the combination of the treatments to inactivate the microbes in LMFs. The application of the pathogen kill step can ensure the safety of LMF thus reducing outbreaks and food recalls. Water activity, temperature, time, product formulation, and a variety of other factors all have an impact on the effectiveness of the treatments. To design the inactivation steps in LMFs, predictive models such as the Bigelow, Weibull, and Kopelman models can be used. Because different treatments are required to inactivate different LMF products, food manufacturers should implement the most effective method of killing the pathogen based on the food produced. Future studies on secondary inactivation models, and applications to process validation, must account for to ensure food safety.

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