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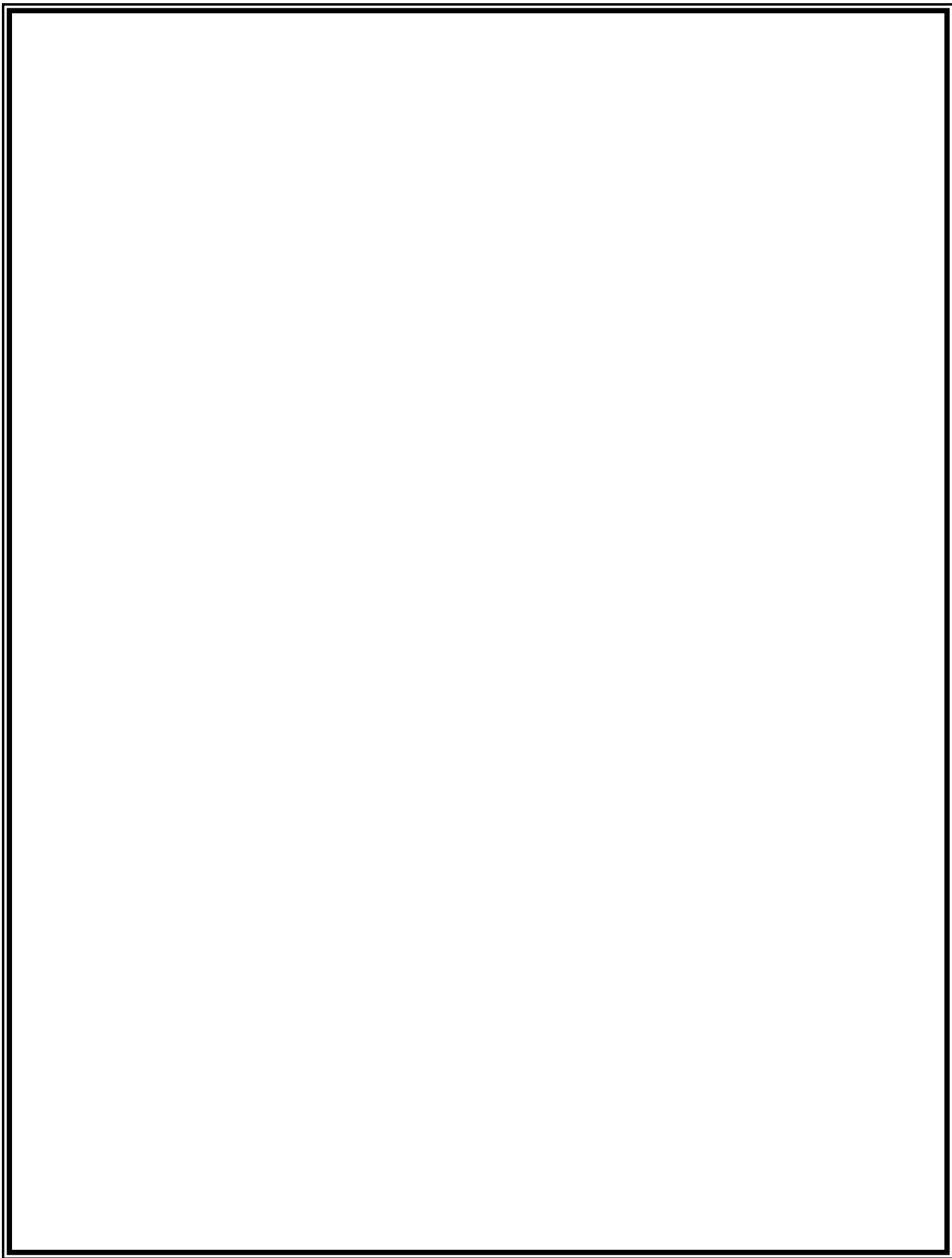
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A_f: Accuracy Factor

ANOVA: Analysis Of Variance

APZ: Acceptable Prediction Zones

a_w: Water Activity

B_f: Bias Factor

BPW: Buffered Peptone Water

CFU: Colony Forming Units

D: Decimal reduction time (min.)

DNA: Deoxyribonucleic acid

GHz: Giga Hertz

hr; heating rates

HSD test: Honestly Significant Difference

Log: Decimal logarithm

MAE: Microwave Assisted Extraction

MW: Microwave

PCA: Plate Count Agar

R²: R-Squared

RMSE: Root Mean Squared Error

RSS: Residual Sum of Squares

SD: Standard Deviation

T: Temperature

t: time

TSB: Tryptone Soy Broth

UHT: Ultra Heat Treatment

W: Watt

δ and p : Weibull model scaling parameters

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Introduction

Introduction

Microwave (MW) technology has been developed and used in a wide range of industrial and domestic applications (Liu et al., 2023). Microwave technology is a modern method of generating thermal energy. It uses the rapid movement and rotation of polar molecules as they rub against each other. This irradiation can cause thermal and electric field effects as well as nonthermal effects in substances, with the former converting electromagnetic energy to thermal energy to produce heat. Non-thermal effects induce rotation and polarization of dipole molecules, subsequently increasing intermolecular collision frequency, resulting in heat production through friction. The heat transfer progresses from the interior to the exterior of the substance (Hu et al., 2021).

A number of industrial applications have emerged from the benefits of using microwaves in the home. They are used to pasteurise, sterilise, dry or thaw food. Microwaves find uses in pasteurisation, sterilisation, drying, or thawing of food products, alongside various conventional thermal methods. Such a support system is often employed across many industries. The use of microwave in the food industry results in reduced processing time and cost, while maintaining high sensory quality and causing minimal changes in nutritional value compared to conventional methods (Guzik et al., 2022; Shaw et al., 2021).

Furthermore, when used in the field of disinfection, microwave irradiation can effectively inactivate micro-organisms and reduce disinfection by-products through the denaturation of enzymes, proteins and membranes and the disruption of cellular metabolic activity. This biophysical effect of microwaves on microorganisms was first observed as early as the 1940s. In the 1960s and 1970s, researchers started contemplating the implementation of microwaves in practical production by studying the sterilization effect of microwaves. They specifically focused on food and wastewater as research subjects (Liu et al., 2023).

The main effect of the electric field is to heat the material. The physical laws governing the heating of dielectric materials by microwaves and the effects of heat on biological systems are well known. The prevailing belief is that a temperature rise due to microwave exposure leads to the deactivation of microorganisms. However, the thermal effect of microwaves is distinct from that of conventional heating. Microwaves can also generate thermal effects, similar to conventional heating, but through a different mechanism utilizing

the dielectric property of molecules. This occurs particularly for polar substances and offers shorter reaction times than those of conventional heating. Conventional heating may introduce heat into the sample from the surface and is comparatively slow. It has been observed that microwaves can eliminate microorganisms at temperatures below the thermal destruction point. (Shaw et al., 2021).

Disinfection targets mainly consist of three categories of microorganisms, namely, bacteria, fungi (including spores), and viruses. Moreover, *Salmonella spp.*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Campylobacter spp.*, and *Listeria monocytogenes* are the primary focus of research on microwave food safety due to their potential as foodborne pathogens. Numerous studies have shown that microwave-assisted disinfection has the ability to inactivate harmful microorganisms within food without compromising the vital components such as wheat germ, milk, carrot juice, and celery juice, as well as major nutrients (Liu et al., 2023).

The sterilisation efficiency of microwave disinfection technology is largely influenced by the type of microorganism. Bacteria, fungi (spores), and viruses differ in their biological type, cell structure, cell size, and mode of proliferation, which consequently affect the inactivation mechanisms.

Escherichia coli and *Staphylococcus aureus* are common organisms found in both humans and animals. They are also capable of causing opportunistic infections in humans. *S.aureus* and *E. coli* are classified as Gram-positive and Gram-negative bacteria, respectively. Gram-positive bacteria possess thicker cell walls in comparison to Gram-negative bacteria, providing greater resistance to environmental stress. Nevertheless, variations exist among diverse Gram-negative bacterial strains (Liu et al., 2023).

Regarding the use of microwave treatment to destroy micro-organisms in food, several factors are involved. These include food moisture and ion content, the specific heat of the food's composition and density, which influence the absorption of microwave energy and modulate the efficiency of the treatment (Hossan and Dutta, 2012; Van Impe et al., 2018).

The presence of dissolved salt reduces the extent to which microwave energy can penetrate through the product, resulting in a temperature gradient that decreases progressively

from the surface. Consequently, microwave heating results in uneven dispersion of heat and a non-uniform temperature profile within the product (Goksoy et al., 1999; Soni et al., 2020; Vadivambal and Jayas, 2010). One of the notable disadvantages of microwave heating is that it may lead to partial inactivation of bacteria in places where the heat intensity is insufficient (Apostolou et al., 2005; Bharti et al., 2022; Heddleson et al., 1996).

Predictive microbiology models describe and quantify the behaviour of foodborne pathogens and spoilage microorganisms in foods under varying environmental conditions. Predictive models are acknowledged as valuable instruments for appraising food safety and quality hazards, and are employed as decision-making tools in regulatory agencies and food industries alike (Perez-Rodriguez and Valero, 2013).

In the field of predictive microbiology, mathematical models are used for the prediction of microbial patterns, taking into account various environmental factors (Stavropoulou and Bezirtzoglou, 2019). The Weibull model is widely used to characterise the survival of micro-organisms. This is due to its adaptability and ease of use in food production (van Boekel, 2002).

Our primary objective via this research was to evaluate and determine the effect of microwave heating and the presence of sodium chloride on the inactivation kinetics of *E. coli* and *S. aureus*. In order to achieve this,

1. Initially, we provide a detailed description of the inactivation kinetics for *E. coli* and *S. aureus* under varying microwaving power levels and sodium chloride concentrations.
2. For the second time, inactivation models predicting microbial survival under dynamic conditions were developed and the effect of NaCl on microwave inactivation kinetics was then investigated.
3. In the final stage of the study, for validation of the developed models, the inactivation kinetics of *E. coli* and *S. aureus* have been simulated with UHT milk.

Bibliographic synthesis

Chapter I. Bibliographic synthesis

I. 1.Generalities on Microwaves

I.1.1. Introduction

Preserving food is imperative to guarantee the safety, quality, and shelf life of food products (Pokharel et al., 2023). Pasteurisation and sterilisation are the most commonly used methods to maintain quality and protect consumers from potential harm of pathogenic germs (Martins et al., 2019).

Sterilization is the process that allows the elimination of all living microorganisms, including those in the vegetative or spore state (Parija, 2023). Pasteurization is a technique that involves the application of moderate heat to destroy pathogens and spoilage microorganisms, as well as some enzymes (Nguyen and Songsermpong, 2022). It is a form of sterilisation that involves the application of heat to foods at a temperature below 100°C. This method is used to eliminate any potential agents of infection or spoilage without affecting the flavour or nutritional value of the liquid. It is widely used for the treatment of milk and other fresh beverages such as fruit juices that are easily contaminated during collection and processing (Parija, 2023).

Generally, traditional methods of preserving food involve subjecting it to high temperatures in order to decrease contamination or microbial load. However, advancements in food processing technology have introduced innovatory preservation techniques that offer significant benefits over traditional methods (Pokharel et al., 2023).

Lajnaf (2023) highlighted two of the latest and modern conservation methods, focusing on non-thermal and thermal techniques as distinct from traditional methods (**Figure 01**).

Heating methods have advanced remarkably, including ohmic heating, induction heating, microwave, and radiofrequency heating. These techniques are described as modern thermal techniques; they generate heat inside food, making them incredibly efficient and energy-dense. Modern thermal techniques have the potential to create safe, nutrient-rich, and organoleptic food that has virtually no side effects from prolonged heating of the product.

Since there is no possibility of any unwanted by-products forming (as it is not subjected to high temperatures) (Habibat and Fapohunda, 2023).

In this present work we are interested in the use of MW radiation since it is being investigated as a technique for reducing microbial populations (Bharti et al., 2022). Microwaves offer an effective method to substitute or complement traditional thermal treatment processes (Viji et al., 2022). Rapid heating and high energy efficiency are the main characteristics of this technology (Sorică et al., 2021).

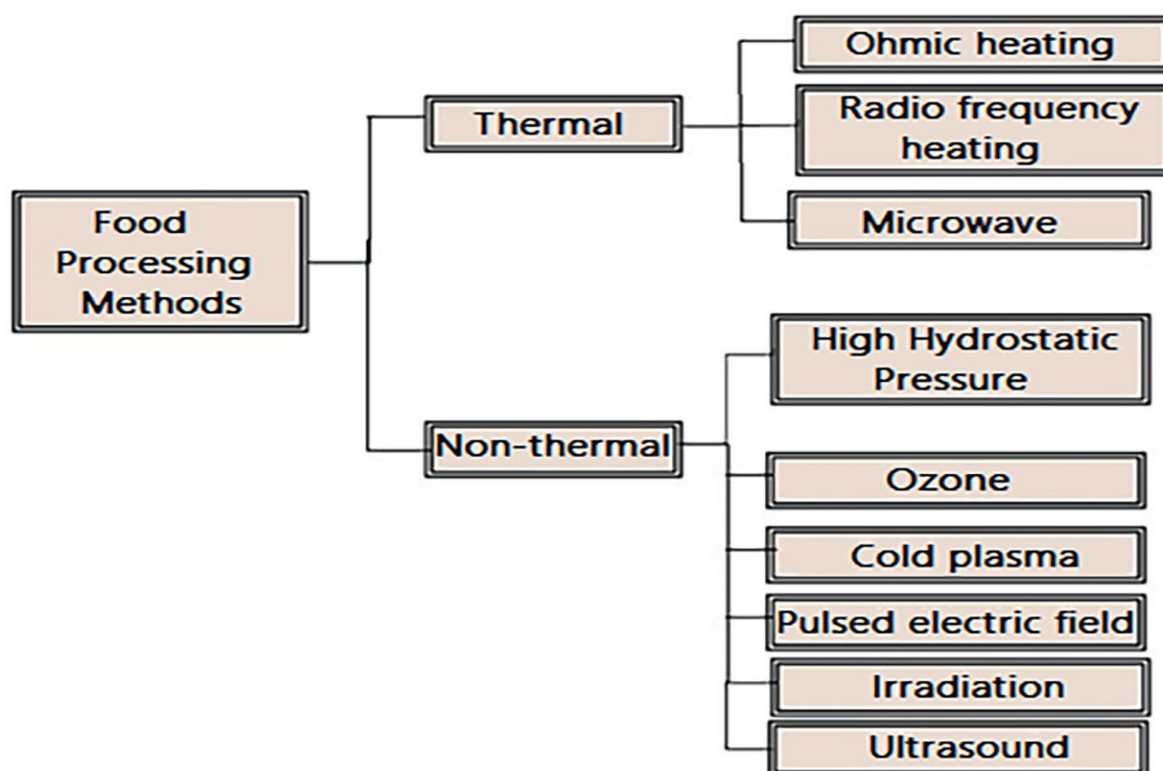


Figure 01. Thermal and non-thermal food processing methods (Lajnaf, 2023).

Despite its potential for various applications, the predominant use of MW technology lies within the food industry. Initially researched in the 1940s, microwave heating of food has gained significance in diverse areas of the food industry over the last few decades (Viji et al., 2022).

MW technology is increasingly utilized in various food processing methods including cooking, blanching, rehydration, thawing, pasteurization, baking, roasting, tempering and drying (Ekezie et al., 2017).

The growing popularity of MW is attributed to their capability to penetrate deeply and cause rapid heating, resulting in significant reduction in processing time, energy efficiency and ease of use (Viji et al., 2022). MW heating has also others several advantages over conventional techniques (Kutlu et al., 2022; Lajnaf, 2023; Guzik et al., 2022; Nguyen and Songsermpong, 2022; López-Salazar et al., 2023; Vignali et al., 2022). The major benefits of using MW processing include:

- Faster heating: High-efficiency volumetric heat generation has been achieved with a success rate of 80% or above;
- Shorter processing time: Requires approximately a quarter of the time of a traditional heating system
- Improved quality of finished product: The high heating rate ensures that the target temperature is achieved rapidly, reducing the thermal effect on the food and minimising adverse effects on the sensory and nutritional properties of the food
- Applications for heat-sensitive, highly viscous and multiphase fluids
- Safe handling is ensured by post-package pasteurisation
- Easy operation, small required space and low energy consumption (high efficiency), reduced noise and low maintenance costs;
- Heating systems can be instantly turned on or off;
- Environmentally friendly processing as the generation of MW does not result in any pollutants or toxic wastes (Guzik et al., 2022).

I.1.2. General aspects and heating mechanism

Many sources, both natural and artificial, emit energy in the form of electromagnetic waves, which are characterised by an electric field and a magnetic field. Depending on their frequency and energy, these waves can be classified as "ionising radiation" (X-rays and gamma rays) or "non-ionising radiation" (ultraviolet, visible, infrared, radio and microwave, low frequencies) (**Figure 02**).

Microwave heating is employed across domestic and industrial settings, utilizing electromagnetic microwave radiation (1-100 GHz) and heat transfer to generate heat in foods (Habibat and Fapohunda, 2023). Their wavelengths range from 1 m to 1 mm and their frequency ranges from 300 MHz to 300 GHz (**Figure 03**) (Sorică et al., 2021). The

microwaves are non-ionizing radiation, lacks the energy necessary to sever molecular bonds or expel electrons from atoms, which is the opposite of ionization. Microwave radiation is insufficiently energetic to ionize food molecules or produce free radicals (Shahi et al., 2021).

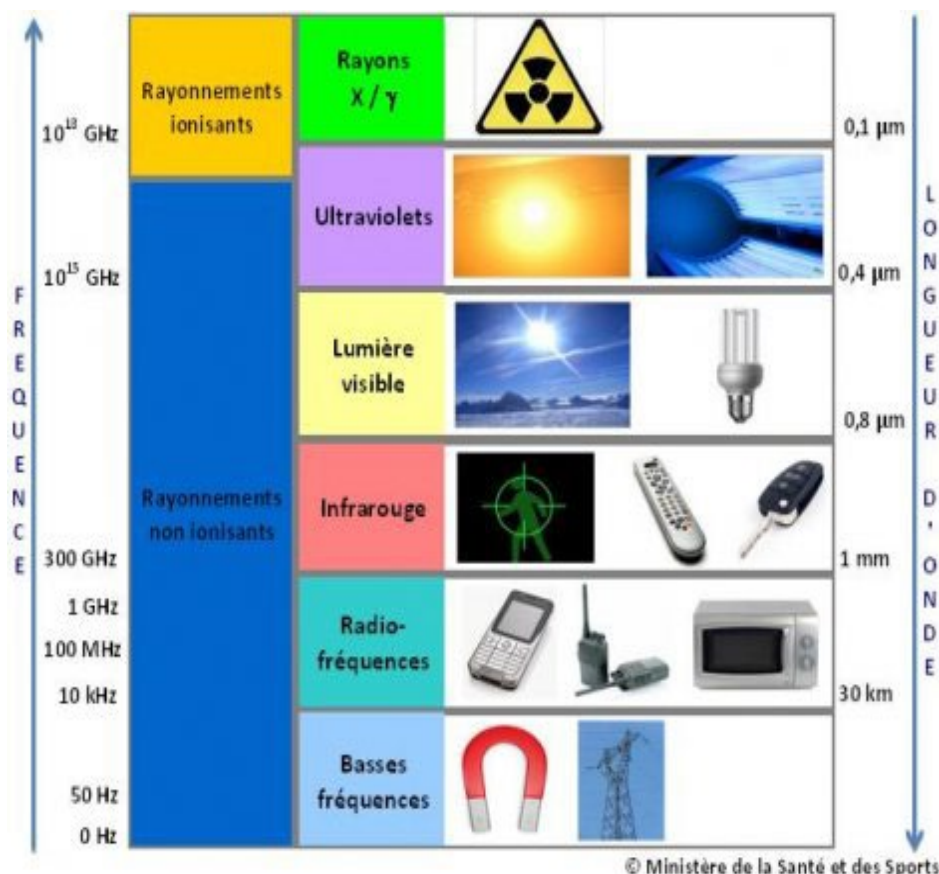
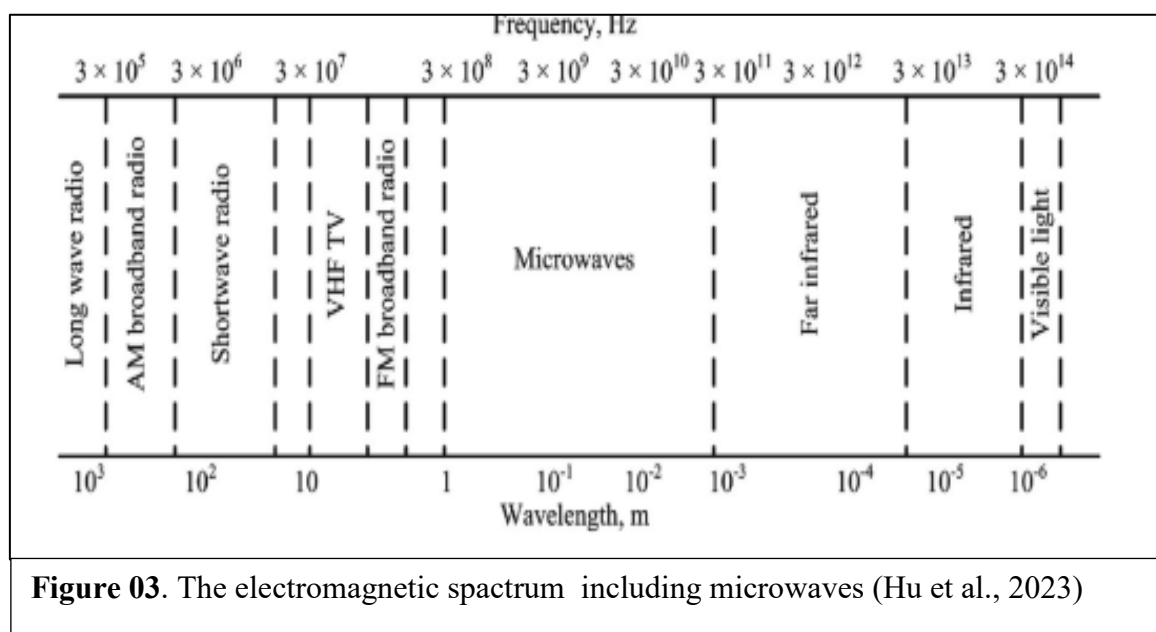


Figure 02. The electromagnetic spectrum and examples of orders of magnitude and associated uses.

MW heating applications are restricted to a few tight frequency bands for industrial, scientific and medical purposes to avoid interference with radio frequencies used for telecommunications. The usual frequency bands are 915 ± 25 MHz and $2,450 \pm 50$ MHz, with penetration depths varying from 8 to 22 cm (corresponding to a wavelength of 32.7cm in air) at 915 MHz and between 3 and 8 cm at 2,450 MHz (with a wavelength of 12.2 cm in air), depending on the level of moisture. The 2,450 MHz frequency is used for domestic MW ovens, while both frequencies are employed for industrial applications (Goyal et al., 2022; Martins et al., 2019).

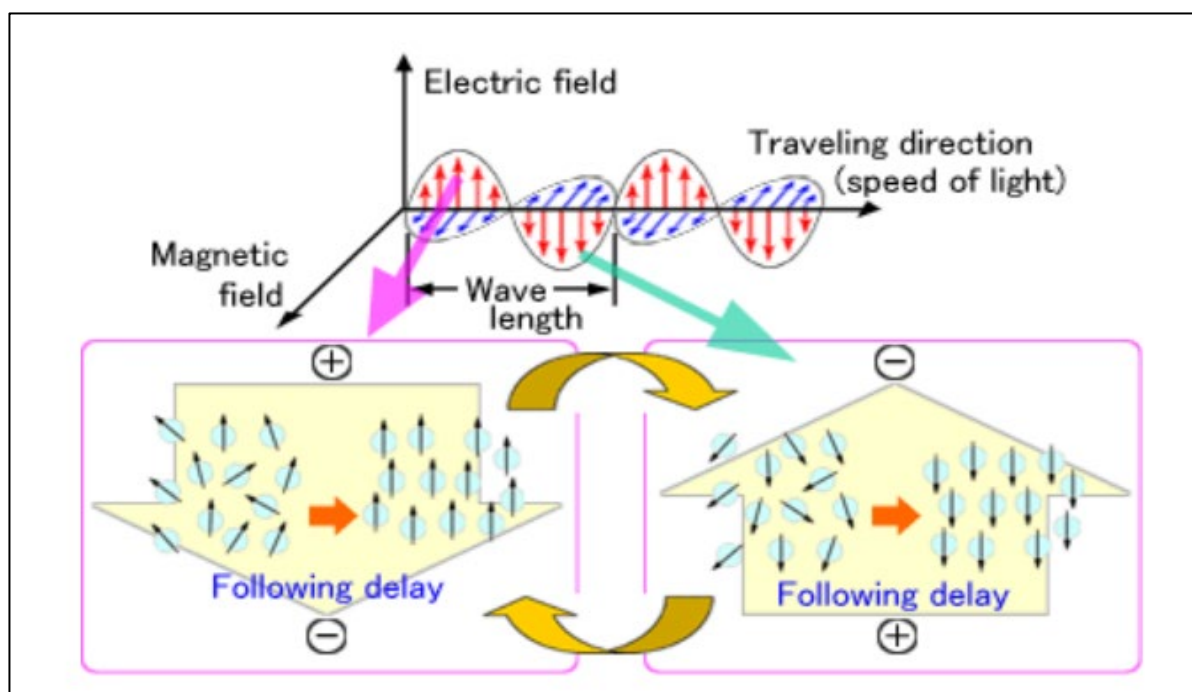
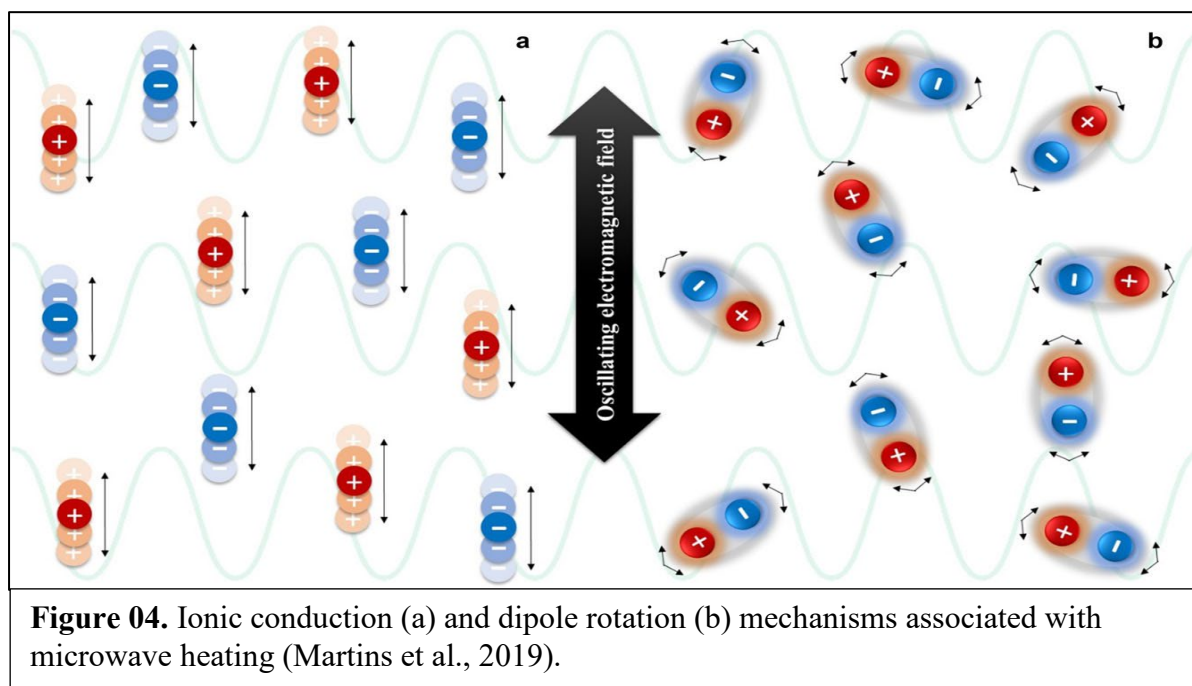
MW heating of food is a multifaceted phenomenon that depends on the dispersion of MWs and their interactions with food determined by their dielectric properties. Heat dissipation is regulated by fundamental mechanisms of heat and mass transfer. Compared to conventional convection and conduction mechanisms, microwaves have the ability to penetrate food, absorb and rapidly convert into heat, resulting in volumetric heat generation and consequently, higher heating rates (Martins et al., 2019). These advantages lead to greater energy efficiency, shorter heating times, and improved sensory and nutritional qualities compared to traditional heating methods (Ekezie et al., 2017).

Two main mechanisms are responsible for the thermal effect of MWs on food: ionic conduction and dipolar rotation (Wang et al., 2022) as illustrated in (Figure 04 and 05). In several cases, these two mechanisms can coexist.



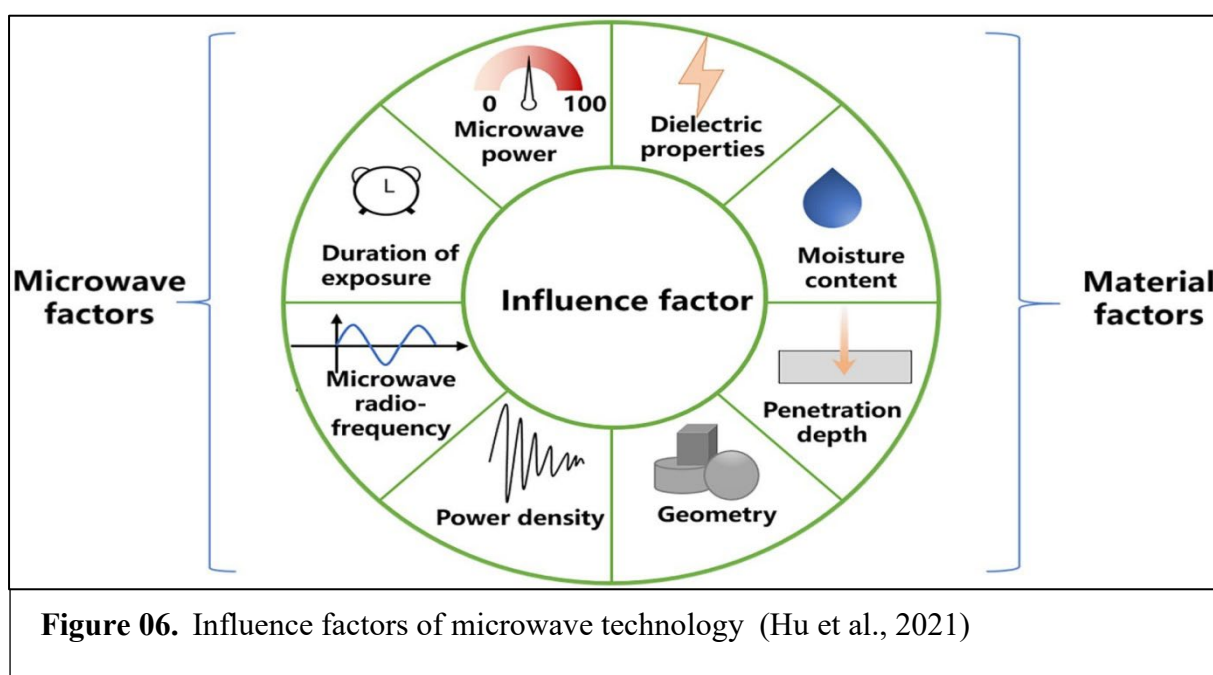
Ionic conduction manifests through the electrophoretic oscillation of ions when applying an alternating electromagnetic field. When exposed to MWs, ionic molecules are induced to flow initially in one direction and then in the reverse direction. This response is triggered by the rapidly changing electromagnetic field signal (Goyal et al., 2022). So a food with a high concentration of ions would have more collisions and consequently a higher temperature than a food with a lower concentration of ions (Ekezie et al., 2017).

The dipolar polarization is related to the alternating movement of polar molecules that possess a dipole moment (either permanent or induced by an electric field) attempting to align with the alternating electric field. When the field decreases, the molecules return to a state of disorder, which releases thermal energy (Martins et al., 2019). Polar liquids, like water, undergo heating via dipole polarization (Goyal et al., 2022).



I. 1. 3. Factors influencing microwave heating

The effectiveness of microwave technology in heating is influenced by various factors categorized as microwave factors and material factors. Microwave factors comprise microwave power, exposure duration and radio frequency and power density. Material factors comprise dielectric properties, moisture, penetration depth, and geometry (Hu et al., 2021) (Figure 06).



I. 1. 3. 1. Dielectric properties

The thermal effect is created by the ability of dielectric materials to absorb MW energy and transform it into heat (Vignali et al., 2022). Dielectric properties are composed of the dielectric constant, a physical parameter that indicates the ability to store electrical energy, and the dissipation factor, an abstract parameter that indicates the ability to convert electrical energy into heat (Nguyen and Songsermpong, 2022). There are several factors that influence microwave heating and its heat distribution, however, the most crucial ones are the dielectric properties and penetration depth (Vignali et al., 2022). The dielectric properties are in turn influenced by various factors such as MW frequency, system composition, and temperature (Tao et al., 2020; Cao et al., 2023).

➤ **MW Frequency**

The dielectric properties of many foods vary significantly with the frequency of the electric field applied. The dielectric constant and loss factors decrease as the frequency increases. The dielectric constant (ϵ') represents the amount of energy that can be stored in a material, whereas the dielectric loss factor (ϵ'') reveals the amount of energy generated that can be absorbed by the material and converted to heat (Kutlu et al., 2022). These characteristics of dielectric materials can be illustrated by the polarization and ionic conduction mechanism (Teseme and Weldeselassie, 2020). Frequency also affects the penetration depth of MW radiation. In general, it is admitted that lower frequencies provide deeper penetration, whereas higher frequencies lead to increased surface heating (Komarov, 2021). Frequencies of 2,450 MHz generally have penetration depths of 0.6 to 1.0 cm, but frequencies of 915 MHz, which have deeper penetration power, are employed in industrial applications (Martins et al., 2019).

➤ **Temperature**

Temperature can significantly affect the dielectric properties and influence the efficiency of the MW heating. The loss factor tends to escalate with the rising temperature at lower frequencies due to ionic conductivity, whereas it decreases at higher frequencies as a result of free water. As the temperature increases, the penetration depth decreases (Martins et al., 2019). The dielectric constant and loss factor decreased with increasing frequency and increased with rising moisture content and temperature (Hu et al., 2021).

➤ **Food composition**

Water is a powerful absorber of MW energy and the primary constituent of most foods. The higher the moisture content, the better is the MW heating, making it a crucial factor to consider. The state of water, whether it is in a free or bound state, has varying effects on the dielectric properties of food materials. In particular, free water molecules are more easily oriented than bound water during the application of dielectric heating. As the quantity of unbound water rises, both relative electrical permittivity and loss factor increase (Boreddy and Subbiah, 2016). Moreover, due to the higher thermal conductivity of ice, the thermal conductivity of the frozen food is higher, whereas the thermal conductivity of the lyophilised food is lower. (Teseme and Weldeselassie, 2020).

Organic materials and salts can impact the dielectric properties of food, depending on their interaction and bonding with water molecules. At MW frequencies, salt solutions serve as conductors, whereby the salts dissolve and create ions that bind water molecules, reducing their polarization. This ultimately leads to a decrease in relative electrical permittivity and an increase in the loss factor (Teseme and Weldeselassie, 2020). For salt concentrations less than 1%, the loss factor decreases with temperature. Conversely, the same factor increases for concentrations above 1% due to the predominance of ionic loss at higher concentrations (Nelson and Datta, 2001). The heat generated during dielectric heating is affected by the moisture, fat, protein, and mineral contents of the product. Carbohydrate and fat exhibit low dielectric properties at microwave frequencies due to the absence of polarisation at microwave frequencies (Teseme and Weldeselassie, 2020).

A large number of studies have investigated the effect of various factors on the dielectric properties of food products. Milk dielectric properties are influenced by factors such as milk type, lactation period, season, breed, feeding practices and compositional changes that occur during the various stages of processing (Hu et al., 2021; Teseme and Weldeselassie, 2020). The dielectric properties of food grains are affected by many factors include; free and bound water content, chemical composition, temperature, particle shape, phase configuration and measurement frequency affect (Teseme and Weldeselassie, 2020; Jones et al., 2022). The chemical compositions of bakery products, principally the moisture content, the content of salt and other minerals, have direct effects on the dielectric properties (Chen et al., 2022). The study investigated the dielectric properties of butter, with and without salt, across the microwave frequency range of 500-3000 MHz. It was determined that the dielectric spectra of unsalted butter varied significantly from those of salted butter as a function of temperature and moisture content. (Ahmed et al., 2007).

➤ **Penetration depth**

The MW causes volumetric heating by penetrating the food matrix. Upon reaching the food's surface, the MWs reflect or penetrate into the food material. MWs that penetrate the food surface contain an initial energy amount that diminishes as they proceed deeper into the food. This phenomenon happens because a portion of the MW energy is absorbed as it travels through the food material (Martins et al., 2019; Hu et al., 2021).

Penetration depth indicates the heating efficiency of a food. The depth of penetration is inversely proportional to the frequency for a material with fixed dielectric properties. Hence, lower frequencies lead to deeper penetration, while higher frequencies cause greater surface heating (Kutlu et al., 2022).

➤ **Physical structure**

The food's shape has an impact on the depth of microwave penetration, heating rate, and uniformity. As product thickness varies, irregularly shaped items are affected. When the food is subjected to MW heating, the temperature at the center increases as the ratio size wavelength decreases. Additionally, smaller particles necessitate less heat than larger particles. Furthermore, maintaining a consistent shape enhances the uniformity of heat distribution in the product. Foods with a spherical or cylindrical shape heat more evenly compared to square ones (Nguyen and Songsermpong, 2022; Martins et al., 2019; Bhatt et al., 2020).

I.1.4. Applications of microwaves in food industry

MW processing technology is regarded as an innovative thermal processing method and is becoming one of the widest applications of MW technology is food technology and engineering (Guzik et al., 2022; Ekezie et al., 2017). The industrial application of MW heating has been extensively researched for various food systems and heat treatment operations in the food processing industry (Martins et al., 2019).

We will only present an overview on the application of MW in extraction processes, thawing, drying and blanching but more importance will be given to microbial destruction.

➤ **Microwave extraction**

Microwave Assisted Extraction (MAE) has been increasingly used for the extraction of natural compounds from plant matrices for about thirty years. MAE is a method to enhance the extraction of bioactive substances and it is founded on the selective heating of polar molecules by MW energy (Destandau and Michel, 2022). Biomolecules and the extraction solvent, subjected to MW radiation, attempt to align themselves with the alternating electromagnetic fields. This is achieved by distortion, molecular movement or physical rotation of the molecular dipoles; the solvent and sample are therefore heated rapidly in

parallel. Extraction rates and extract quality are influenced by the dielectric properties of the food plant and the solvent, the solubility of the bioactive compounds in the solvent and the temperature (Nguyen and Songsermpong, 2022; López-Salazar et al., 2023) .

The quick and homogeneous heating of a sample allows a brief extraction time and a reduction in solvent consumption and waste, MAE is consequently considered to be an environmentally and human friendly method that saves time, solvent and energy (Nguyen and Songsermpong, 2022; Destandau and Michel, 2022)

➤ **Microwave thawing**

Thawing is defined as the opposite of a freezing process. Compared to conventional techniques, modern thawing methods can significantly reduce the time of thawing. The main objective of defrosting with a microwave is to reduce the time required for defrosting (Erdogdu et al., 2019).

MW-assisted thawing is the fastest method for thawing frozen food products, such as frozen meat, fish, vegetables, fruit, butter or juice concentrate. The electromagnetic waves are directed toward the sample through a waveguide with the absence of conductors or electrodes. (Nguyen and Songsermpong, 2022)

Microwave thawing provides many advantages in terms of reduced processing times, energy saving, improved nutritional quality and acceptance in some foods such as meat products and frozen fruit (Peng et al., 2022). MW treatment has also preserved some of the characteristic flavour components that are most acceptable to consumers (Liu et al., 2020).

➤ **Microwave drying**

Drying is a most ancient method like, solar drying or hot-air drying, for preserving food that reduces water activity. Due to the low water level, the activity of microorganisms/enzymes is reduced and the product is easier to transport as its weight is reduced. Conventional techniques typically require an extended drying period, subsequently reducing the nutrient content of foods (such as vitamins, minerals, or bioactive compounds) (Nurhaslina et al., 2022).

To enhance product quality and cut down on production expenses and energy usage, alternative drying processes are being investigated. MW drying is an energy-saving method

(Kutlu et al., 2022) where water molecules absorb the energy quickly and the drying time is reduced (Guzik et al., 2022). Volumetric heating in MW drying generates vapours within the product, which lead to an internal pressure gradient, forcing the water out of it (Nguyen and Songsermpong, 2022; Soni et al., 2020).

➤ **Microwave blanching**

Blanching is a pre-treatment process used to preserve the quality of food by inactivating enzymes (peroxidase, polyphenol oxidase, and pectinases). This method reduces the volume of food by expelling trapped air, decreasing the amount of microorganisms, and eliminating unwanted undesirable color, odor and flavour (Guzik et al., 2022; Nguyen and Songsermpong, 2022). Recent research indicates that blanching fruits and / or vegetables in the MW results in smaller reductions in nutrient content compared to blanching using traditional methods (Kutlu et al., 2022).

MW blanching is a more effective process because it uses high temperature / short time treatment of solid foods through volumetric heating. This is crucial in preserving heat sensitive nutrients, vitamins, and other bioactive compounds. The main benefits are speed of operation, energy savings, precise process control and faster start-up and shut-down times (Muthukumarappan and Swamy, 2019). MW blanching has also a direct impact on the texture of food products, particularly their hardness properties (Kutlu et al., 2022).

➤ **Microwave sterilization / pasteurization**

Food-borne illnesses resulting from bacterial contamination have garnered significant attention from both the media and food scientists alike. The use of microwave sterilisation for food is attributed to the heat produced, the by the type of food and the frequency of use, as well as the temperatures achieved across different parts of the item (Soni et al., 2020).

MW heating has been recognised as a good alternative to conventional heat treatment for producing high quality dairy products with extended shelf-life. MW heating has proven to be highly effective in eradicating pathogenic/spoiling microorganisms and their spores, and also inactivation of enzymes, thereby preserving fresh characteristics of dairy products (Martins et al., 2019).

Table I. Potential advantages of microwave processing

MW technique	Advantages	References
Sterilization Pasteurization	Enhances quality, storage time and safety	(Shaw et al., 2021; Martins et al., 2019; Muñoz et al., 2023)
Thawing	Improves quality, less drip loss, reduced textural damage and discoloration of frozen foods	(Peng et al., 2022; Liu et al., 2020)
Drying	Produces instant foods with short processing time Prevents oxidation, maintains good color, texture, flavor and nutrition of dried products. Produces highest quality of dried products.	(Mandliya et al., 2023)
Blanching	Uses less energy and short time to inactivate enzymes in food components, maintains color of products.	(Guzik et al., 2022)
Extraction	Saves time and energy, achieves high retention of nutrients, increases extracted bioactive compounds	(Destandau and Michel, 2022; López-Salazar et al., 2023)

I. 1. 5. Effect of microwave heating on nutrition and sensory quality

Microwave heating typically involves high temperatures for shorter periods, resulting in enhanced nutritional and sensory characteristics of the end products (Contreras et al., 2017). This is the consequence of internal heat generation and volumetric heating in the MW heating system. Microwavable food products offer superior quality as a result of the quicker heating and high energy efficiency (Nguyen and Songsermpong, 2022).

Feng et al. (2020), were investigated how four cooking methods (steaming, microwaving, baking, and frying) affect the sensory and digestibility of sturgeon steak that had undergone under a vacuum pre-treatment during cold storage (0-25 days). The findings revealed that the digestibility of the steaming and microwaving groups (ranging from 80.34% to 90.12%) was significantly greater than that of the other treatment groups ($p < 0.05$).

Dry microwave blanching was investigated as an alternative to hot water blanching of Indian gooseberry (*Phyllanthus emblica*) to reduce its nutrient losses, especially ascorbic acid, and to facilitate seed removal. MW blanching showed better nutrient retention than heat MW blanching with easier seed removal. The use of MW radiation for blanching of other fruits has been demonstrated (Mandliya et al., 2023).

Peng et al. (2022) have compared MW thawing and ultra-high-pressure thawing to traditional thawing methods (air thawing and water thawing) as references. Evaluation of thawing time, quality attributes, and sensory scores of frozen mangoes were done. Compared to traditional methods, innovative thawing methods can extensively shorten thawing time. The thawing time was further decreased with the increase in MW power. Additionally, MW thawing enhanced the quality of mangoes in terms of less color change and drip loss and reduced loss of firmness and vitamin C content.

It should be noted that despite the advantages and the wide use of MW, it has disadvantages. However, it has a number of limitations, such as dry heating and dehydration of treated foods. Non-uniform temperature distribution to hot and cold spots in microwave-heated products was observed mainly in solid and semi-solid products.

Thawing food is a typical example of the non-uniformity of the MW process and one of its limitations: the surface heats up more quickly and may degrade, while at the same time there may still be ice in some parts of the product (Soni et al., 2020).

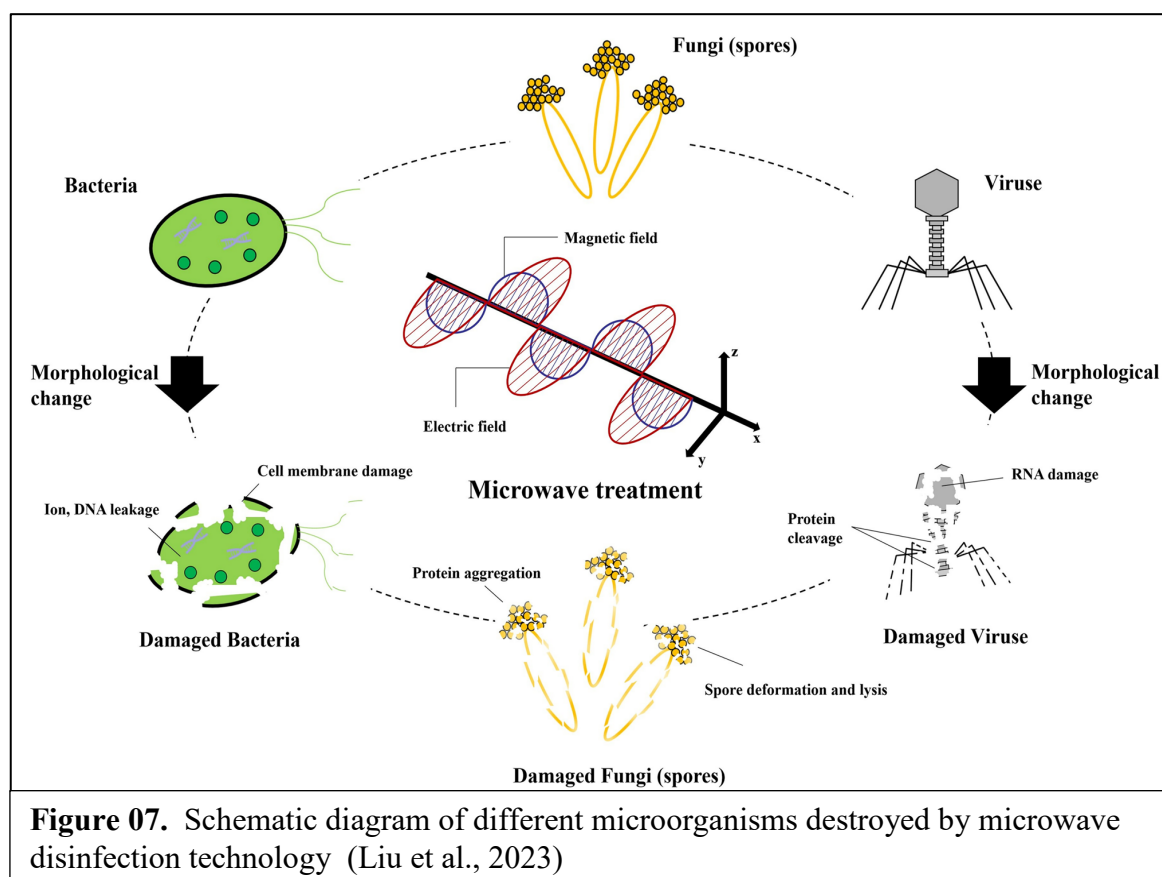
The limited of empirical data required for MW heating modelling, energy and manufacturing cost analysis, the need for technical expertise to understand and minimise heat leakage, and the high initial investment are also considered to be limitations of the technology. Further researches are needed to assess the manufacturing costs of MW heating and to compare them with conventional thermal processes (Vignali et al., 2022).

I.2. Microbial destruction by MW

I.2.1. Introduction

MW radiation has been utilized in food pasteurization and sterilization procedures since the 1970s (Kutlu et al., 2022; Stanley and Petersen, 2017). MW energy has been investigated widely as a potential alternative energy source for sterilisation. Experimental studies have shown that the heat generated by the irradiation, in combination with the electric field formed by the microwaves, causes the death of microbial cells (Shahi et al., 2021).

Microwave-assisted pasteurization destroys pathogenic microbes and some enzymes using microwaves at a sub-lethal temperature. Selective heating, electroporation, cell membrane damage or magnetic field coupling are proposed to explain the microbial destruction or enzymatic destruction by microwaves at sub-lethal temperatures (**Figure 07**).



The theory of selective heating proposes that microwaves selectively heat microorganisms causing them to reach higher temperatures than the surrounding fluid, leading to their rapid destruction. According to the theory of electroporation, the electrical potential

difference across the cell membrane generates pores, leading to the release of cellular materials. In the theory of cell membrane rupture, the application of voltage across the membrane causes rupturing. The magnetic field coupling theory suggests that the coupling of electromagnetic energy with critical molecules, such as protein or DNA, disrupts the internal components of the cell (Nguyen and Songsermpong, 2022)

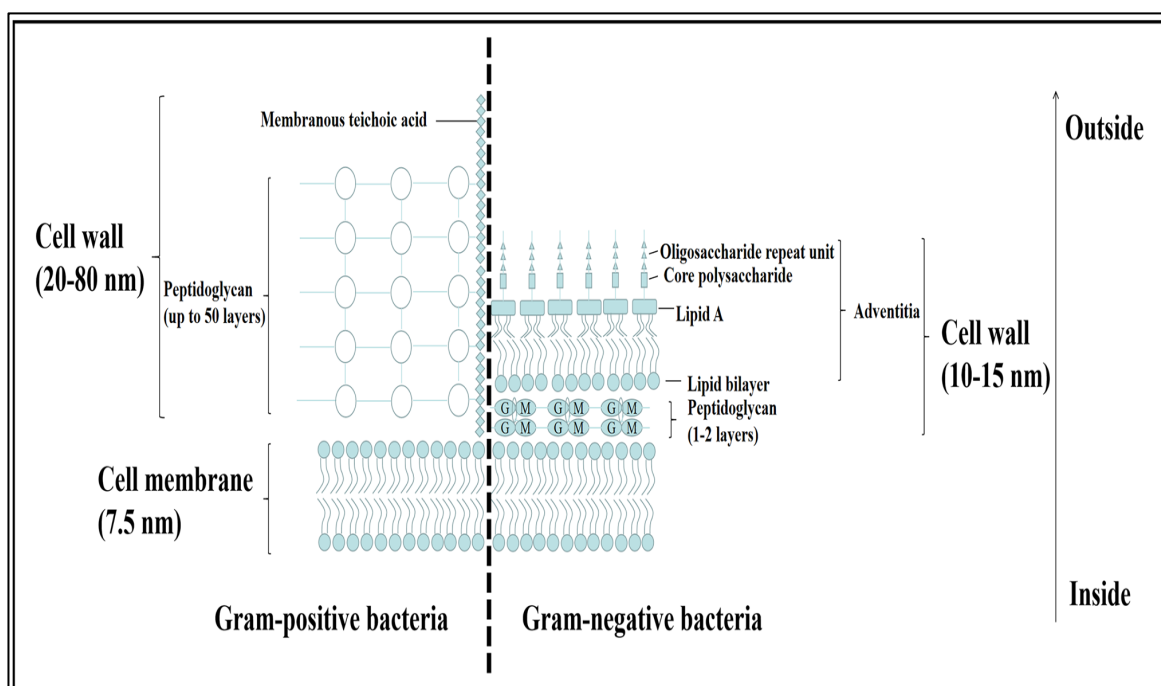


Figure 08. Gram negative and gram positive membrane cell (Liu et al., 2023)

As showed in **Figure 08**, Gram-positive bacteria like *Staphylococcus aureus* possess more thick and strong cell walls, due to the additional presence of peptidoglycan layer makes destruction of cell membrane difficult compared to Gram-negative bacteria like *Escherichia coli*. The cell membrane in Gram positive bacteria can provide better protection against environmental stress (Liu et al., 2023; Shaw et al., 2021).

Microwave-assisted sterilisation relies on the pH of food products, as it is linked to the thermal resistance of bacterial spore formers. Foods with a high acidity and ($\text{pH} \leq 4.6$) such as canned fruit juice, fruits, and pickled vegetables, can be treated between 80 and 90°C for a certain period of time, while low acid food products ($\text{pH} > 4.6$) require a temperature of approximately 121°C for a certain period to attain commercial sterility (Nguyen and Songsermpong, 2022).

II.2. 2. Destruction mechanism by MW

MW processing is mainly responsible for the inactivation of micro-organisms and enzymes due to heating increase (Van Impe et al., 2018). About the destructive mechanism, the effect of MW in destroying micro-organisms is the subject of debate; thermal effect and non-thermal effect are implicated.

The thermal effect

Thermal radiation heating: Heat is first transferred to media such as a gas or liquid. Next, the external heat of the heated media is transferred to objects. In addition, the media being heated and the target objects to be reached are not the same (Xiong et al., 2021).

Microwave heating makes use of the ability of some types of compounds (liquids or solids) to convert electromagnetic energy into heat. The transfer of energy occurs through dielectric losses. This is in contrast to the conduction and convection processes observed in conventional heating. The amount of heating depends on the dielectric properties of the molecules. This also differs from conventional heating. These properties mean that the absorption of the radiation and the heating can be carried out in a selective manner. Microwave irradiation is fast and volumetric, heating the entire material simultaneously (De la Hoz et al., 2005).

The thermal effect is caused by an increase in the temperature of the structure intracellular and extracellular under the influence of MW (Kubo et al., 2020).

The thermal effect involves the denaturation of enzymes, proteins, and nucleic acids through heat generated by electromagnetic waves and food interaction. Enzymes act as catalysts for biochemical reactions in the living cells of micro-organisms. As proteins, their primary, secondary, and tertiary structures are denatured when exposed to high temperatures. Thus, the active sites of the enzymes are lost and some biochemical activities essential for the survival of the micro-organism are reduced. Moreover, nucleic acids, typically DNA, are also damaged at high temperatures (Kubo et al., 2020).

Non-thermal effect

The literature suggests that the effects of exposing a prokaryotic biosystem to microwave radiation cannot be entirely explained by the increase in temperature. The interaction of microwave radiation with microorganisms suggests the existence of specific (or non-thermal) effects (Shamis et al., 2012).

In order to obtain more information on the mechanisms of action of microwaves, particularly in terms of non-thermal effects, numerous studies have been carried out on the interactions between microwaves and certain cellular constituents (Banik et al., 2003).

Non-thermal radiation heating: Internal heat is generated directly on target objects. There is no need for an additional medium to be used to transfer the heat. The heat transfer medium and the target objects are in the same state (Xiong et al., 2021).

Shamis et al. (2011), tested the effects of microwaving on *E. coli* at sub-lethal temperatures (<40 °C) at a frequency of 18 GHz. Immediately after microwave exposure, *E. coli* cells were found to have a significantly different cell morphology compared to negative controls (cells appeared shrunken and dehydrated), as determined by scanning electron microscopy (SEM). However, this MW effect appeared to be transient, as 10 minutes after treatment the cell morphology appeared identical to that of untreated controls. They also suggested that pores were formed in the cell membrane. However, cell viability tests showed that microwave treatment under these conditions was not bactericidal.

A non-thermal effect on microorganisms and enzyme inactivation is mainly caused by the electric field. Non-thermal effects affect the integrity of cell membranes and release intracellular proteins in microorganisms (Guo et al., 2020). Under the influence of MW, the cell membrane is ruptured, leading to the formation of pores, increased permeability and ultimately an irreversible loss of cellular integrity (Nguyen and Songsermpong, 2022).

MW sterilisation is widely used to ensure food safety. By determining the optimum application conditions during sterilisation, a short processing time and superior product quality can be achieved, making it suitable for use across a diverse range of food products (Kutlu et al., 2022; Vignali et al., 2022).

Table II summarises some of the work that has been carried out to assess the effect of microwaves on microbial inactivation in certain foods. In addition, MW sterilised products do not show non-enzymatic browning or develop a surface crust, presenting potential advantages in specific applications (Shahi et al., 2021).

Table II. Examples of microwave applications in food technology (Guzik et al., 2022).

Material	MW process	MW system	Results	References
Fresh carrots	Pasteurisation	915 MHz, 1400 W, For 3 min and 10 min	Microwave-assisted pasteurisation has reduced the total processing time (by 50%), has reduced the cooking values* and has improved the quality and uniformity of the products in comparison with conventional pasteurisation.	(Peng et al., 2017)
Durum wheat fettuccine	Pasteurisation	915 MHz, 4.5 kW	Microwave treatment has a weakening effect on the pasta strands. It increases the processes of retrogradation. Significant differences in the percentage of gelatinised starch compared to conventionally prepared pasta	(Joyner et al., 2016)
Strawberry purée (Senga Sengana)	Pasteurisation	2450 MHz; 2000 W, 90 and 120°C for 10 s	Nutrient degradation was faster when pasteurised using microwaves compared to conventional pasteurisation. After storage, 43 and 34% of polyphenols, 89 and 58% of anthocyanins and 57 and 52% of antioxidant capacity were lost for microwave and conventional pasteurisation respectively.	(Marszałek et al., 2016)
Green beans (Phaseolus vulgaris L.)	Pasteurisation	915 MHz	Microwave pasteurisation causes 9.2% loss of ascorbic acid compared to conventional heating, resulting in 15.3 %, 28.3 % and 33.9 % of chlorophyll a, respectively.	(Qu et al., 2021)
Orange juice-milk beverages	Pasteurisation	At 65 °C for 60 s	After 28 days of storage, the microwaved sample had a higher ascorbic acid activity compared to traditional heating.	(Martins et al., 2021)

Table II. Examples of microwave applications in food technology (continued) (Guzik et al., 2022).

Material	MW process	MW system	Results	References
Fruit beverages	Pasteurisation	2450 MHz; 490 W	Enzymatic activity is completely inactivated. Sensory panel shows adverse colour changes	(González-Monroy et al., 2018)
Mexican sauces	Pasteurisation / Sterilisation	915 MHz; 2450 MHz; 5800 MHz	The frequency of microwave irradiation of denser liquids should be 915 MHz.	(Hernandez-Gomez et al., 2021)
Cow's milk	Pasteurisation	Frequency unknown; 160–480 W, 40–80 s	Vitamin C losses during treatment are reduced by increasing the thickness of the milk layer (from 3–5 cm).	(Bai et al., 2015)
Vegetables packs (broccoli, pepper, sweet potato)	Pasteurisation	915 MHz, 3500 W, processing time 4–7 min	Sterility was achieved, colour of broccoli degraded (pheophytinisation), when stored at 30 °C for at least 60 days, but largely retained in pepper and sweet potato	(Koskineemi et al., 2013)
Chicken components (drumettes)	Pasteurisation	2450 MHz, 900 W, 10–80 s	Elimination of <i>Listeria monocytogenes</i> after 60 s and at a temperature above 74°C.	(Zeinali et al., 2015)
Jalapeño pepper (<i>Capsicum annuum</i> L.) Coriander leaves (<i>Coriandrum sativum</i> L.)	Sterilisation	2450 MHz, 1200 W, till reaching 63 °C in the samples	5.12 and 4.45 log reduction in <i>Salmonella Typhimurium</i> in jalapeño pepper and coriander leaves respectively.	(De La Vega-Miranda et al., 2012)
Cow's milk	Heating	2455 MHz; 100, 200, 300, 400, 496 (~500), and 600 W	Total plate count and yeast count reduced by approximately 5 log ₁₀ CFU/cm ³ after microwave treatment.	(Kapsándi et al., 2020)

Table II . Examples of microwave applications in food technology (continued) (Guzik et al., 2022).

Material	MW process	MW system	Results	References
Salmon (<i>Salmo salar</i>) and whiting (<i>Merlangius merlangus</i>)	Cooking	2450 MHz; to internal temperatures of 50 and 70 °C, 2.2 and 5.2 min for whiting, and 1.5 and 3.1 min for salmon, respectively	Microwave cooking of fish fillets reduced the number of <i>Escherichia coli</i> O157:H7 by 4 log cfu/cm ² , the number of <i>Staphylococcus aureus</i> by 2.78 log cfu/cm ² and the number of <i>Listeria monocytogenes</i> by 2.78 log cfu/cm ² .	(Ulusoy et al., 2019)
Fresh apple juice	Pasteurisation	2450 MHz; 600 W and 720 W, treatment times: 5, 10, 15, 20, 25 s	Microwave treatment reduces pathogens by up to 7 log cycles.	(Mendes-Oliveira et al., 2020)
Pehlitz	Sterilisation	850, 1700, 2550, 3400, 4250 and 5100 W, treatment times: 30, 40, 50 s.	Microbial inactivation even reached 98.05% after microwave sterilisation.	(Fan et al., 2020)
Red pepper (<i>Capsicum annuum L.</i>)	Sterilisation	treatment times: 15, 30, 45, 60, 75 s	An overall log reduction of 8.85 in the total number of bacteria and a 7.37 log reduction in the number of yeasts and moulds after microwave sterilisation compared to the natural microbial flora.	(Shirkole et al., 2020)
Green beans	Pasteurisation	915 MHz; with a total microwave power of 18.7 kW	Microwave pasteurisation resulted in a reduction of 9.0 log CFU/g, compared with high hydrostatic pressure.	(Inanoglu et al., 2021)
Carrot slices	Pasteurisation	200, 400, 600 W, time up to 250 s	At 600 W, the highest level of pathogen reduction was reported (approx. 90%).	(Roohi and Hashemi, 2020)
Vegetables packs (broccoli, pepper, sweet potato)	Pasteurisation	915 MHz, 3500 W, processing time 4–7 min	Sterility was achieved, colour of broccoli degraded (pheophytinisation), when stored at 30 °C for at least 60 days, but largely retained in pepper and sweet potato	(Koskineniemi et al., 2013)

I.3. Predictive microbiology

I. 3 .1. Introduction

Predictive microbiology was developed in the early 20th century when Bigelow et al. introduced a log-linear model to describe the kinetics of bacterial death (Petruzzi et al., 2022). It aims to combine traditional microbiological knowledge with knowledge from mathematics, statistics, information systems and technology to describe microbial behaviour and prevent food spoilage and food-borne diseases (Fakruddin et al., 2011).

The establishment of log-linear microbial death kinetics by Bigelow et al. (1920), Bigelow (1921) and Esty and Meyer (1922) was the first example of a predictive model that found extensive application in the food industry (Fakruddin et al., 2011). It could be defined as the application of research related to the quantitative microbial ecology of food (Fakruddin et al., 2011). Predictive microbiology could be defined as the application of research related to the quantitative microbial ecology of food (Fakruddin et al., 2011).

Predictive microbiology is a fundamental aspect of food microbiology; its objectives are to determine the responses of a given micro-organism by combining mathematical models with experimental data under specific environmental conditions, and to simulate a priori the growth/inactivation of a population based on known characteristics of a food matrix (Petruzzi et al., 2022).

Nowadays, predictive microbiology is usually referred to as a tool/theory applied to the improvement of food safety and quality and there are many models and statistical tools dealing with pathogens, toxins, spoilage micro-organisms, thermal and non-thermal processes (Bevilacqua et al., 2015).

I. 3. 2. Classification

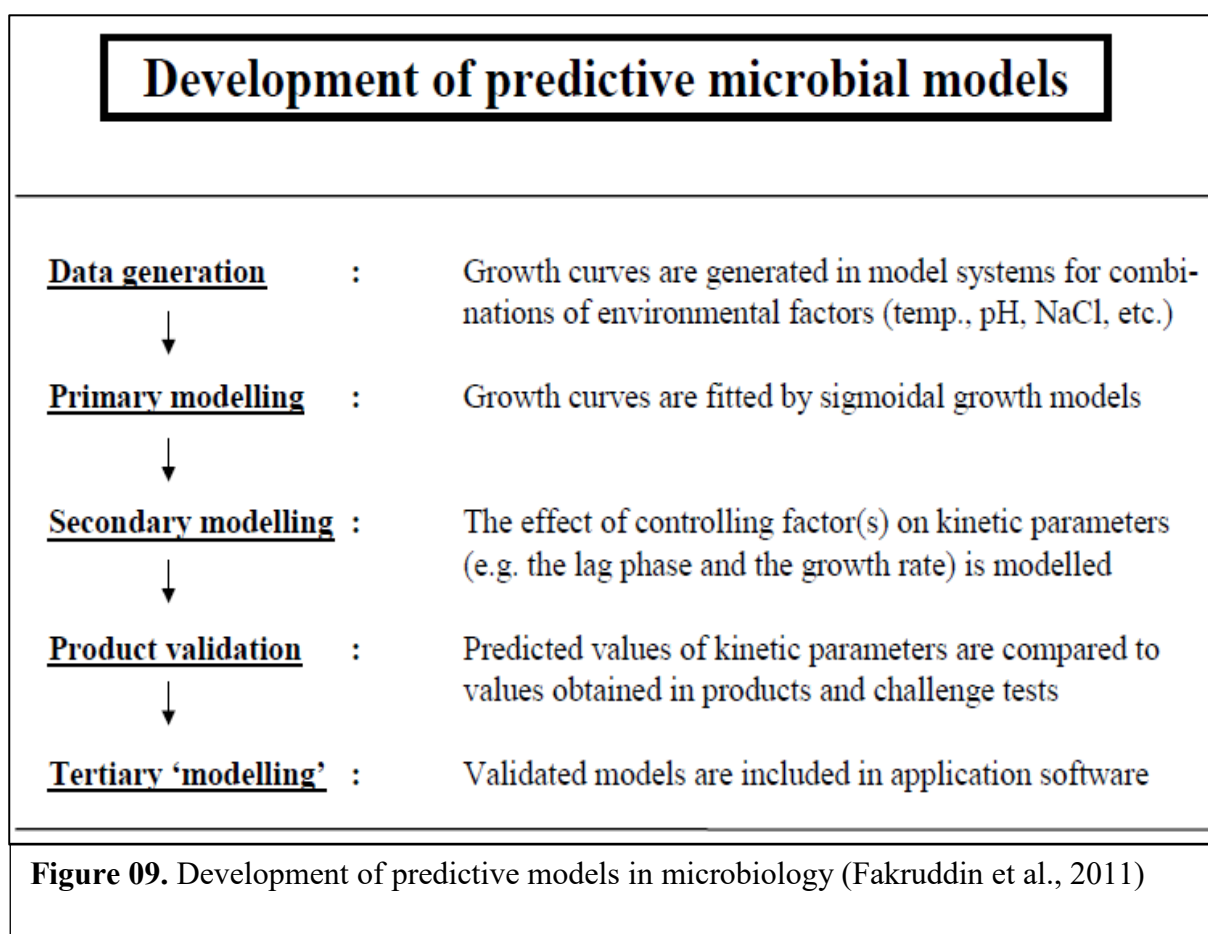
In predictive microbiology, mathematical models are utilised to predict microbial patterns as they are affected by various environmental influences (**Stavropoulou and Bezirtzoglou, 2019**). There are various methods to categorise models, according to the event (growth or destruction), the theoretical basis (empirical models or mechanistic functions), the dependent variables and the input conditions (**Figure 09**): primary, secondary and tertiary models (Fakruddin et al., 2011).

A classification of the models using growth rates would be as follows (Stavropoulou and Bezirtzoglou, 2019; Fakruddin et al., 2011; Petruzzi et al., 2022):

1. Kinetic models of microbial growth or destruction, taking growth rate as the dependent variable,
2. Probability models, typically utilising the logit methodology (expressing the probability of growth or mortality under specific conditions),
3. Empirical models are first or second order polynomials used to describe data with mathematical equations (curve fitting). However, such models have a major disadvantage when it comes to prediction precision, which can be greatly reduced by extrapolation,
4. Mechanistic or deterministic models. They have a robust theoretical basis and can serve to understand and explore phenomena beyond biological processes.

The classical three-level scheme is another classification of models in predictive microbiology based on the dependent variable (primary vs. secondary models) or the objectives (interpolation or prediction; primary/ secondary vs. tertiary models) (Petruzzi et al., 2022).

1. First-order models are based on microbial growth over time (Stavropoulou and Bezirtzoglou, 2019). These models include mortality models, D-values, destruction/ survival and growth rate values, and estimates of lag time based on the concept that microbial growth can be described by a sigmoid (Pradhan et al., 2019).
2. A secondary model describes the influence of environmental conditions (pH, temperature, a_w , nutrients, antimicrobials, etc.) on the kinetic parameters evaluated by the primary model (growth rates, lag phases, inactivation rates, etc.) (Stavropoulou and Bezirtzoglou, 2019).
3. Finally, in tertiary models, computer tools are employed to simulate the growth of microorganisms a priori (Stavropoulou and Bezirtzoglou, 2019).



Developing a predictive model necessitates meticulous planning and execution of the experimental design to assess the impact of both intrinsic and extrinsic factors (**Figure 10**). A top-notch experimental design guarantees that the data collection process produces reliable and high-quality results. Furthermore, adequate planning and organization of the experiment play a pivotal role in ensuring the collected data meets the necessary criteria for studying microbial behavior and quality (da Silva et al., 2023).

Some of the applications of predictive microbiology are listed in **Table III** (Fakruddin et al., 2011).

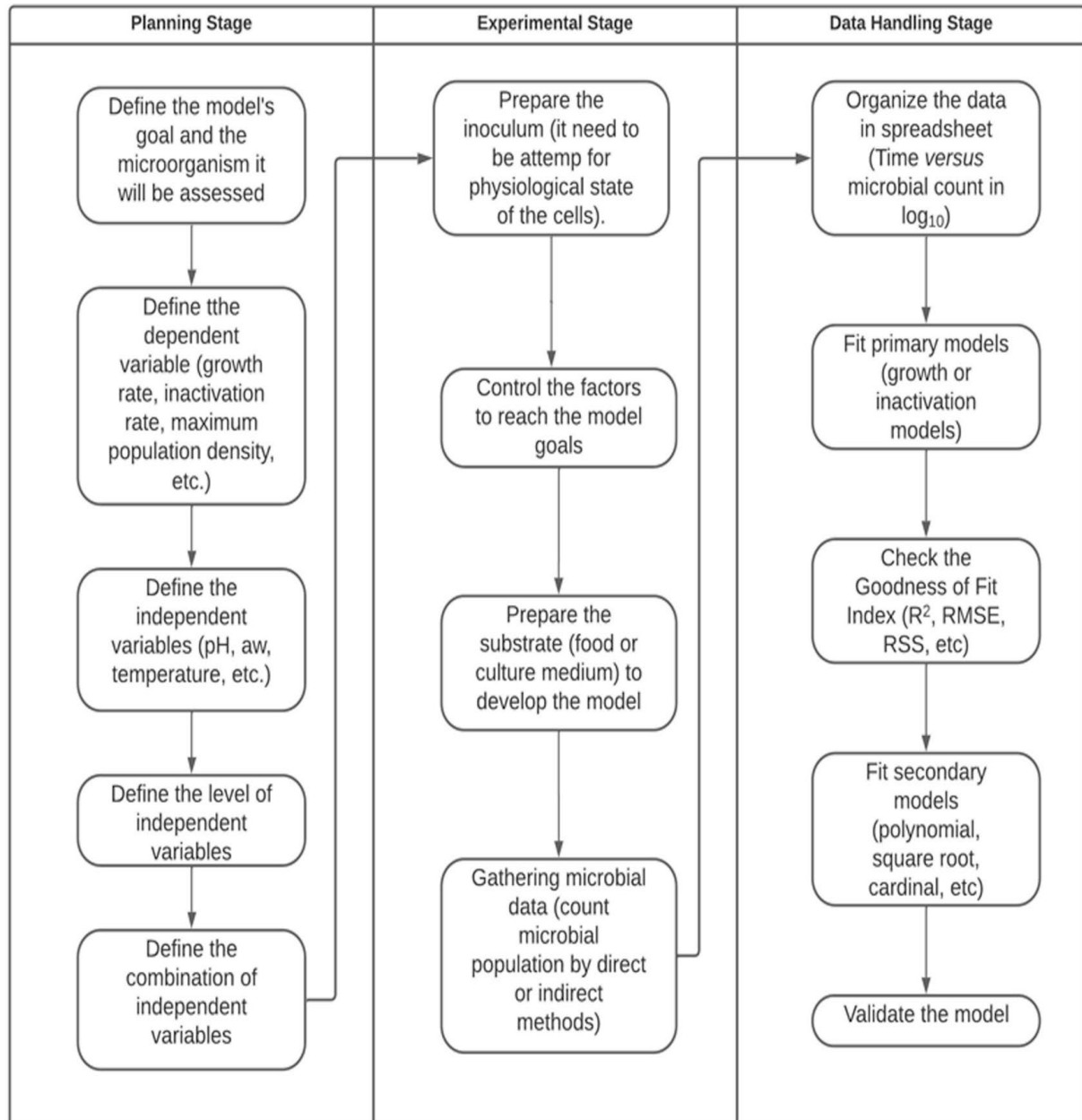


Figure 10. Step-by-step guide for developing a predictive model (da Silva et al., 2023)

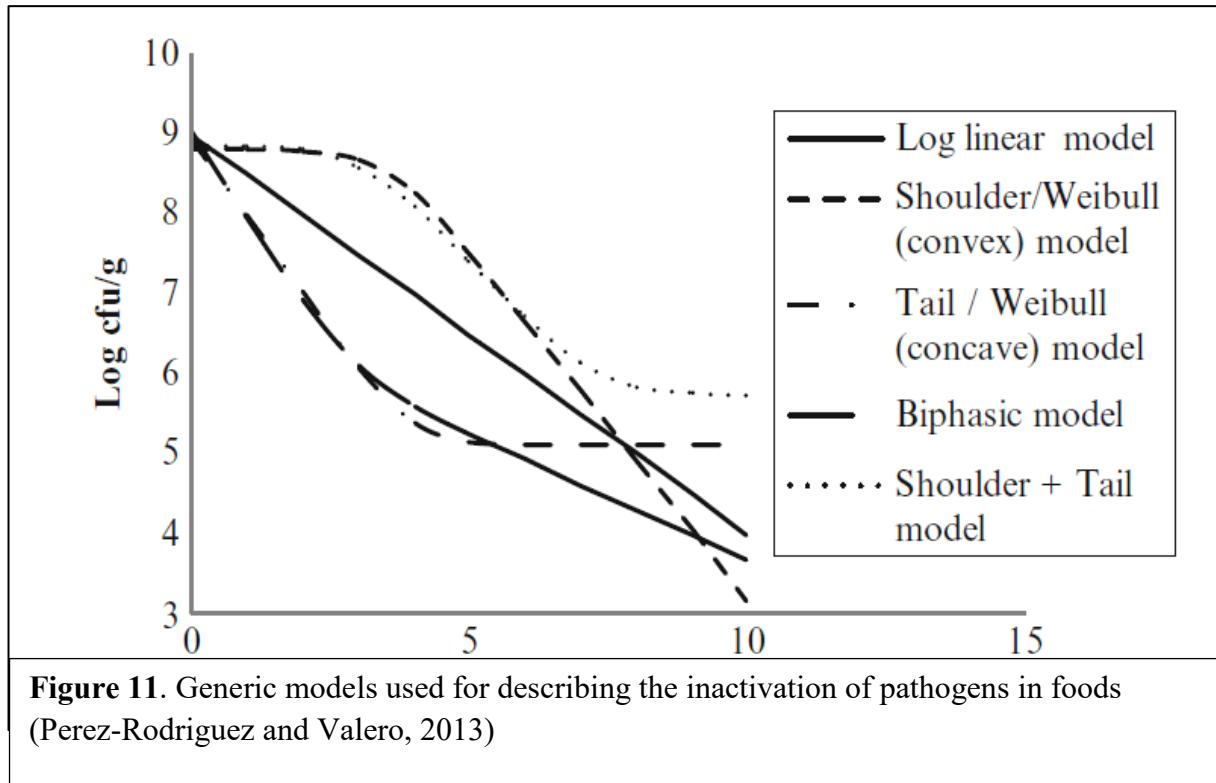
Table III. Applications of predictive microbiology (Fakruddin et al., 2011)

Area of Application	Example
Hazard Analysis Critical Control Point (HACCP)	Preliminary analysis of the hazards Critical control point identification and establishment corrective measures Assess the significance of the interaction between variables
Risk assessment	Estimating changing microbial counts in a production chain Assessing exposure to a specific pathogen
Microbial Shelf life studies	Predicting the growth of specific foodborne pathogens Predicting the growth of specific foodborne pathogens
Product research and development	Impact of changing product composition on food safety and spoilage Impact of processing on food safety and spoilage Assessment of the impact of out-of-specification circumstances
Temperature function integration and hygiene regulatory activity	The impact of temperature in the cold chain on safety and spoilage
Education	Safety education, especially for non-technical people
Design of experiments	Number of samples to prepare Definition of the sampling interval

I. 3.3. Inactivation Models

The most widely used models to characterize the inactivation of foodborne pathogens are proposed in literature (**Figure 11**). Geeraerd et al. (2005) describe nine different types of primary inactivation models:

1. Classical log linear curves.
2. Curves displaying a so-called shoulder before a log-linear decrease are apparent.
3. Curves displaying a so-called tail after a log-linear decrease.
4. Survival curves displaying both shoulder and tailing behaviour.
5. Concave curves.
6. Convex curves.
7. Convex/concave curves followed by tailing.
8. Biphasic inactivation kinetics.
9. Biphasic inactivation kinetics preceded by a shoulder.



I. 3.3. 1. Primary model

Primary models assess the reaction of microorganisms over time for a particular set of circumstances. The survival curve that ensues may be linear or non-linear. To describe inactivation behavior, various equations have been developed by several authors for this purpose: The linear model, Weibull model and Non-linear models with shoulder and / or tail. (da Silva et al., 2023).

➤ Linear Model / Bigelow Model

The term "log-linear" pertains to the model as many inactivation data indicate a close alignment with a straight line (Dolan and Mishra, 2013). The first model, introduced around a century ago, represents a log-linear inactivation as shown in equation (1) (Van Boekel, 2021):

$$\log_{10}N = \log_{10}N_0 - \frac{t}{D} \quad (1)$$

Parameter D represents decimal reduction time, $\log_{10}N_0$ and $\log_{10}N$ the logarithm of the number of micro-organisms at time zero and time t , respectively. Sometimes, the survival ratio $S = N/N_0$ is modeled as in Equation (2):

$$\log_{10} \frac{N}{N_0} = \log S_t = - \frac{t}{D} \quad (2)$$

S_t is the momentary survival ratio.

One drawback of this model is that it tends to focus on thermal treatments rather than innovative than innovative preservation techniques (Perez-Rodriguez and Valero, 2013).

Plotting $\log D$ -values against temperature results in a reciprocal slope that represents the z -value. This z value indicates the temperature that is required to reduce D by a factor of 10, resulting in an increase in the rate of destruction by a factor of 10 (Perez-Rodriguez and Valero, 2013). D and z are represented by equation (3) and (4) as following:

$$D = D_{\text{ref}} 10^{\frac{T_{\text{ref}} - T}{z}} \quad (3)$$

$$z = \frac{T_{\text{ref}} - T}{\log D - \log D_{\text{ref}}} \quad (4)$$

Where D_{ref} is the D -value at the reference temperature, T_{ref} (the usual T_{ref} is 121.1°C). D -values may be affected by the organism's type (strain), treatment temperature, cell physiological state, pH, fat, and a_w content.

➤ The Weibull model

The traditional D value of first-order kinetics is inadequate for quantification of bacterial heat resistance for non-log linear survival curves. A straightforward model based on the Weibull cumulative function is used to describe the non-logarithmic kinetics of microorganisms (Couvert et al., 2005).

Over time, several other models have been proposed as log-linear behaviour is not commonly observed: a concave or upward concavity of curves was frequently observed. The Weibull model presented by **equation 5 and 6** is quite flexible as it can describe both log-non-linear and log-linear behaviour (Van Boekel, 2021).

The model is built by two parameters, the scale parameter δ (time) and the dimensionless shape parameter p . The logarithm of the scale parameter δ depends linearly on

temperature; however, this relationship for parameter p is not so well established. The shape parameter accounts for upward concavity of a survival curve ($p < 1$), a linear survival curve ($p = 1$), and downward concavity ($p > 1$) (**Figure 12**). Therefore, if $p = 1$ no biological variation is assumed (each cell is equally susceptible to be destroyed) (Perez-Rodriguez and Valero, 2013). For the traditional case where the survival curve, originated from a first order, is linear p equal 1 and the δ parameter correspond to the classical D value (Couvert et al., 2005).

Different forms of this model were presented in literature, however the decimal logarithm equation 5 and 6, seems more suitable to describe the non-logarithm linear survival curves (Mafart et al., 2002; van Boekel, 2002):

$$\log N_t = \log N_0 - \left(\frac{t}{\delta}\right)^p \quad (5)$$

$$\log_{10} \frac{N}{N_0} = \log S_t = -\left(\frac{t}{\delta}\right)^p \quad (6)$$

N is the number of microorganisms (CFU/mL) surviving the treatment at time t and N_0 is the initial number of microorganisms (CFU/mL).

This variation in parameters allows the Weibull model to be more flexible than the linear model based on D-values. For example, the behaviour of cells may be differently if the cells have been adapted to certain stress conditions in food or if they have been grown in grown under 'ideal' laboratory conditions (Perez-Rodriguez and Valero, 2013).

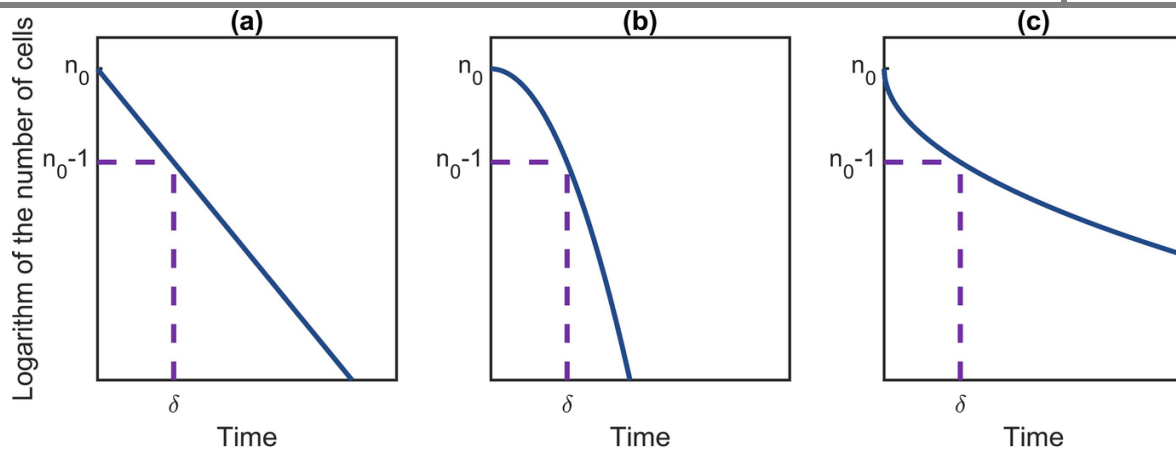


Figure 12. Weibull inactivation models for which the initial number of cells (n_0), population level after a one decimal reduction ($n_0 - 1$), and the rate parameter (δ) are indicated. The subfigures represent cases with a different shape parameter p : (a) a linear model with $p = 1$, (b) a concave model with $p > 1$, and (c) a convex model with $p < 1$ (Akkermans et al., 2020).

➤ Shoulder/tail model

Shoulder/tail models are founded on the presence of a shoulder or lag phase (prior to inactivation) and a tail region (following the inactivation treatment) (Perez-Rodriguez and Valero, 2013).

Geeraerd et al. (2000) reported at least four different reasons for a shoulder appearing in a dying kinetic (Bevilacqua et al., 2015):

- Microorganisms are arranged into clumps. The shoulder is the time before all but one organism in such clumps has died.
- Cells have a tendency to counterbalance the effect of a lethal treatment, so the shoulder is the period when cells are able to re-synthesise a critical component, and death only occurs when the rate of destruction exceeds the rate of synthesis.
- A shoulder could describe the protective effect of the medium or of some of its components (fats, proteins) on the cells.
- A shoulder could describe a cumulative injury that needs to occur before the cell is inactivated.

Nonlinear models with a shoulder and tail (equation 7) characterise a period of time prior to inactivation and a residual population after treatment, the tail region (da Silva et al., 2023).

$$N = N_0 e^{-k_{\max} \cdot t} \frac{e^{k_{\max} \cdot Sl}}{1 + (e^{k_{\max} \cdot Sl} - 1) \cdot e^{-k_{\max} \cdot t}} \quad (7)$$

With N is the cell concentration after a treatment time $t_{(s)}$, N_0 is the initial cell concentration, $k_{\max}$ is the maximum inactivation rate, and Sl is the shoulder length (the length of the lag phase).

I. 3.3 .2. Secondary Models

Secondary Models detail the effects of several variables on microbial behaviour in food. These models are built upon primary models, which describe microbial growth, survival, or inactivation as a function of environmental factors like temperature, pH, and water activity. Secondary models take into account other factors that influence microbial behaviour. These include other microorganisms, the components present in the food and any chemical or physical treatments used (da Silva et al., 2023).

Secondary models include competitive growth models describing the development of multiple microorganisms in a food matrix, and hurdle models describing the effect of preservation factors on microbial growth/survival. Further secondary models encompass models for the influence of preservatives, thermal processing, and modified atmosphere packaging on microbial growth. These models can use either kinetic or probabilistic approaches to describe the influence of intrinsic and extrinsic factors on microbial growth, survival or inactivation (da Silva et al., 2023; Stavropoulou and Bezirtzoglou, 2019).

I. 3.4. Goodness Fit

Once the mathematical model has been refined, it is crucial to test it to ensure its accuracy in real-world conditions, which is the final step in the modelling cycle (Guillard et al., 2016).

Goodness of fit indices are used to assess the fit of a model. Certain metrics, including R^2 , RSS, and RSME, can supply distinct information regarding the accuracy and precision of the model's predictions (da Silva et al., 2023; Perez-Rodriguez and Valero, 2013).

➤ **R-Squared (R^2)**

R^2 is a statistical parameter that indicates how well the model fits the data, i.e. it assesses the performance of a security or index (dependent variable) in relation to a given comparative index (independent variable). R^2 adjusted indicates the proportion of variance in the dependent variable that can be accounted for by the independent variable.

$$R^2 = \frac{\sum_{i=1}^n (\hat{y}_i - \bar{y})^2}{\sum_{i=1}^n \frac{(y_i - \bar{y})^2}{(n-1)}} \quad (8)$$

With y_i observed value, $\hat{y}$ is the predicted value and n is the number of data points or observations.

➤ **RSS**

The residual sum of squares (RSS), also known as the sum of squared errors (SSE), determines whether the regression model accurately fits the given data set. It measures the variability between the observed data values and their predicted values by the regression model.

$$RSS = \sum_{i=1}^n (y_i - f(x_i))^2 \quad (9)$$

Where y_i = the i^{th} value of the variable to be predicted, $f(x_i)$ = predicted value of y_i , n = upper limit of summation

➤ **RMSE**

RMSE (Root Mean Squared Error) is an absolute error measure that squares deviations to avoid positive and negative deviations cancelling each other out. This measure also tends to overestimate significant errors, which can be helpful in identifying methods with these errors.

$$RMSE = \sqrt{\frac{\sum_{i=1}^n \left[\left(\log \frac{N}{N_0} \right)_{\text{observed}} - \left(\log \frac{N}{N_0} \right)_{\text{predicted}} \right]^2}{n - p}} \quad (10)$$

Where n and p are the numbers of replications for each experiment and p is the number of parameters for each equation.

1. 3. 5. Limitations of model

Some limitations to predictive microbiology that need to be taken into account cited below (Fakruddin et al., 2011):

- The models are not extrapolable outside the ranges (e.g. $T^{\circ}\text{C}$, a_w) in which they were deduced. The models do not model microbial behaviour because they are derived by fitting observed data.
- The models tend to predict faster growing rates than are observed. This makes them fail-safe, but they can be overly conservative. The reason for this is that the models are usually carried out in laboratory media and although they are validated in food, they may not be widely used in the food industry.
- Several workers have also pointed out that models derived under static conditions may not be applicable to fluctuating conditions, i.e. those in which where, during the life of the product, environmental conditions such as temperature, pH, gaseous atmosphere and water activity change

I. 3. 6. Model validation

The validation of models is an essential step in the modelling process. A validation process, usually consisting of experimental confirmation of the predictions using some quantitative technique, must precede the application of a model.(Perez-Rodriguez and Valero, 2013)

To validate models for foodborne pathogens, compare predicted and observed values and calculate the residual (observed minus predicted). Primary, secondary (initial number, maximum population density), and tertiary models predicting log numbers can be evaluated using the residual to compare their predicted and observed values. Rather, the relative residual is used to compare the observations and predictions obtained from secondary models that predict lag time (prediction-observation) or growth rate (observation-prediction) (Oscar, 2023).

Validation of foodborne pathogen models is essential to give users confidence in the reliability of the predictions (Fakruddin et al., 2011). Testing data, model performance and model validation criteria facilitate objective, comprehensive, unbiased, accurate and unbiased validation (Oscar, 2005; Oscar, 2020) . Validation of models is crucial as it can highlight any data gaps and prediction problems that can be addressed through further research (Oscar, 2005).

However, there are only a small number of studies that apply these criteria to the validation of models. The aim of validation is not the rejection of models, but rather the refinement of models to improve their performance (Boleratz and Oscar, 2022).

➤ Bias factor (B_f), and Accuracy factor (A_f)

The model's predictive effectiveness was assessed by computing the performance indicators, Bias Factor (B_f) and Accuracy Factor (A_f), as shown in equations (11) and (12).

$$B_f = 10^{\left[\Sigma \log \frac{(pred)}{obs} \right]} \quad (11)$$

$$A_f = 10^{\left[\Sigma \frac{\log pred}{obs} \right]} \quad (12)$$

Ross (1996) were proposed criteria for the validation of growth rate prediction models based on these factors. The criteria state that growth rate models with a B_f between 0.9 and 1.05 are considered good, models with a B_f between 0.7 and 0.9 or 1.06 and 1.15 are considered acceptable, and models with a B_f less than 0.7 or greater than 1.15 are considered unacceptable (Portela et al., 2019).

The prediction bias (B_f) and accuracy (A_f) factors are the most widely used measures of performance of predictive models for food pathogens. Nevertheless, B_f and A_f possess limitations that can lead to inaccurate model assessments. As a result, APZ method was to establish for measuring model performance that can overcome the limitations of B_f and A_f (Oscar, 2005).

➤ **The acceptable prediction zones (APZ)**

The acceptable prediction zones (APZ) technique was created to overcome the shortcomings of conventional methods. It has demonstrated superior performance to traditional methods and is the sole approach that implements standards for test data, model performance, and model validation (Oscar, 2005).

The APZ method sets out criteria for three categories of data: (1) data that depends on others; (2) data that is independent for interpolation; and (3) data that is independent for extrapolation. These criteria necessitate validating dependent data before validating interpolation and validating interpolation before validating extrapolation (Oscar, 2005; Oscar, 2020). This last process is optional but important because it can save time and money by identifying independent variables for which a new model is not required (Oscar, 2023).

The APZ method establishes criteria for the evaluation of the performance of three different types of models: (a) secondary models for lag time; (b) secondary models for growth rate; and (c) primary, secondary and tertiary models for log number.

The test data and validation criteria are identical across all three types of models, although prediction errors and APZ vary depending on the model type (Boleratz and Oscar, 2022; Oscar, 2005; Oscar, 2020; Oscar, 2023). The APZ was comprised between -1 and $+0.5$ log CFU/mL (Desriac et al., 2019). The APZ had to be doubled in width in the fail-safe direction since more error tolerance can be allowed in that direction when the model is utilized to meet the food safety standards (Ross et al., 2000).

Matériel & Methods

Chapter II. Materiel & Methods

II.1. Bacterial cultures

Escherichia coli and *Staphylococcus aureus* were obtained from the Spanish Type Culture Collection (CECT, Burjassot, Valencia). They were stored at -80°C in cryovials containing beads and cryopreservatives.

The experimental protocol (**Figure 13**) applied in this study was as follows:

- The first step consisted to revive the bacterial strains (*E.coli* and *S. aureus*). Three days before the test, one bead of each bacterial strain was transferred to a tube containing 9 mL of tryptone soy broth (TSB, Oxoid, UK) and incubated at 37 °C for 24 h.
- A second subculture was prepared for each strain by inoculating tubes containing 9 mL of TSB with 0.1 mL of the initial subcultures and incubating under the same conditions followed in the first step (37 °C for 24 h).
- Finally, a third subculture of each bacterial suspension was prepared and incubated at 37°C for 14 hours to reach the early stationary phase.
- The resulting final subcultures were centrifuged for 10 minutes at 4000 r/min and the precipitate was then resuspended in 10 mL of phosphate-buffered saline (PBS, Oxoid).
- A standardisation of the inoculum concentration was achieved via microbial counts on Plate Count Agar (PCA agar) in Petri dishes, resulting in the number of bacteria expressed as colony-forming units CFU /mL. A microbial count has been carried out to standardise the concentration of the inoculum to approximately one. 10^8 - 10^9 CFU / mL.

Enumeration of colonies was performed using an automated colony counter Flash & Go (IUL Instruments, Barcelona, Spain).

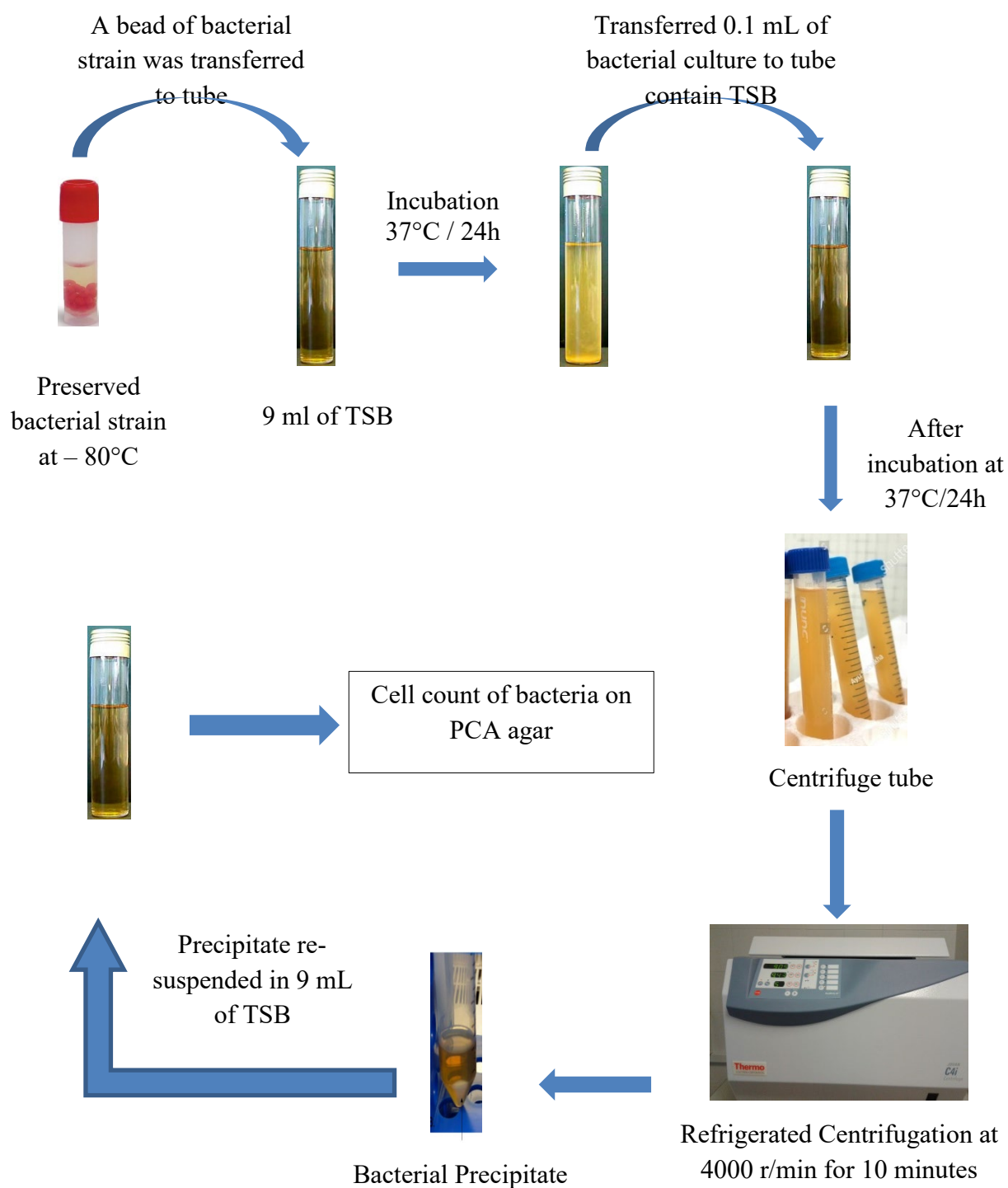


Figure 13. Revivification of *Escherichia coli* and *Staphylococcus aureus*

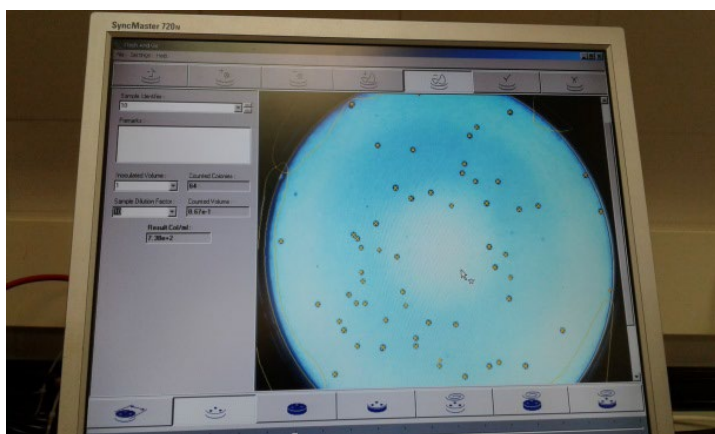


Figure 14. Flash & Go automated colony counter

The Flash & Go is a compact, automated colony counter represented in **figure 14**. It is designed for both pour plates and spiral spreading. It is capable of counting all types of colonies on a range of media, and its high definition colour camera, which is connected to an external PC. It can even detect and the computer captures and analyses images of the agar plates, saving the results in a Microsoft spreadsheet (Excel format). The screen displays the count of colonies and the number of CFU per mL (Mahapatra et al., 2009).

II.2. Preparation of media and addition of salt solutions

The study was carried out on buffered peptone water (BPW, NaCl content 5 g/L and pH 7.2±0.2 at 25 °C). Sodium chloride was included in the BPW (Oxoid) at levels of 3.5% and 8.5% (w/v). To reach a salt level of 3.5% and 8.5%, 30g and 30g of NaCl are added respectively to one litre of BPW.

To carry out the microbial destruction by microwave, three series of flasks were prepared. Each series of flasks is distinguished to effect a bacterial destruction at a well-defined concentration (0.5%, 3.5% and 8.5%). The prepared flasks contained 50 mL of BPW were sterilised by autoclaving at 121°C for 20 minutes.

II.3. Microwave treatments

Microwave treatments were performed in a commercial microwave oven (**Figure 15**) with a frequency of 2450 MHz, output power between 100 and 800 W, Oven cavity Dimensions are 30.6 x 20.1 x 32.2 cm and volume of 20 L (Samsung M1727, Food Science and Technology International Korea).



Figure 15. Microwave used to perform experiments on bacterial destruction (Samsung M1727, 2450 MHz)

II.3.1. Determination of thermal histories

Power levels of 450, 600 and 800 W were applied in the present study. The first step was the determination of thermal histories. For each selected power, the temperature was measured every 5 seconds until the maximum required temperature was reached (70 and 80 °C). This choice was made taking into account the lethal temperatures for *E. coli* and *S. aureus*, which are 70 °C and 65 °C, respectively. Temperatures were measured with a food thermometer equipped with a special probe (HI 95809-1, Hanna Instruments). These measurements were taken from centre of exposed solutions after mixing.

Irradiation times were fixed According to NaCl concentrations of the treated solutions. At 0.5% NaCl, irradiation times were adjusted to 70 s, 50 s, and 40 s for 450 W, 600 W, and

800 W, respectively. For the concentrations of 3.5% and 8.5% NaCl, the microwave treatment times were 100 s (450 W), 70 s (600 W) and 50 s (800 W).

II.3.2. Bacterial destruction

For microwave processing, the flasks were inoculated with 1 mL of the previously prepared reconstituted suspensions of *E coli* and *S aureus*. Then, the flasks were positioned at the centre of a domestic microwave oven and exposed to microwave radiation.

The volume treated by microwave was 50 mL contained in a 500 mL bottle with a diameter of 8.5 cm and a thickness of less than 2 cm, to prevent a temperature gradient in the microwave heated medium.

After each time interval treatment, the flasks were immediately placed in ice containers for cooling. Then they were subjected to microbiological analysis.

II.4. Microbial analyses

After each microwave treatment, the solutions were cooling immediately, then, the microbiological counting was undertaken on Petri dishes.

Series of dilutions was carried out in physiological water (0.9% w/v) taking 1 ml from each treated vial. All dilutions were plated in duplicate and plates were incubated at 37 °C for 24 h before enumeration. The enumeration was performed on PCA agar (Oxoid) following by incubation of plates agar at 37°C for 24 hours. After incubation as shown in **Figure 16**, the enumeration of colonies was performed using an automated colony counter Flash & Go (IUL Instruments, Barcelona, Spain).

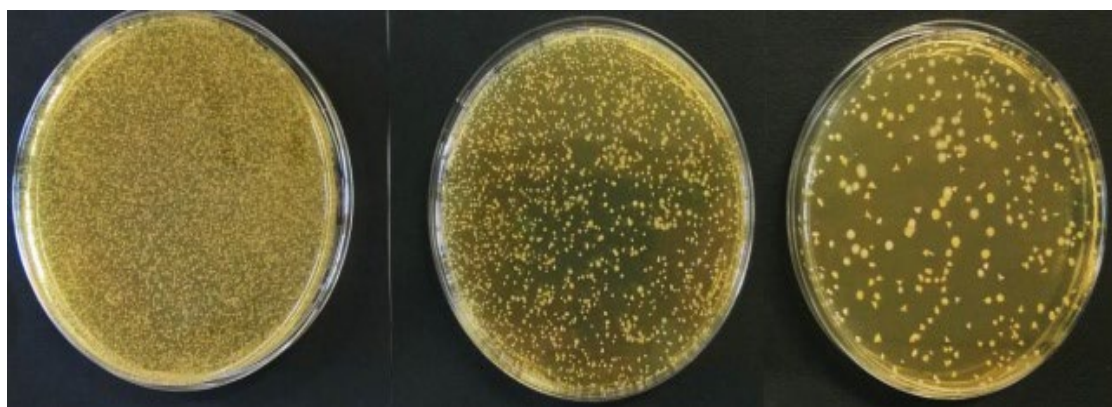


Figure 16. Enumeration colonies on Petri dishes after microwave treatment

II.5. Modeling of survival curves

In modelling, a residual population density ($\log N_{\text{res}}$) of 0.1 log CFU/ml was assumed if colonies were not observed.

A Weibull model was selected to model the survival of *E. coli* and *S. aureus* as a function of microwave intensity and salt concentrations. First, the classical two-step procedure was carried out, followed by a one-step fitting to account for the impact of non-isothermal treatments.

Survival models were constructed in the first stage of the analysis, which varies depending on the microwave power level, applied in 450, 600, and 800 W, and salt concentrations between 0.5, 3.5, and 8.5%. Fitting was performed using R 4.2.1 (R Core Team, 2020) and R package “nls Microbio” (Baty and Delignette-Muller, 2017).

The equation 13 (Mafart et al., 2002) is used by the model that describes destruction profiles without tails, while the model that fits destruction profiles characterized by a tail uses **equation 14** (Albert and Mafart, 2005).

$$\text{Log } S_{(t)} = \left[\frac{t}{\delta} \right]^p \quad (13)$$

$$S_{(t)} = (N_0 - N_{\text{res}}) 10^{\left[-\left(\frac{t}{\delta} \right)^p \right]} + N_{\text{res}} \quad (14)$$

The estimated parameters represented the time of the first decimal reduction (δ , [sec]) and the shape parameter (p , [-]) (van Boekel, 2002). N_0 and N_{res} are the initial and residual microbial populations (CFU/mL), $S_{(t)}$ the survival population of each microorganism at time t .

For the secondary modelling, the heating rates (hr, [°C/sec]) were calculated by determining the slope of the temperature and time regressions at each microwave treatment. The impact of temperature on the Weibull model parameter, δ , was formulated using the following **equation 15**:

$$\log \delta = a * hr + b \quad (15)$$

Where a and b are the coefficients to be estimated and hr is the heating rate ($^{\circ}\text{C}/\text{sec}$). When necessary, the natural logarithm ($\ln$) was applied to the first term of the equation to improve the fitting of the model.

Finally, a comprehensive Weibull model was developed by a one-step procedure by combining the primary and secondary models and setting a fixed value of p for each salt concentration. The values of p per microorganism and salt concentration were compared using an F-test to check for possible differences in model fitting, and the value with the best fit was selected for each salt concentration and microorganism.

The predictive models were evaluated for accuracy in describing the observed experimental data using the determination coefficient R^2 and root mean squared error (RMSE). R^2 was employed to assess overall prediction precision.

II.6. Comparison of inactivation kinetics

To obtain a direct comparison of inactivation kinetics, the time required to achieve 1, 2, 3 and 4 log reductions (t_x , [sec]) was calculated from the fitted parameters of the global Weibull models (Buchanan et al., 1993). To estimate t_x , we applied the subsequent **equation 16**:

$$t_x = \delta * (x)^{\frac{1}{p}} \quad (16)$$

Normal distribution and homogeneity of variances were tested using Shapiro-Wilk test and Levene test, respectively.

II.7. The goodness of fit

All experiments were carried out in triplicate in independent experiments with a duplicate set of samples. The goodness of fit of each model was assessed using several statistical parameters, such as the coefficient of determination (R^2) and the root-square mean error (RSME). A better model fit is indicated by lower RMSE values. The Coefficient of Determination and RMSE were calculated using Excel. A coefficient of determination (R^2) of 1 indicates a perfect fit between the model and the experimental data. The R^2 and RSME was calculated as follows:

$$RMSE = \sqrt{\frac{\sum_{i=1}^n \left[\left(\log \frac{N}{N_0} \right)_{observed} - \left(\log \frac{N}{N_0} \right)_{predicted} \right]^2}{n - p}} \quad (17)$$

Where n and p are the numbers of replications for each experiment and p is the number of parameters for each equation.

$$R^2 = \frac{\sum_{i=1}^n (\hat{y}_i - \bar{y})^2}{\sum_{i=1}^n \frac{(y_i - \bar{y})^2}{(n - 1)}} \quad (18)$$

With y_i observed value, $\hat{y}$ is the predicted value and n is the number of data points or observations.

II.8. Model validation

The models generated in the broth culture underwent validation using independent data acquired from UHT milk. Two microwave treatments were evaluated at 800 W and 600 W, respectively. For this purpose,

- 50 mL of milk was poured into 500 mL glass flasks and inoculated with 1 mL of bacterial suspension for each bacterial strain (*E. coli* and *S. aureus*) to reach a concentration of 10^6 to 10^7 CFU/mL.
- Microwave heating was tested at two power levels- 600 W for 40 seconds and 800 W for 30 seconds.
- Before commencing microbiological analysis, the samples were placed in an ice bath at regular intervals to prevent residual heating.
- Subsequently, 1 mL aliquots were subjected to microwave irradiation, serially diluted with 9 mL of physiological water, and inoculated on PCA Agar (Oxoid). The plates were then incubated for 24 hours at 37°C.
- After incubation, a colony counter, the Flash & Go, was employed for cell counting purposes.

To evaluate model validation, we followed (Oscar, 2005) recommendation and used the Acceptable Prediction Zone (APZ) index. The APZ estimates the percentage of residuals falling within the -1 to 0.5 log CFU/mL range. Additionally, we calculated the validation

indices for *E. coli* and *S. aureus* using Ross's (1996) (Ross, 1996) proposed metrics - the Accuracy Factor (A_f) and the Bias Factor (B_f). The A_f measures the deviation of the observed data from model predictions, whereas the B_f evaluates the model's tendency to underestimate or overestimate the observed growth. Higher values of A_f and B_f indicate better alignment between the experimental data and model predictions.

Additional performance evaluation indices were computed to compare prediction accuracy between different models used in the selected pasteurization treatments. The Accuracy Factor (A_f) and Bias Factor (B_f) assess the ability of a model to forecast microorganism response under specific stressors such as radio frequency and heat. As suggested by Ross (1996), these factors were calculated as follows:

$$B_f = 10^{\left[\frac{\sum \log \left(\frac{\text{pred}}{\text{obs}} \right)}{n} \right]} \quad (19)$$

$$A_f = 10^{\left[\frac{\sum \log \frac{\text{pred}}{\text{obs}}}{n} \right]} \quad (20)$$

Where n is the number of observations for each used for the calculation. An accuracy and bias factor equal to 1 means a perfect agreement between the predicted and the observed value (Keklik et al., 2012; Ross, 1996).

II.9. Statistical analysis

The results are presented as the mean $\pm$ SD (standard deviation) of three independent experiments. Statistical analysis was performed with SPSS. Statistical tests were used to obtain the p -value was ANOVA followed by Tukey's HSD test. Differences at $p < 0.05$ were considered statistically significant.

Results & Discussion

Chapter III. Results & Discussion

III.1. Measurement of temperature

An experimental heating curve was obtained for each target pathogen, power levels and salt concentrations used in the study (**Figure 17**). Microwave treatment temperature curves at each power level, showing how treatment times shorten with increasing power.

The temperature of the cell suspensions, recorded separately after microwave irradiation, increased from the initial 23 to 24° C for all applied conditions to almost 70 to 80° C. The time required for reaching these temperatures according to the different concentrations of NaCl and the microwave powers applied are shown in **Table IV**.

Table IV. Temperature and time recorded during microwave treatment of *E.coli* and *S.aureus* suspensions

Salt level	0.5% NaCl		3.5% NaCl		8.5% NaCl	
Power (W)	t (sec)	T (° C)	t (sec)	T (° C)	t (sec)	T (° C)
450 W	70	82.5	100	83.4	100	71.6
600 W	50	76.7	70	86.2	70	76.8
800 W	40	76.4	50	83	50	76.5

t represents time in seconde

T represents Temperature in ° C

According to the results shown in **Table IV**, the temperature rise is influenced by the power applied and the exposure time. As the power is increased, the heating time becomes shorter. On the other hand, the effect of the NaCl content in the irradiated medium has a slowing effect on microwave heating, so as the content increases, the heating time increases.

Kapcsándi et al. (2020), were studied the microwave radiation on the microbiological parameters of raw cow's milk at different power levels (100, 200, 300, 400, 496 (~500), and 600 W). The the authors were observed a linear relationship between power level and treatment time, indicating a strong relationship between the power applied and the heating rate at the power levels studied.

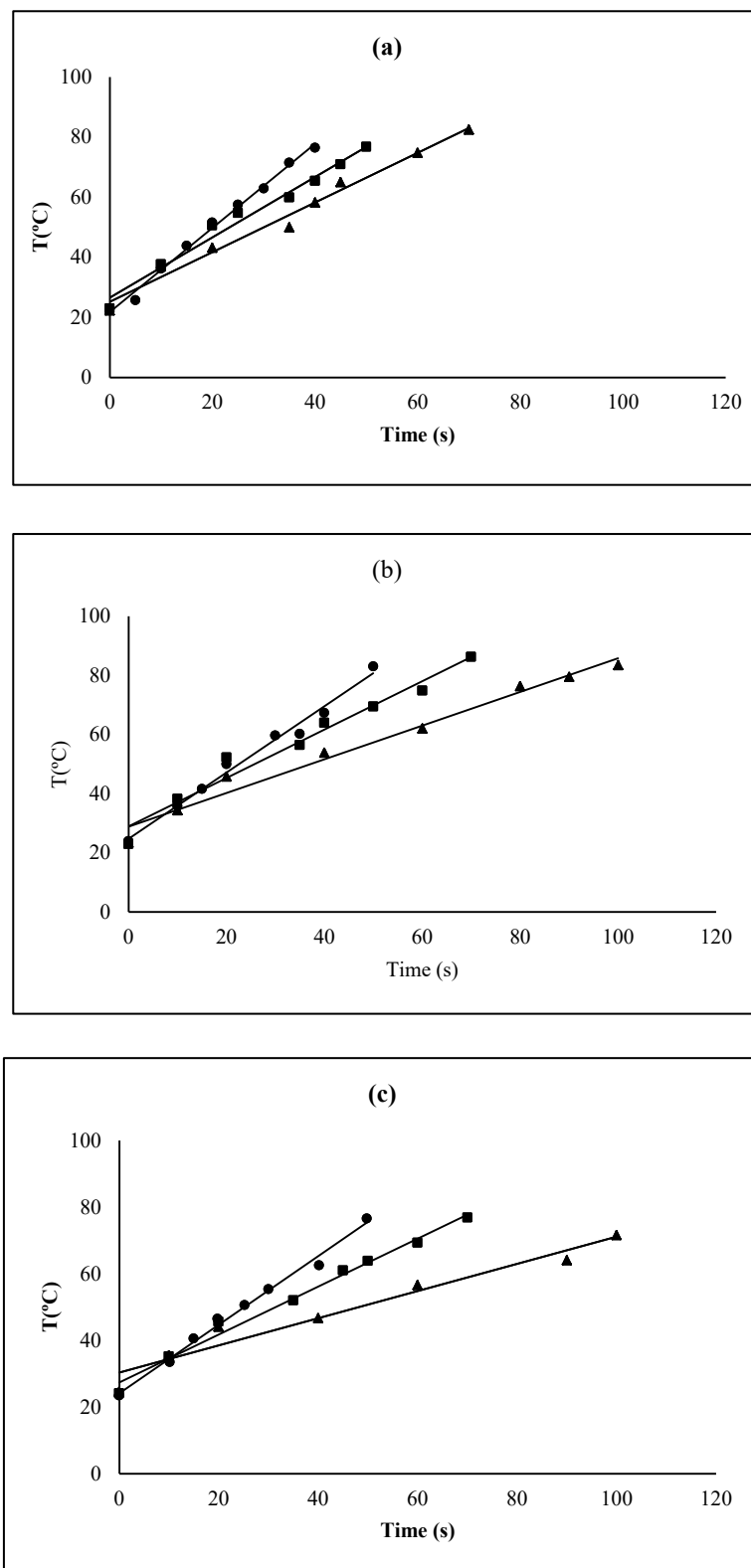


Figure 17. Experimental heating curve was obtained for each target pathogen, power levels and salt concentrations used. With (a) 0.5% NaCl, (b) 3.5% NaCl and (c) 8.5% NaCl. ● 800 W ■ 600W ▲ 450 W

According to Chandrasekaran et al. (2013), the addition of a small amount of salt to the pure water significantly increases the heating rate, as the defects in the water network are weakened by the large negative ions that slosh around. Diluted salt water heats up much faster than pure water. This is due to the weakening of the hydrogen-bonded water network by the large salt ions, including Cl⁻ (Tanaka and Sato, 2007).

Dissolved salt significantly reduces the penetration depth of incident microwave energy. This means that more of the microwave energy is absorbed closer to the outer surface of the container (Anantheswaran and Liu, 1994).

III. 2. Effect of MW heating on inactivation of *E.coli* and *S. aureus* in PBW

Figure 18 and 19 illustrate survival curves for *E. coli* and *S. aureus* in PBW during MW treatment at the specified power levels of 450 W, 600 W and 800 W, which heated the samples to final temperatures of approximately 70 °C for *E.coli* and 65 °C for *S. aureus*, respectively. Given the sample size (50 ml of sample in glass flask of 500 ml and 86 mm diameter) and the heat penetration characteristics of the MW radiation, it is reasonable to assume that temperatures in the sample were uniform.

Bacterial reduction tended to accelerate with increasing exposure time to MW for both *E. coli* and *S. aureus*. Microbial inactivation started after 10 s of MW heating at 450 W and 600 W respectively, and after 5 s at 800 W for *E. coli* at 0.5 % of salt concentration. For *S. aureus* at the same salt concentration, inactivation started after 30 s of MW heating at 450 W, 25 s at 600 W and 20 s at 800 W. These reflect that *E. coli* (Gram negative) was more sensitive to MW irradiation than *S. aureus* (Gram positive). This is explained by the thick structure of the cell wall in gram-positive bacteria therefore they are more resistant to heat and other physical stresses.

According to Valero et al. (2014), at 300 W and 800 W, respectively, 80 and 40 seconds were necessary to achieve reduction of *S. enterica serovar Enteritidis* in potato omelettes by over 4 log units using microwave heating.

Achieng et al. (2022) investigated the efficacy of high-power, short-duration MW treatment of rough rice using 915 MHz frequency for microbial inactivation, while preserving rice quality. MW irradiation treatment using powers of 16, 18, and 20 kW and exposure

durations of 1, 2, and 3 minutes indicated the potential for high-power microwaves to effectively inactivate microorganisms on rough rice within just 3 minutes.

Kang et al. (2023) examined the impact of the 915 MHz MW heating power level on ground pork's bactericidal mechanism and the inactivation of foodborne pathogens (*E. coli* O157:H7, *S. Typhimurium*, *L. monocytogenes*, and *S. aureus*). The authors were found that heating rate and antibacterial effect were proportional to the power level. They also reported that the reduction of Gram-positive bacteria was less pronounced than Gram-negative bacteria under MW treatments. This suggests that Gram-positive bacteria are more resistant to microwave heating treatment than gram-negative bacteria.

This conclusion was also noted by Yang et al. (2023). In accordance with the findings of those studies on the effectiveness of microwave inactivation of bacteria, it has been demonstrated that the rate of inactivation is proportional to the level of microwave power.

Figure 18. Fitting of the Weibull modified models (solid lines) to the observed survival data ($\log S(t)$) (symbols) of *E. coli* subjected to microwave treatments. Dashed lines represent the evolution of temperature ($^{\circ}\text{C}$) during the microwave treatments.

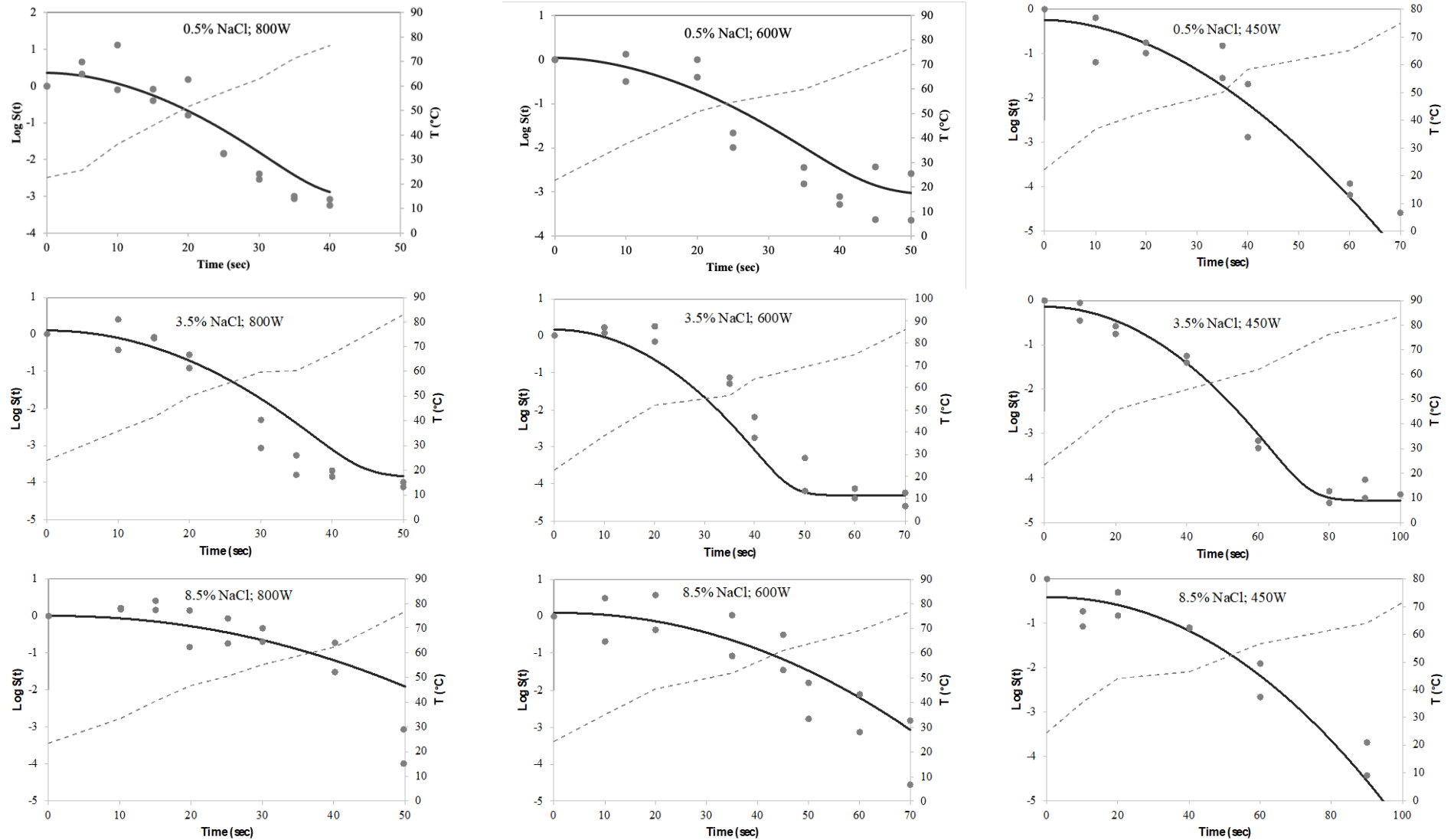
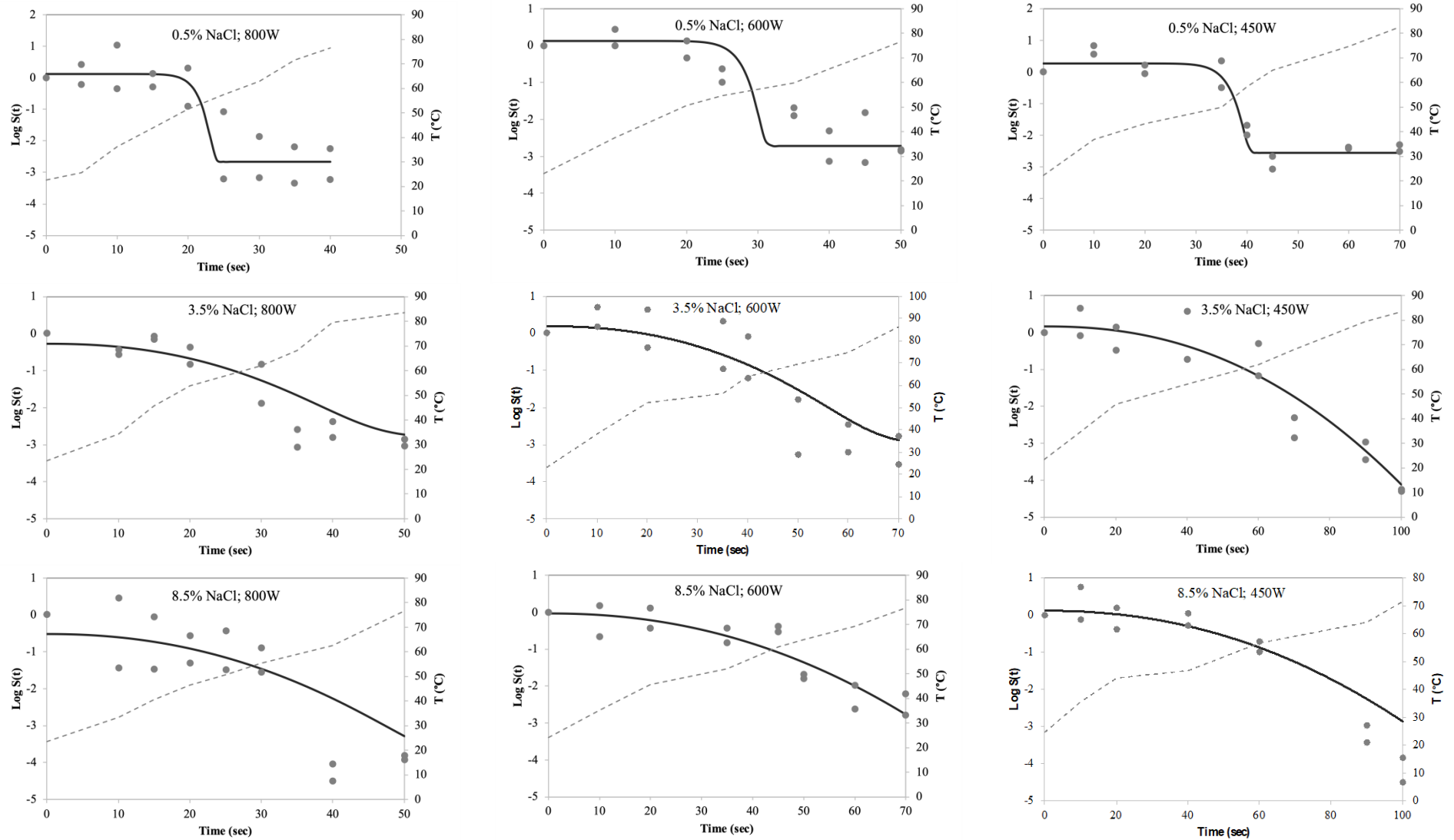


Figure 19. Fitting of the Weibull modified models (solid lines) to the observed survival data ($\log S(t)$) (symbols) of *S. aureus* subjected to microwave treatments. Dashed lines represent the evolution of temperature ($^{\circ}\text{C}$) during the microwave treatments.



III.3. Modeling of survival curves

An important feature of the survival curves for all power levels is their lack of linearity. It is therefore necessary to use non-linear models in order to accurately describe the effect of treatment time and temperature on the kinetic parameters (Mendes-Oliveira et al., 2020). The non-log-linear kinetics of microorganisms are defined by a basic model derived from the Weibull cumulative function (Couvert et al., 2005). This is why, in our study, the *E. coli* and *S. aureus* survival curves, at the different treatments, were modeled through Weibull model (**Figures 18 and 19**), considering the dynamic temperature profile of MW treatments. The survival curves for both microorganisms were convexly shaped, with a shoulder region preceding an acceleration of the decay rate, with or without a tailing effect.

A shoulder in the heat inactivation kinetics was observed, indicating that bacterial resistance declined gradually after the application of heat treatment (Desriac et al., 2019). And a tail effect is considered to be a sub-microbial population that does not undergo any significant subsequent inactivation. (Geeraerd et al., 2005).

According to Bermudez-Aguirre and Niemira (2023), Several factors impact microbial inactivation, including the intensity of stress variation, the physiological state of the microbial population in relation to the growth phase (stationary vs. exponential) and the microorganism's stress adaptation. Microbial inactivation curves exhibit concavities, shoulders, and tailings. The equation based on the Weibull distribution often performs better in fitting the survivor's curve than first-order kinetics or linear inactivation. This mathematical technique is founded on a microbial population's resistance to certain stress and follows the Weibull distribution patterns. This model accounts for the varying resistance of individual cells in a given population.

Yang et al. (2023) indicated that the Weibull model was a more flexible model for describing and predicting microbial inactivation in the non-isothermal Continuous flow microwave processing treatments.

The effect of environmental factors (like presence of presence of NaCl) on Weibull model parameters, the shape parameter " p " and the scale parameter " δ ", was investigate. The estimated parameters together with the goodness of fit indices are represented in **Table V**. The δ values varied according to the MW power and the concentration of NaCl. The results

showed that the δ values decreased with increasing MW powers. As illustrated, increasing of MW power induce increasing of temperature, this is reflected in an acceleration of the heating rate and thus produce a decreasing of δ values (note that δ is the time when $\log_{10}S(t) = -1$) this reflected by acceleration of bacterial inactivation by MW. In other hand, the addition of NaCl in the heated solutions, affect the δ values. As shown in the results, the higher the concentration of the salt, the longer the value of δ .

It is common for the shape parameter (p -value) not to be dependent on temperature (Buzrul, 2022). While the p -value varied according to the degree of convexity of each survival curve. In our study, The p value does not equal 1 under any of the test conditions. This indicate that using first-order kinetics for calculating D -values is unsuitable for the study of thermal inactivation kinetics and is only a special case of the Weibull model ($p = 1$) (Breniaux et al., 2023). Thus, in order to limit the number of parameter values in the equation as much as possible, the survival curve was refitted taken into account a constant value of p . The p value chosen was estimated from the best fit of the experimental values obtained at different concentrations of sodium chloride for each studied power level. This was underlined by several authors in previous studies (Hassani et al., 2005; Kernou et al., 2021; van Boekel, 2002; Zhang et al., 2018; Breniaux et al., 2023).

As expected, the secondary model developed to describe the heating and recovery environmental influence on δ value estimates. Thus, secondary models describing the linear relationship between the δ values and the heating rates (hr) have been developed as follows in **table VI**. A one-step approach was followed by introducing the equations (**table VI**) into the Weibull primary model in order to predict bacterial inactivation under varying powers and salt concentrations. For the shape p parameter of the Weibull model, no secondary model could be developed. Therefore, the equation for the one-step modelling used the overall mean p parameter estimates. In contrast to the secondary models a temperature-dependency of the log transformed parameter δ was captured by the model generated in the one-step fitting.

The determination coefficients (R^2 values) for *E. coli* varied from 0.917 to 0.983. In the case of *S. aureus*, R^2 values varied from 0.908 to 0.983 (**Table V**). And the $RMSE$ calculated from the models were ranged from 0.074 to 0.501 and from 0.154 to 0.842 for *E. coli* and *S. aureus*, respectively. The Weibull models described accurately the complete survival curves profiles of *E. coli* and *S. aureus* obtained, demonstrating their applicability as reported in other studies (Greenacre et al., 2003; Valdramidis et al., 2005; Yang et al., 2023).

Table V. The estimated parameters together with the goodness of fit indices of *E. coli* and *S. aureus* obtained under MW treatments at 0.5%, 3.5% and 8.5% of NaCl.

Microorganisms	NaCl	MW power	Hr (°C/sec)	p	δ	R ²	RMSE
<i>E. coli</i>	0.5%	800	1.344	1.830	19.573	0.911	0.274
		600	1.076		23.487	0.869	0.327
		450	0.860		28.222	0.932	0.251
	3.5%	800	1.180	2.016	22.147	0.844	0.501
		600	0.902		27.756	0.960	0.176
		450	0.600		35.472	0.980	0.074
	8.5%	800	1.066	2.091	36.772	0.843	0.468
		600	0.752		40.299	0.823	0.460
		450	0.470		45.590	0.963	0.147
<i>S. aureus</i>	0.5%	800	1.34	13.90	21.764	0.809	0.392
		600	1.08		28.332	0.851	0.293
		450	0.86		36.955	0.934	0.158
	3.5%	800	1.18	2.274	29.981	0.865	0.292
		600	0.90		39.306	0.832	0.433
		450	0.60		52.752	0.910	0.261
	8.5%	800	1.07	2.157	30.870	0.769	0.842
		600	0.75		43.911	0.841	0.154
		450	0.47		60.259	0.960	0.486

Table VI. Secondary model the linear relationship between the δ values and the heating rates (*hr*)

Bacterial strain	[NaCl]	Equations
<i>E. coli</i>	0.5	$\log \delta = -0.337 (\pm 0.116) * \ln(hr) + 1.395(\pm 0.023)$
	3.5	$\log \delta = -0.360 (\pm 0.078) * hr + 1.769(\pm 0.072)$
	8.5	$\log \delta = -0.114 (\pm 0.038) * \ln(hr) + 1.573(\pm 0.018)$
<i>S. aureus</i>	0.5	$\log \delta = -0.515 (\pm 0.058) * \ln(hr) + 1.493(\pm 0.011)$
	3.5	$\log \delta = -0.423 (\pm 0.050) * hr + 1.976(\pm 0.046)$
	8.5	$\log \delta = -0.487 (\pm 0.013) * hr + 2.009(\pm 0.010)$

III.4. Influence of NaCl concentration on microbial inactivation during MW treatment

The effect of NaCl on the effectiveness of MW treatments on *E. coli* and *S. aureus* was observed on the time to require a certain reduction level (t_x , s), as shown in **Table VII**. The t_x values changed depending on the MW power and the NaCl concentration, such as the applied power increases, the t_x decreases for both bacterial strains. This means an acceleration of the bacterial destruction by the increase of the applied MW powers. On the contrary, increasing the concentration of NaCl induced an increase of t_x which resulted in a slowing down of the bacterial inactivation. This could be explained by the presence of NaCl which would offer a protection of the microorganisms against the temperatures generated by the different MW powers applied (Heddleson et al., 1993).

Previous studies have found that the response of microorganisms depends on microwave power and time. The rate of microbial inactivation could be related to temperature increase. (Fan et al., 2020; Guo et al., 2020). In other part, Several factors affect the effectiveness of microwave heating, including frequency of radiation, food composition, material size, salt and moisture content, and temperature and material density (Espitia et al., 2023).

High salt content has been found to increase conduction losses at lower frequencies, which has a greater impact on the loss factor. Additionally, the interaction between molecules and compounds in the medium can affect the dielectric properties of the sample. For instance, depending on the size and charge of the dissolved ions, salt may bind free water molecules, causing water polarization and subsequently lowering the dielectric constant (Espitia et al., 2023)

According to Martins et al. (2019), organic materials and salts can impact the dielectric properties of food materials, which largely depend on their interaction with each other and with water molecules. At MW frequencies, salt solutions serve as conductors; the salts dissolve and create ions that bind with water molecules, reducing their polarization and thus reducing the relative permittivity and increasing the loss factor. Temperature and salt content increased the loss factors. However, the dielectric constants were not significantly affected. Ionic conductivity was the major factor at low frequencies. The power penetration

depth increased with moisture content, but decreased with temperature, frequency, and salt content (Uan et al., 2004)

Ionic compounds, such as sodium chloride (NaCl), are recognized to be responsible for increased temperature rise and cell inactivation. Ionic solutions have higher electroconductivity and electric properties that are significantly closer to those of conductors than pure water (Watanabe et al., 2000). When ionic salts are dissolved in water, the relaxation time falls at low concentrations and gradually increases, affecting the structure of the water. It has been suggested that the ions' presence leads water to undergo a structural breakdown process. The water molecules that are coordinated to the ions are rotationally locked; however, the uncoordinated water molecules do not suffer as strong intermolecular hydrogen bonding effects, and as a result, they have shorter relaxation durations in this more free state. The effects are reversed at higher concentrations, and the relaxation frequency of water in concentrated salt solutions is higher than that of pure water. This is probably because the water molecules are more ordered in the presence of many ions (Grant and Halstead, 1998). Significant differences ($p < 0.05$) were obtained between power levels and NaCl concentrations for each microorganism.

Table VII. Predicted values for the time to achieve a 1, 2, 3 and 4 logarithmic reductions of *E. coli* and *S. aureus* at the different microwave treatments and NaCl concentrations.

Microorganism	Power level	NaCl%	t _{1D} (sec)	t _{2D} (sec)	t _{3D} (sec)	t _{4D} (sec)
<i>E. coli</i>	800W	0.5	19.57	28.58	35.67	41.75
		3.5	22.15	31.23	38.19	44.05
		8.5	36.77	51.23	62.19	71.36
	600W	0.5	23.49	34.30	42.81	50.09
		3.5	27.76	39.14	47.86	55.20
		8.5	40.30	56.14	68.15	78.21
	450W	0.5	28.22	41.22	51.44	60.19
		3.5	35.47	50.02	61.17	70.55
		8.5	45.59	63.51	77.10	88.48
<i>S. aureus</i>	800W	0.5	21.76	23.01	23.70	24.19
		3.5	29.98	40.66	48.60	55.15
		8.5	30.87	42.57	51.37	58.70
	600W	0.5	28.33	29.96	30.85	31.49
		3.5	39.31	53.31	63.72	72.31
		8.5	43.91	60.55	73.07	83.50
	450W	0.5	36.95	39.08	40.24	41.08
		3.5	52.75	71.55	85.51	97.04
		8.5	60.26	83.10	100.28	114.59
<i>E. coli</i>	800W	0.5	14.63	21.36	26.66	31.20
(UHT milk)	600W	0.5	18.95	26.72	32.67	37.69
<i>S. aureus</i>	800W	0.5	14.37	15.10	15.55	15.88
(UHT milk)	600W	0.5	20.89	28.34	33.87	38.43

The highest t_x values corresponded to 8.5% and 450 W treatments, ranging from 45.59 to 88.48 s for *E. coli* and from 60.26 to 114.59 s for *S. aureus* (**Table VII**). It was also shown that *S. aureus* was more resistant to the applied treatments than *E. coli* regardless the NaCl concentration and power level ($p < 0.05$). Then, it seems that using moderate heat powers and increasing NaCl concentrations may induce a protection for the microorganisms against heat (Heddleson et al., 1993).

Some research work has been carried out to try to determine the effect of salt on microbial destruction by microwave. Dealler and Lacey (1990) prepared mashed potato mixed with various concentrations of ionic compounds (NaCl, KCl, NH_4Cl , and sodium glutamate) and was irradiated under MWs at 1250-MHz, they discovered that increasing concentrations of the ionic compounds added suppressed the temperature increase at the core part.

Fujikawa et al. (1992) exposed a solution of *E. coli* cells in a glass beaker to MW irradiation at various concentrations of NaCl and found that increasing the NaCl content inhibited bacterial death.

Heddleson et al. (1993) investigated the relationship between the quantity of NaCl present in saline solutions, the temperatures that were reached, and the level to which *Salmonella spp.* might be inactivated. They reported that sodium chloride serves a dual effect in determining their survival by producing a thermal stress of microbial cells and a temperature change in the broth media. This suggests that sodium chloride plays a role in both aspects of this process. They also observed that salts in solutions, when present in appropriate quantities, may cause a reduction in the penetration depth of MWs. This will result in more energy being absorbed closer to the surface of the solution.

Morozov et al. (2017) used different concentrations of NaCl to demonstrate the protection of bacteria from the thermal damage caused by SHF (super high frequency: frequencies fall within the MW band) at 50 and 60 °C. The results demonstrated a clear effect of cell defense against damage. At different temperatures, the efficiency of the protection against thermal damage produced by NaCl relies on the degree of the heat exposure, being the highest resistance obtained at 60 °C and 7.5% NaCl.

In summary, the dielectric properties of liquids are dependent on their constituents, such as the fat content, the salt content, the solid content and others (Liu et al., 2018; Martins

et al., 2019). Additionally, the dielectric characteristics can impact the microwave penetration depth parameter. As the penetration depth decreases, it becomes easier to reach the specified temperature and vibration frequency (Salazar-González et al., 2012), resulting in more efficient heating and disinfection. The dielectric loss was observed to fluctuate with the concentration and types of salts in liquids (Navarkhele et al., 1998).

Researchers studying microwave disinfection have observed that the uniformity of electromagnetic fields also affects the efficiency of disinfection for liquid media. The temperature distribution was influenced by the properties of the disinfection object (dielectric and thermal parameters) and the microwave heating system's features (such as frequency, power, and application system geometry). Furthermore, the power of the microwave was a crucial factor in disinfecting liquid media (Liu et al., 2023).

III. 5. Effect of MW heating on inactivation of *E.coli* and *S. aureus* in UHT milk

The survival curves of *E. coli* and *S. aureus* in UHT milk during MW treatment at the specified power levels of 600 W and 800 W are represented in **Figures 20 and 21**. The samples were heated to reach a final temperature of approximately 70 °C for *E.coli* and 65 °C for *S. aureus*, respectively.

The rate of heat transfer and microbial heat resistance during processing can be influenced by a number of factors such as pH, fat, moisture and protein content (Contreras et al., 2017). These parameters may influence the inactivation effect of heat by reducing the water activity of the food and therefore need to be characterised ((Van Asselt and Zwietering, 2006).

It can be seen that for *E. coli* there was a slight decrease over time until a reduction of about 3 log CFU/mL was reached at 800 and 600 W. In contrast, *S. aureus* population remained within 1 log reduction during the first 15 s treatments, showing a linear decay afterwards, being the total reduction ca. 4 log CFU/mL for 800 and 600 W treatments.

The survival curves of *E. coli* and *S. aureus* were convex, with a shoulder preceding acceleration in decay rate, with or without tailing. A Weibull model was used to model the survival curves in the different treatments conditions (800 W and 600 W). For both *E. coli* and *S. aureus*, there was a trend towards an acceleration of bacterial reduction with increasing exposure time to MW.

The estimated parameters together with the goodness of fit indices are represented in **Table VIII**. The δ values varied according to the MW power, the results showed that the δ values decreased with increasing MW powers. As shown, an increase in MW power causes an increase in temperature, which is reflected in an acceleration of the heating rate and thus a decrease in δ values, resulting in an acceleration of bacterial inactivation by MW in UHT milk.

Table VIII. Estimated parameters fitted of *E. coli* and *S. aureus* from MW treatments in UHT milk and goodness of fit indices.

		Hr	δ	p	R^2	R^2 adjusted	RMSE
<i>E. coli</i>	600W	0.91	26.23	2.71	0.97	0.96	0.24
	800W	0.81	22.75	10.03	0.87	0.84	0.56
<i>S. aureus</i>	600W	0.91	15.70	2.53	0.97	0.96	0.21
	800W	0.81	16.08	1.99	0.86	0.85	0.49

The determination coefficients (R^2 values) for *E. coli* were 0.87 at 800 W and 0.971 at 600 W. In the case of *S. aureus*, R^2 values were 0.86 at 800 W and 0.98 at 600 W (**Table VIII**). And the *RMSE* calculated from the models were 0.56 at 800 W and 0.24 at 600 W in the case of *E. coli* and 0.49 at 800 W and 0.21 for *S. aureus*. According to these results, the Weibull models accurately described the full profiles of the *E. coli* and *S. aureus* survival curves that were obtained, demonstrating the applicability of these models.

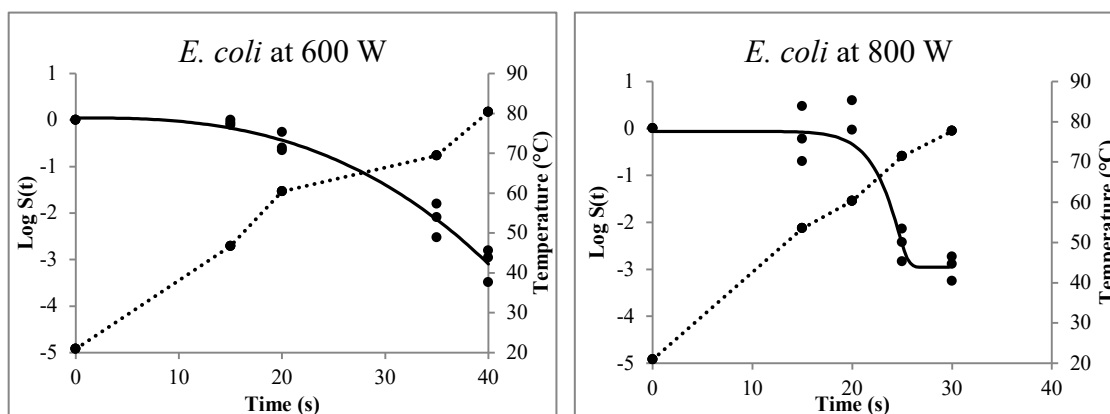


Figure 20. Fitting of the Weibull modified models (solid lines) to the observed survival data ($\log S(t)$) (symbols) of *E. coli* subjected to microwave treatments in UHT milk at 800 W and 600 W. Dashed lines represent the evolution of temperature ($^{\circ}\text{C}$) during the microwave treatments.

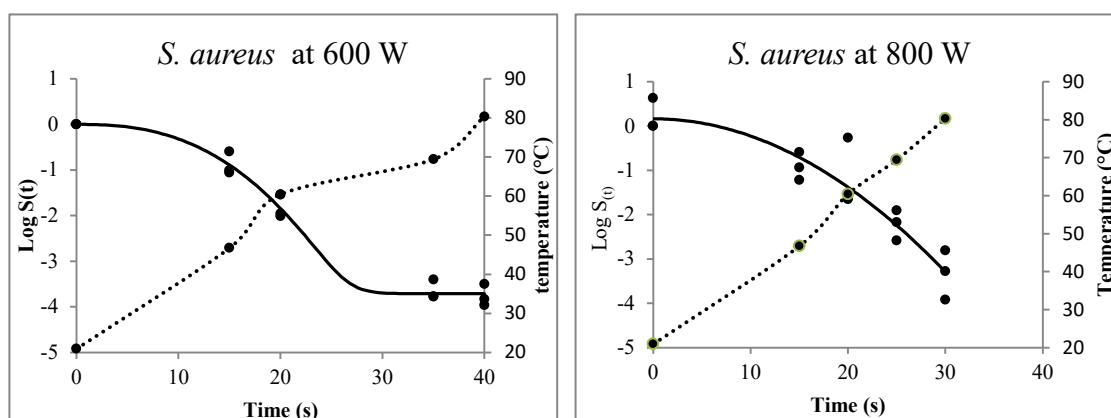


Figure 21. Fitting of the Weibull modified models (solid lines) to the observed survival data ($\log S(t)$) (symbols) of *S. aureus* subjected to microwave treatments in UHT milk at 800 W and 600 W. Dashed lines represent the evolution of temperature ($^{\circ}\text{C}$) during the microwave treatments.

Pasteurisation must extend the shelf life of food products while maintaining product quality. Minimising quality losses and at the same time providing an adequate process with the desired sterility is one of the challenges for the canning industry. In this sense, MWP offers an alternative to reduce food quality losses compared to traditional treatment. Microwave volumetric heating can be extremely beneficial for liquids such as milk, solving the problem of fouling of heat transfer surfaces existing in the conventional process (Lespinard et al., 2019).

Manjunatha et al. (2012) were studied the effect of MWH (2,450 MHz/1,200 W) on the thermal resistance of bacterial cultures (*E. coli*, *Salmonella spp.*, *S. aureus*, *P. aeruginosa*, *B. subtilis*) in whole milk, skim milk, milk concentrates and fresh cheese. In whole milk and skimmed milk, the time required for the complete destruction of the vegetative cells was 40 s, with the exception of *E. coli* and *B. subtilis*, both of which were effectively destroyed at 50 s. In the case of concentrated milk, the complete destruction of the vegetative cells was achieved after 60 s. The same time was necessary for fresh cheese. However, 50 s was sufficient for *Salmonella spp.* They conclude that among the five bacteria cultures tested, *S. aureus* and *P. aeruginosa* were the most sensitive to MW irradiation, and *B. subtilis* culture was the most resistant.

Rasooly et al. (2014), tested whether microwaves would inactivate the toxicity of Shiga toxin 2 (Stx2: toxin produced by toxigenic *E. coli*) added to 5% reconstituted non-fat milk administered to monkey kidney Vero cells. Heating Stx2-added milk in a microwave

oven at a 10% duty cycle (30-second cycle) for a total energy of 165 kJ, or thermal heating (pasteurisation), which is widely used to kill pathogenic bacteria, did not destroy the biological effect of the toxin in Vero cells. On the other hand, conventional heating of the milk to 95 °C for 5 min or with increased microwave energy of 198 kJ reduced the Stx2 activity. They observed that a high degree of sensitivity of the bacterial cells to MW. Lower intensities result in higher percentages of damaged cells progressively dying during refrigerated storage (up to 24h), reaching reductions greater or equal to 5 log₁₀ cycles.

Tremonte et al. (2014) were observed that after 72 h during refrigeration, Microbial populations in raw milk increased to reach values of around 8.0 log CFU/mL for *Pseudomonas spp*, 6.5 log CFU/mL for yeasts and up to 4.0 log CFU/mL for *Enterobacteriaceae*. The boiling treatment applied to chilled milk samples after 72 h resulted in complete decontamination, but had a negative impact on the nutritional quality of the milk, as evidenced by a drastic reduction in whey proteins. Microwave treatment at 750 W for 75 s resulted in a partial inactivation of the micro-organisms in the milk. Treatment with microwaves at 900 W for 75 s produced a microbiological decontamination similar to that obtained by boiling, while preserving the whey protein content of the milk. The requirement to boil raw milk from vending machines before consumption is fully justified by the microbiological characteristics of raw milk observed in this study. However, the nutritional value of milk is drastically reduced by domestic boiling. Microwave treatment, provided that the process variables are standardised for safe domestic use, could be a good alternative to boiling.

Ali et al. (2015) were investigated the effect of MW treatment on the microbiological quality of raw milk, and the effect of these treatments on *E. coli*, *S. aureus* and *B. cereus* after inoculation into raw milk, compared with heat treatments such as direct flame or boiling water for different times. MW treatment reduced microbial load in raw milk almost as much as direct flame treatment at all different temperatures used (55, 80, and 90°C) and more than boiled water treatment. The results of this study show the possibility of preserving the nutritional value of raw milk by MW heating for short periods of time, without any negative consequences compared with the other heat treatments.

In spite of its microwave characteristics, the major drawback of MW heating is the irregularity of the temperature profile within the milk during heating, as the presence of cold zones is of concern for the microbial safety of the treated product (D'Incecco et al., 2021).

Coronel et al. (2003) studied the temperature profile within milk using a prototype tubular MWH plant. The researchers found that the 'cold spots' were closer to the walls of the tube, while the 'hot spot' was near the centre of the tube. In general, compared to batch systems, continuous flow systems proved to be more efficient in terms of heat distribution.

III.6. Model validation

The Weibull models have been validated using UHT milk at two power levels (800 and, 600 W) for *E. coli* and *S. aureus*. The fitted survival curves are represented in **Figures 22 and 23**.

The Weibull models have been validated using UHT milk at two power levels (800 and, 600 W) for *E. coli* and *S. aureus*. To accurately assess the goodness of fit of the Weibull models, the APZ was calculated by the differences between predicted and observed values. The graphical representations are shown in **Figures 24 and 25**. It was seen that for *E. coli*, 84.30% of the data fell inside the APZ (+1 to -0.5 log CFU/mL), while more variability was observed for *S. aureus* since 72.41% were within the APZ. This variability can be associated to the increased resistance of *S. aureus* to the different MW treatments and NaCl applied. However, it was considered that in both cases, the Weibull models provided a reasonable description of the observed data.

As additional validation indices, the A_f and B_f values were calculated to assess models' performance. Regarding *E. coli*, $B_f=1.033$ and $A_f=1.195$, showing a slight overestimation of microbial inactivation. For *S. aureus* $B_f=0.925$ and $A_f=1.104$ so that it was evidenced an under-estimation of microbial reductions, which can be seen at 600 W (**Figure 24 and 25**).

However, the model was validated in a matrix of different nature as the culture medium, such as UHT milk. As it is presented in a form of emulsion, the physical properties and the different nutritional composition would lead to limit the applicability of the model. However, as shown with the obtained validation indices, the model can be effectively applied for this matrix to predict microbial fate at different salt concentrations and MW treatments.

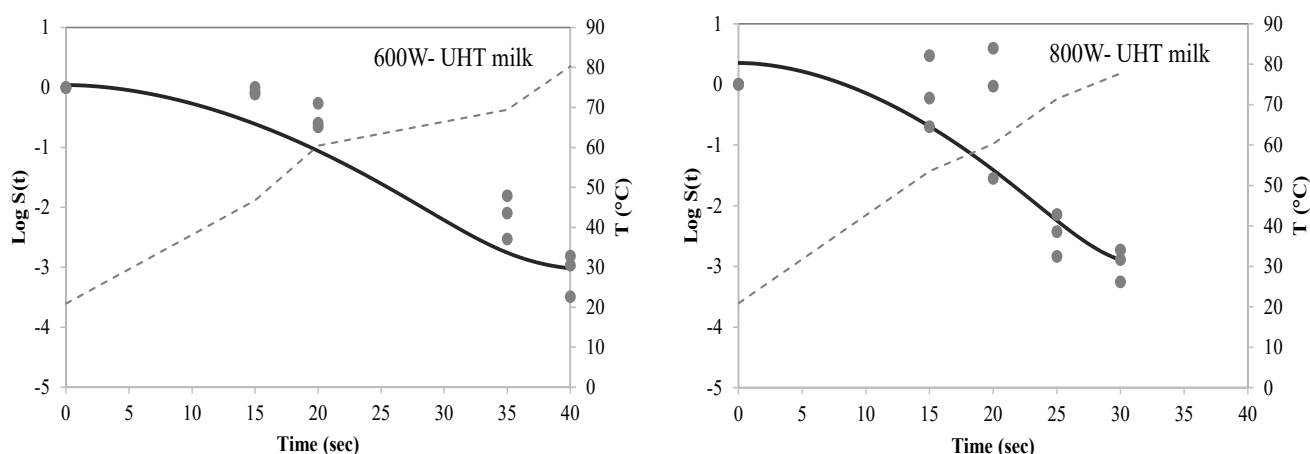


Figure 22. Validation of the Weibull modified models (solid lines) to the observed survival data (log S(t)) (symbols) of *E. coli* subjected to microwave treatments in UHT milk at 800 W and 600 W. Dashed lines represent the evolution of temperature (° C) during the microwave treatments.

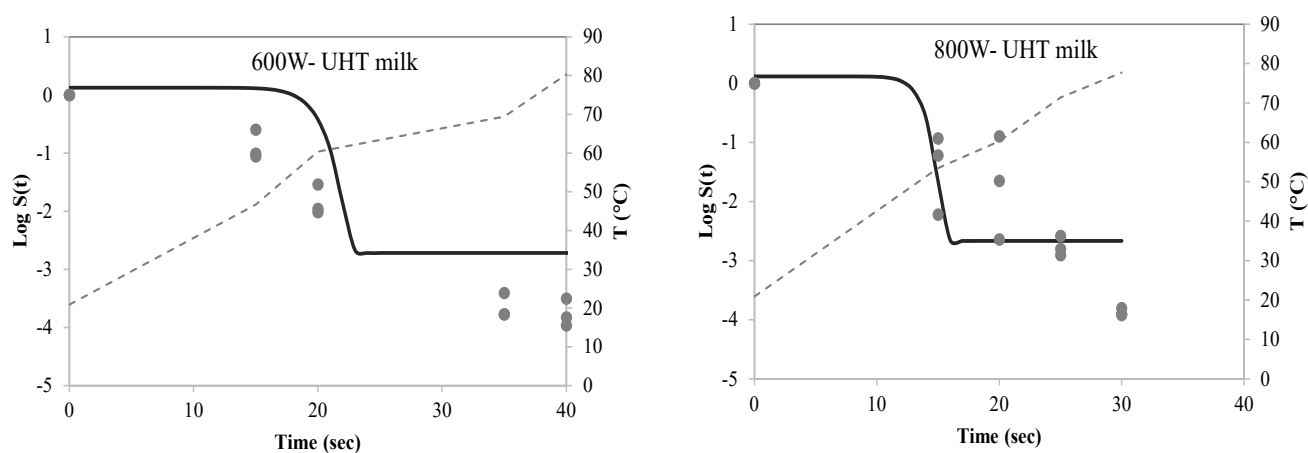


Figure 23. Validation of the Weibull modified models (solid lines) to the observed survival data (log S(t)) (symbols) of *S. aureus* subjected to microwave treatments in UHT milk at 800 W and 600 W. Dashed lines represent the evolution of temperature (° C) during the microwave treatments.

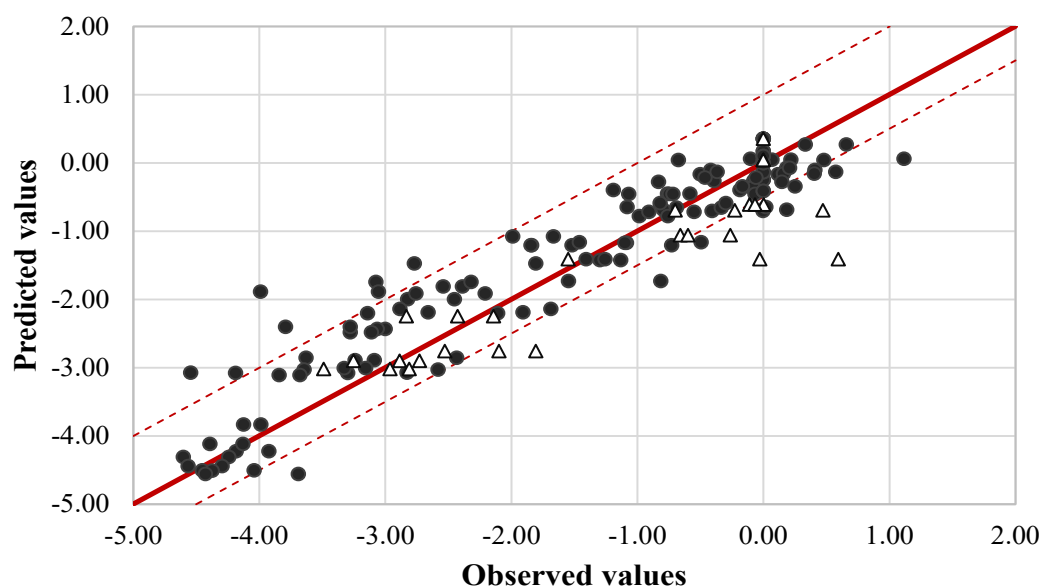


Figure 24. Graphical representation of observed vs predicted values for model data (black circles) and validation data in UHT milk (white triangles) for *E. coli* at the different microwave treatments. The equivalence line is represented by the solid red line, while the dotted lines define the acceptable prediction zone (+1 to -0.5 log CFU/mL).

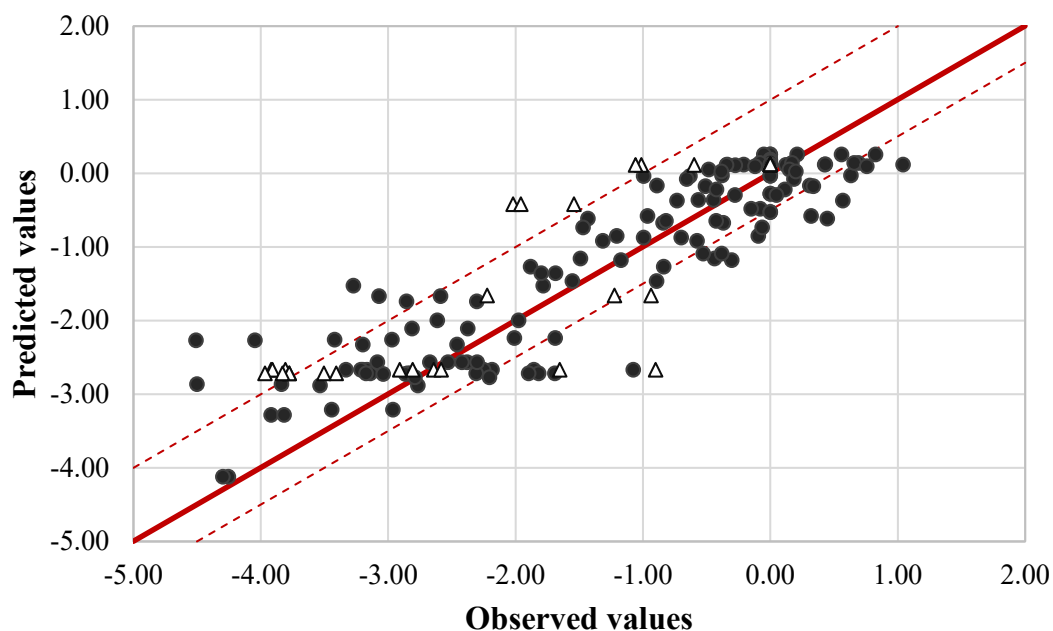


Figure 25. Graphical representation of observed vs predicted values for model data (black circles) and validation data in UHT milk (white triangles) for *S. aureus* at the different microwave treatments. The equivalence line is represented by the solid red line, while the dotted lines define the acceptable prediction zone (+1 to -0.5 log CFU/mL).

Conclusion

Conclusion

The benefits of using microwaves in the home have led to a number of industrial applications. Microorganisms can be effectively inactivated by microwave irradiation. Many environmental conditions have a significant effect on the assessment of the dielectric properties of food, therefore efficiency of microwave disinfection technology. As well as environmental factors, In addition to environmental factors, the sterilisation efficiency of microwave disinfection technology is largely influenced by the type of micro-organism.

It was with this in view that we carried out our study on "Modelling microwave microbial inactivation kinetics". the main aim of this study was to investigate the influence of microwaves and the presence of sodium chloride on the inactivation kinetics of *E. coli* and *S. aureus*.

In the first part of this study, the inactivation kinetics for *E. coli* and *S. aureus* at different microwave power levels and sodium chloride concentrations are described in detail. This part was performed using Peptone-buffered water (PBW) as a model medium to eliminate the influence of external variables (different components of the matrix to be treated, such as proteins, fats, etc.)

Bacterial destruction was performed at three NaCl concentrations of 0.5%, 3.5%, and 8.5% (w/v) and three microwave powers (450, 600 and, 800 W). For each power level and salt concentration, a temperature profile was generated. Then, for microwave processing, the flasks of 500 mL contain 50 mL of PBW inoculated with one of prepared suspension bacterial, and exposed to microwave radiation.

The results indicated that rising microwave power levels (linked to the increasing of the irradiation time) and lowering salt concentrations resulted in higher microbial inactivation, with the δ values time for reaching a first decimal reduction) for *E. coli* at 800 W and 0.5% NaCl being 19.57 s as comparison, *S. aureus* experimental results exhibited greater variability because it was more resistant to microwave treatments.

The effect of NaCl on the effectiveness of MW treatments on *E. coli* and *S. aureus* was observed on the time to require a certain reduction level (t_x , s). the t_x value decreases for both bacterial strains when the applied power increases. On the contrary, increasing the concentration of NaCl induced an increase of t_x . This means an acceleration of the bacterial

destruction by the increase of the applied MW powers and a slowing down of the bacterial inactivation with increased salt concentration.

In the second part, inactivation models predicting microbial survival under dynamic conditions were developed and the effect of NaCl on microwave inactivation kinetics was then investigated. A Weibull model was selected to model the survival of *E. coli* and *S. aureus* as a function of microwave intensity and salt concentrations. The Weibull models described accurately the complete survival curves profiles of *E. coli* and *S. aureus* obtained.

In the final stage of the study, for validation of the developed models and the inactivation kinetics of *E. coli* and *S. aureus* have been simulated with UHT milk. The validation of results of inactivation under MW treatment and presence of salt was indicated through Acceptable Prediction Zones (APZ) Method. The result indicated that the Weibull models provided a reasonable description of the observed data.

The model was also validated in a real matrix: UHT milk. The physical properties and different nutritional composition of UHT milk would limit the applicability of the model. However, the model can be effectively applied to this matrix to predict microbial fate at different salt concentrations and MW treatments, as shown by the validation indices obtained.

In the light of the above, it can be concluded that the results obtained and the patterns generated can be used as decision support tools to establish specific instructions for microwave treatments to ensure food safety.

As a perspective, and with the aim of completing the study, other elements would be needed that are currently lacking in our work:

- Modelling of inactivation kinetics of *E. coli* and *S. aureus* for NaCl concentrations in food products
- To optimise the best sterilisation/pasteurisation conditions for food by studying and modelling the effect of different food components at different microwave power levels.
- To study the effect of the treatment on the quality parameters of the milk (organoleptic and sensorial qualities).
- To model microwave heating kinetics for saline solutions and UHT milk.
- To determine the effects of microwaves on the different components of the bacterial cell of *E. coli* and *S. aureus*.

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Appendix

Composition of the cultures media

Plate Count Agar

Component	Amount
Agar	9.0 g/L
Dextrose	1.0 g/L
Tryptone	5.0 g/L
Yeast extract	2.5 g/L

PBS (Phosphate Buffered Saline)

Component	Amount	Concentration
Sodium chloride	8 g	0.137 M
Potassium Chloride	0.2 g	0.0027 M
Sodium Phosphate Dibasic	1.44 g	0.01 M
Potassium Phosphate Monobasic	0.245 g	0.0018 M

Buffered Peptone Water

Component	Amount
Peptone	10,00 g
Sodium chloride	5,00 g
Disodium phosphate, anhydrous	3,57 g
Monopotassium phosphate, anhydrous	1,50 g

Abstract

In the current investigation, we evaluated the effectiveness of microwave irradiation treatments combined with salt concentration against *Escherichia coli* and *Staphylococcus aureus*. To model microbial survival during heating treatments, we utilized a one-step Weibull equation that takes into account the non-isothermal profiles. Three different concentrations of sodium chloride (0.5%, 3.5%, and 8.5% w/v) were exposed to three levels of microwave power (450 W, 600 W, and 800 W) for testing purposes. The models developed were validated by utilising the determination coefficient (R^2), root mean squared error and the acceptable prediction zone in conjunction with external data acquired from UHT milk. Based on the obtained results, it can be inferred that raising the microwave power levels leads to an increase in effectiveness. The reduction of salt concentrations led to a greater microbial inactivation. For *E. coli*, the δ value (time for achieving a first decimal reduction) was 19.57 s at 800 W and 0.5% NaCl. However, experimental data for *S. aureus* exhibited a higher degree of variability due to its increased resistance to microwave treatments. These results demonstrate the relationship between salt concentration and microbial inactivation. The models obtained and generated can be utilised as decision-making tools to establish precise recommendations on microwave treatments, thereby ensuring the safety of food.

Key words: Dynamic inactivation, *Escherichia coli*, hurdle technology, *Staphylococcus aureus*, Weibull models

Résumé

Dans la présente étude, nous avons évalué l'efficacité des traitements d'irradiation par micro-ondes combinés à la concentration en sel contre *Escherichia coli* et *Staphylococcus aureus*. Pour modéliser la survie microbienne pendant les traitements de chauffage, nous avons utilisé une équation de Weibull en une étape qui prend en compte les profils non isothermes. Trois concentrations différentes de chlorure de sodium (0,5%, 3,5% et 8,5% w/v) ont été exposées à trois niveaux de puissance de micro-ondes (450 W, 600 W et 800 W) à des fins de test. Les modèles développés ont été validés en utilisant le coefficient de détermination (R^2), l'erreur quadratique moyenne et la zone de prédiction acceptable en conjonction avec des données externes acquises à partir de lait UHT. Sur la base des résultats obtenus, on peut déduire que l'augmentation des niveaux de puissance des micro-ondes conduit à une augmentation de l'efficacité. La réduction des concentrations en sel a conduit à une plus grande inactivation microbienne. Pour *E. coli*, la valeur δ (temps nécessaire pour obtenir une première réduction décimale) était de 19,57 s à 800 W et 0,5 % de NaCl. Cependant, les données expérimentales pour *S. aureus* ont montré un degré de variabilité plus élevé en raison de sa résistance accrue aux traitements par micro-ondes. Ces résultats démontrent la relation entre la concentration en sel et l'inactivation microbienne. Les modèles obtenus et générés peuvent être utilisés comme outils d'aide à la décision pour établir des recommandations précises sur les traitements par micro-ondes, garantissant ainsi la sécurité des aliments.

Mots clés : Inactivation dynamique, *Escherichia coli*, technologie hurdle, *Staphylococcus aureus*, modèles de Weibull

ملخص

في التحقيق الحالي، قمنا بتقييم فعالية علاجات الإشعاع بالموجات الدقيقة جنباً إلى جنب مع تركيز الملح ضد الإشريكية القولونية والمكورات العنقودية الذهبية لنمذجة البقاء الميكروبي أثناء علاجات التدفئة، استخدمنا معادلة ويبل المكونة من خطوة واحدة والتي تأخذ في الاعتبار الاعتبار الملامح غير الحرارية. تعرضت ثلاثة تركيزات مختلفة من كلوريد الصوديوم (0.5 % و 3.5 % و 8.5 % وزن/فولت) لثلاثة مستويات من طاقة الميكروويف (450 واط و 600 واط و 800 واط) لأغراض الاختبار. تم التحقق من صحة النماذج التي تم تطويرها من خلال استخدام معامل التحديد، وخطأ تربيع متوسط الجذر ومنطقة التنبؤ المقبولة جنباً إلى جنب مع البيانات الخارجية المكتسبة من حليب المعقم. بناءً على النتائج التي تم الحصول عليها، يمكن استنتاج أن رفع مستويات طاقة الميكروويف يؤدي إلى زيادة الفعالية. أدى انخفاض تركيزات الملح إلى زيادة التعطيل الميكروبي. بالنسبة للإشريكية القولونية كانت القيمة دالتا (وقت تحقيق أول تخفيض عشري) 19.57 ثانية عند 800 واط و 0.5 % من الملح. ومع ذلك، أظهرت البيانات التجريبية للمكورات العنقودية درجة أعلى من التباين بسبب مقاومتها المتزايدة لعلاجات الميكروويف. توضح هذه النتائج العلاقة بين تركيز الملح والتعطيل الميكروبي. يمكن استخدام النماذج التي تم الحصول عليها وإنتاجها كأدوات لاتخاذ القرار لوضع توصيات دقيقة بشأن المعالجات بالموجات الدقيقة، وبالتالي ضمان سلامة الغذاء

الكلمات الرئيسية: التعطيل الديناميكي، الإشريكية القولونية، تقنية الحواجز، المكورات العنقودية الذهبية، نماذج ويبل