U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
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Kinetics of Microbial Inactivation for Alternative Food Processing Technologies

Microwave and Radio Frequency Processing

(Table of Contents)

Scope of Deliverables

The use of microwave and radio frequencies to heat food for commercial pasteurization and sterilization in order to enhance microbial safety is discussed here. Although not under FDA regulations, use of microwave technology to enhance microbial food safety in the home is also discussed briefly. Mechanisms of heating food and destroying pathogens, and the validation of industrial processes are also discussed, followed by conjecture on handling deviations during industrial processing. This document summarizes information obtained through published literature and personal contacts with industry, academia, and government.

Although radio frequency is covered whenever possible, very little information on radio frequency heating for commercial pasteurization or sterilization of food is available in the published literature and no commercial facility for this purpose could be located. The microbial inactivation mechanisms of radio frequency heating are also quite similar to those of microwave heating. Thus, this document refers mostly to microwave processing with the implicit assumption that the principles are generally applicable to radio frequency. Specific information on radio frequency is included whenever available.

1. Introduction

1.1. Definition, Description and Applications

1.1.1.Definition

Microwave and radio frequency heating refers to the use of electromagnetic waves of certain frequencies to generate heat in a material (Metaxas 1996; Metaxas and Meredith 1988; Roussy and Pearce 1995). The frequencies allocated by the Federal Communications Commission (FCC) for the purposes of heating are listed in Table 1. Typically, microwave food processing uses the 2 frequencies of 2450 and 915 MHz. Of these two, the 2450 MHz frequency is used for home ovens, and both are used in industrial heating. It is worthwhile to note that outside of the United States, frequencies of 433.92, 896 and 2375 MHz are also used.

Radio frequency heating in the United States can be performed at any of the 3 frequencies listed in Table 1. As mentioned earlier, there is not much commercial use of these frequencies for food pasteurization or sterilization, although they are used in baking and other processes in the food industry. An overview of food and chemical processing uses of radio frequency can be seen in Kasevich (1998) and Minett and Witt (1976).

Table 1. Frequencies assigned by the FCC for industrial, scientific, and medical use.

	Frequency
Radio	13.56 MHz ± 6.68 kHz
	27.12 MHz ± 160.00 kHz
	40.68 MHz ± 20.00 kHz
Microwaves	915 MHz ± 13 MHz
	2450 MHz ± 50 MHz
	5800 MHz ± 75 MHz
	24125 MHz ± 125 MHz

1.1.2.How the microwaves and radio frequencies generate heat

Heating with microwave and radio frequency involves primarily 2 mechanisms--dielectric and ionic. Water in the food is often the primary component responsible for dielectric heating. Due to their dipolar nature, water molecules try to follow the electric field associated with electromagnetic radiation as it oscillates at the very high frequencies listed in Table 1. Such oscillations of the water molecules produce heat. The second major mechanism of heating with microwaves and radio frequency is through the oscillatory migration of ions in the food that generates heat under the influence of the oscillating electric field.

The rate of heat generation per unit volume, *Q*, at a particular location in the food during microwave and radio frequency heating can be characterized by (Buffler 1993; Datta and Anantheswaran 2000)

$$Q = 2\pi f \varepsilon_0 \varepsilon'' E^2$$

where *E* is the strength of electric field of the wave at that location, *f* is the frequency (Table 1) of the microwaves or the radio frequency waves, ε_0 the permittivity of free space (a physical constant), and ε'' is the dielectric loss factor (a material property called dielectric property) representing the material's ability to absorb the wave. Not apparent from the above equation, there is another dielectric property called the dielectric constant that affects the strength of the electric field inside the food. The dielectric properties depend on the composition (or formulation) of the food, moisture and salt being the two primary determinants of interest (Mudgett 1994; Datta and others 1994). The subsequent temperature rise in the food depends on the duration of heating, the location in the food, convective heat transfer at the surface, and the extent of evaporation of water inside the food and at its surface.

1.1.3.Advantages of microwave and radio frequency processing

Microwave and radio frequency heating for pasteurization and sterilization are preferred to the conventional heating for the primary reason that they are rapid and therefore require less time to come up to the desired process temperature. This is particularly true for solid and semi-solid foods that depend on the slow thermal diffusion process in conventional heating. They can approach the benefits of high temperature-short time processing whereby bacterial destruction is achieved, but thermal degradation of the desired components is reduced. This is illustrated in Fig. 1 for typical time-temperature histories of microwave and conventional heated processes.

Microwave and radio frequency heating may be relatively more uniform than conventional heating, depending on the particular heating situation (Datta and Hu 1992); however, heating uniformity is hard to predict. Figure 2 illustrates a scenario in which microwave heating is spatially more uniform than conventional heating and helps demonstrate the reasoning behind it. The information shown in Fig. 2 is computed from mathematical models of a conventional and a comparable microwave heating process for a solid for input parameters given in Datta and Hu (1992). Figure 2a shows that the range of temperatures reached by the 2 processes are approximately similar (as read from the horizontal axes) at the heating times shown. The vertical axis shows the cumulative volume fractions of the food associated with a temperature, that is, for any temperature, the value on the curve signifies the volume fraction of food that has temperatures at or below this value. Figure 2b shows that the range of F_0 values (signifying time-temperature histories) are quite different for the same conventional and microwave heated food as in Fig. 2a, for which temperatures are approximately similar. The conventional heat process shows a much larger spread of F_0, which primarily signifies its tremendous non-uniformity of temperatures and long processing times leading to significant over-processing of the surface regions of the food.

Other advantages of microwave and radio frequency heating systems are that they can be turned on or off instantly, and the product can be pasteurized after being packaged. Microwave and radio frequency processing systems also can be more energy efficient.

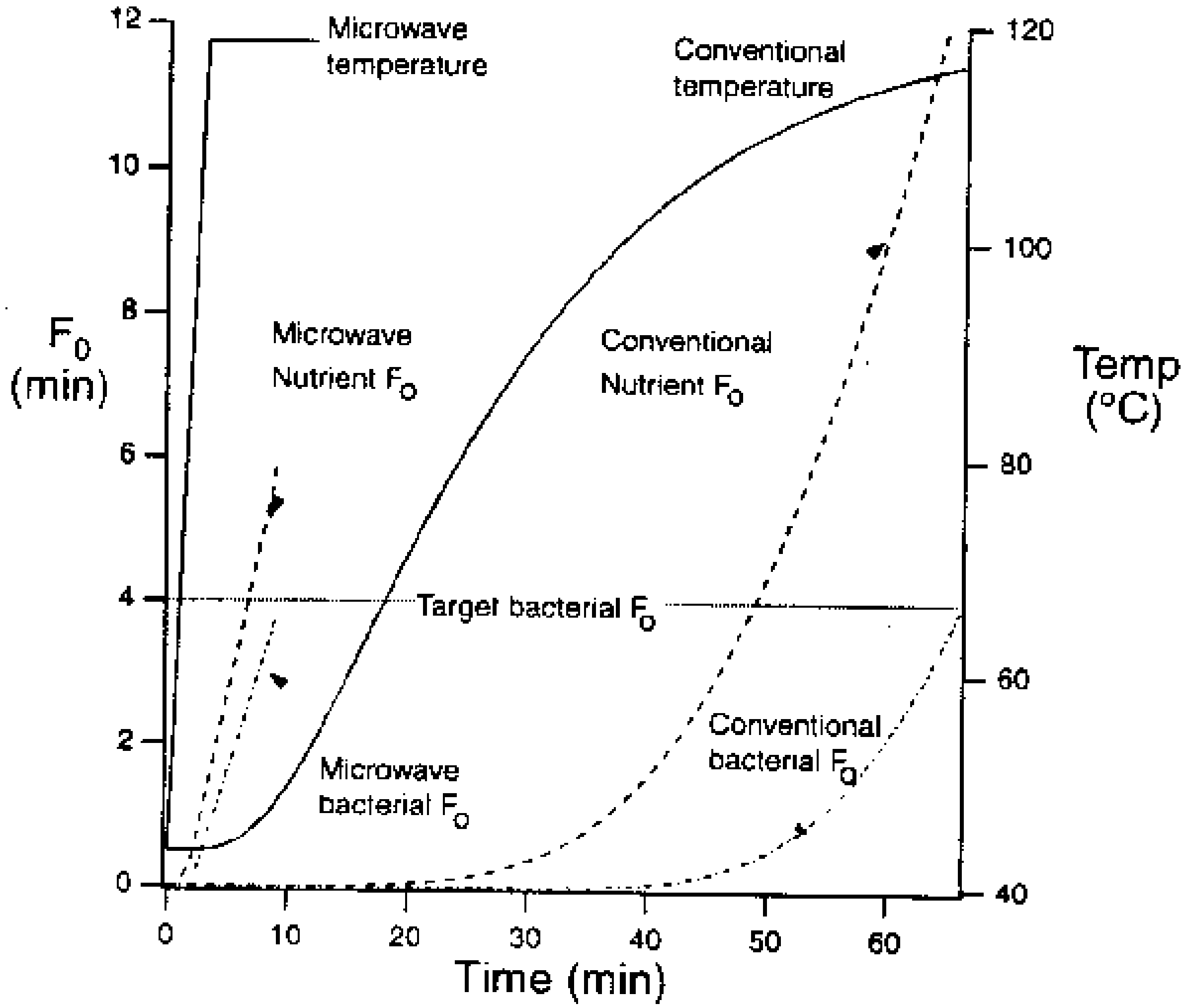

Figure 1. Quality parameters for microwave and conventional heating compared using computed values for typical heating situations (Datta and Hu 1992). F_0 represents the accumulated lethality (see Section 4.)

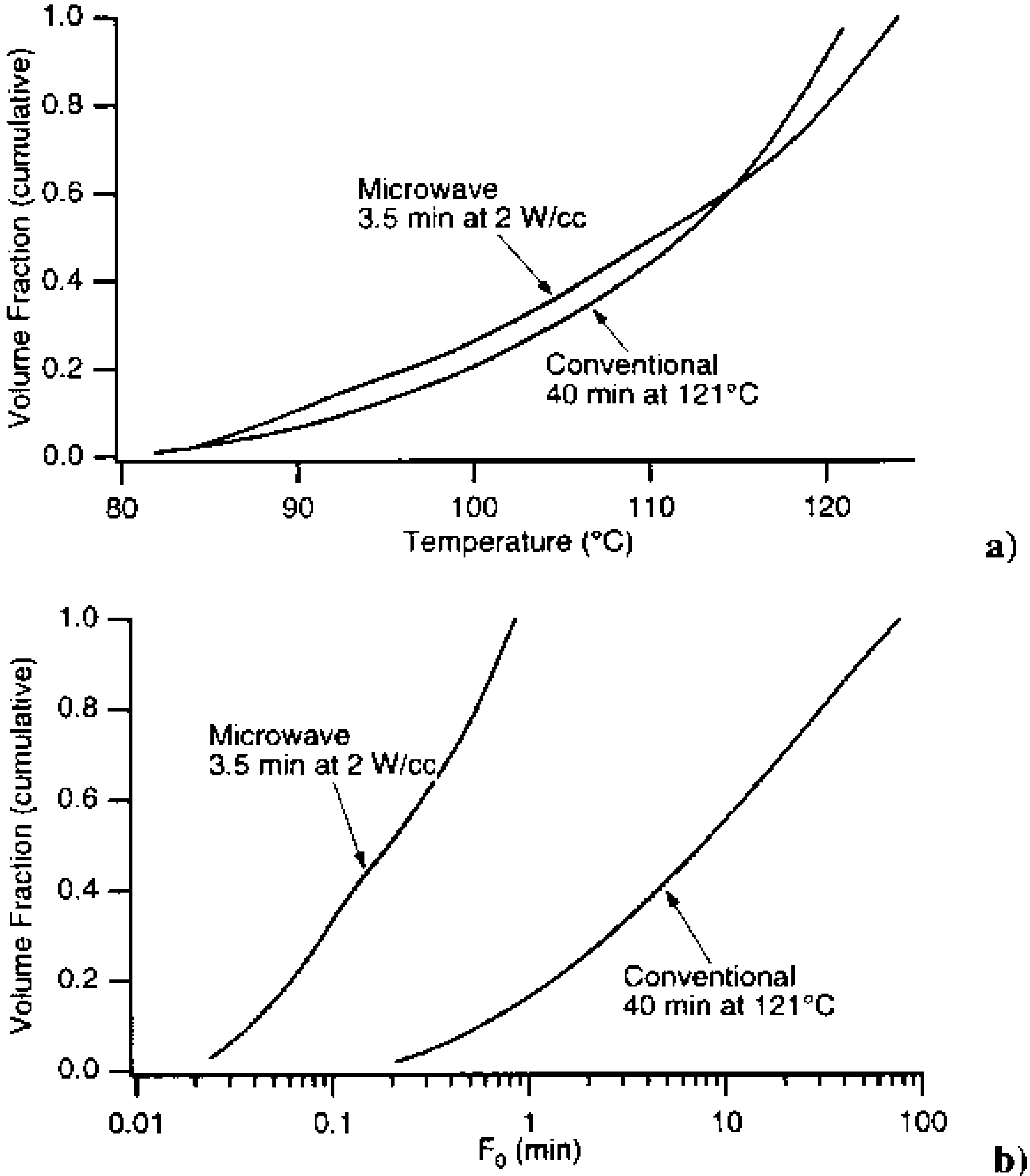

Figure 2. Illustration of how F_0 values (b) are typically quite different for microwave processing against conventional processing even when the range of temperatures are similar (a). From Datta and Liu (1992).

1.1.4.Industrial pasteurization and sterilization systems

Industrial microwave pasteurization and sterilization systems have been reported on and off for over 30 y (Jeppson and Harper 1967; Kenyon and others 1970; Mudgett and Schwartzbenrg 1982; Decareau 1985; Schlegel 1992; Harlfinger 1992; Anonymous 1996;

Tops 2000). Studies with implications for commercial pasteurization and sterilization have also appeared for many y (Proctor and Goldblith 1951; Hamid and others 1969; Knutson and others 1988; Burfoot and others 1988; 1996; Kudra and others 1991; Cassanovas and others 1994; Villamiel and others 1997; Zhang and others 1999). Early operational systems include batch processing of yogurt in cups (Anonymous 1980) and continuous processing of milk (Sale 1976). A very significant body of knowledge has been developed related to these processes. As of this writing, 2 commercial systems worldwide could be located that currently perform microwave pasteurization and/or sterilization of foods (Akiyama 2000; Tops 2000). As a specific example, ± company (Tops 2000) produced over 13 million ready meals in 1998 and have installed a newly designed system in 1999. Although continuous microwave heating in a tube flow arrangement has been studied at the research level, no commercial system is known to exist for food processing.

Commercial radio frequency heating systems for the purpose of food pasteurization or sterilization are not known to be in use, although it has been researched over the y (Bengtsson and Green 1970; Houben and others 1991; Wig and others 1999). The primary advantage of improved uniformity of heating was shown for in-package sterilization of foods in large packages using radio frequency at 27.12 MHz, although enhanced edge heating continued to be an issue (Wig and others 1999).

Implementation of a microwave sterilization process can vary significantly among manufacturers. Unlike conventional heating, the design of the equipment can more dramatically influence the critical process parameter--the location and temperature of the coldest point. This uncertainty makes it more difficult to make general conclusions about processes, process deviations, and how to handle deviations. For example, in one implementation (Harlfinger 1992) the process design consisted of heating, equilibration, holding, and cooling stages. The equilibration stage between heating and holding was to equilibrate the temperatures and avoid non-uniformities within the product. Hot air temperature and time are the factors controlling the equilibration process. All 4 stages are done under pressure to reach sterilization temperatures. The parameters recorded for the process were delivered power, temperature, pressure, speed, and cycle time.

Another system (Tops 2000) consists of microwave tunnels with several launchers in relation to the number of products (ready meals). Microwave-transparent and heat-resistant trays are used with shapes adapted for microwave heating. Exact positioning of the package is made within the tunnel and the package receives a pre-calculated, spatially varying microwave power profile optimized for that package. The process consists of heating, holding and cooling in pressurized tunnels. The entire operation is highly automated (see monitoring of process deviations later).

For in-package pasteurization or sterilization, packaging materials need to be microwave transparent and have a high melting point. Also, because metal reflects microwaves, packages with some metal component can considerably change the food temperatures (critical process factor). In some situations, metals have been deliberately added to the package to redistribute microwave energy to achieve increased uniformity of heating. The most common packages that have been tried are individual pouches made of microwave transparent rigid films such as polypropylene with an ethylene vinyl alcohol (EVOH) barrier or a polyethylene terephtalate (CPET) film.

1.1.5.Shelf-life extension at home

Almost every U.S. household owns a microwave oven that uses microwaves at a frequency of 2450 MHz. Reheating in a microwave oven, which the FDA does not regulate, is perhaps the most widespread use of the microwaves and has been known to involve serious microbiological safety issues. Researchers have also reported the use of home microwave ovens for pasteurization or for increasing shelf-life (Chiu and others 1984; Knutson and others 1988; Thompson and Thompson 1990).

1.1.6.Future processes

Many techniques have been tried to improve the uniformity of heating. These include rotating and oscillating the food package (O'Meara and others 1977), providing an absorbing medium (such as hot water) surrounding the product (Stenström 1974; Ohlsson 1991; Lau and others 1998), equilibrating after heating (Fakhouri and Ramaswamy 1993), and cycling the power. In the past, success of these processes has been limited due to the tremendous dependence of temperature and its distribution on food and oven factors. Use of the 915 MHz and radio frequencies to improve uniformity of heating may have potential for the future (Lau and others 1999b; Wig and others 1999). Future possibilities to improve the uniformity of heating include variable frequency microwave processing and phase control microwave processing. Although these 2 techniques have been applied to microwave heating of non-food materials, they are yet to be applied to food in any significant way. Combinations of microwave and conventional heating in many different configurations have also been used to improve heating uniformity. The critical process factor in combination heating or any other novel processes would most likely remain the temperature of the food at the cold point, primarily due to the complexity of the energy absorption and heat transfer processes.

In the future, microwaves may be combined with conventional heating or chemical treatments for surface treatment, for example, meat processing (KSU 1999) or food contact surface (Anonymous 1996).

1.2. Summary of Mechanism of Inactivation

The energy absorption from microwaves and radio frequency can raise the temperature of the food high enough to inactivate microorganisms for effective pasteurization or sterilization. A number of studies have proven that the thermal effect is the essential contributor to the destruction of microorganisms (Goldblith and Wang 1967; Rosén 1972; Fujikawa and others 1992) as well as the degradation of vitamin B_1, thiamin (Welt and Tong 1993).

Since the beginning of microwave processing, there has been controversy about the possible non-thermal (also called "athermal") effects of microwave processing (these are effects unrelated to the lethality caused by the heat) (McKinley 1936; Burton 1949; Cross and Fung 1982; Fung and Cunningham 1980). Researchers have repeatedly reported non-thermal effects, although detailed discussions on those mechanisms are difficult to locate in the literature. As many as 4 separate effects have been proposed--selective heating of microorganisms, electroporation, cell membrane rupture, and cell lysis due to electromagnetic energy coupling. These mechanisms are discussed later in more detail; however, the general consensus (Heddleson and Doores 1994) is that the reported non-thermal effects are likely to be due to the lack of precise measurements of the time-temperature history and its spatial variations.

1.3. Summary of Microbial Inactivation Kinetics

Since the studies reporting non-thermal effects have been inconclusive, only thermal effects are presumed to exist. Thus, microbial inactivation kinetics for microwaves are essentially the same as the inactivation kinetics of conventional thermal processing. The microwave non-thermal effects have been reported to add to the destruction of microorganisms. Thus, ignoring the possible non-thermal effect can only provide an extra safety factor. To date, there do not appear to be any microwave-resistant foodborne pathogens.

1.4. Summary of Critical Process Factors

Since the thermal effect is the sole lethal mechanism assumed in this processing technology, time-temperature history at the coldest location will determine the safety of the process. Both the magnitude of time-temperature history and the location of the cold points are functions of the composition (ionic content, moisture, density, and specific heat), shape, and size of the food, the microwave frequency, and the applicator (oven) design. Time is also a factor in the sense that, as the food heats up, its microwave absorption properties can change significantly and the location of cold points can shift (Fig. 3).

1.5. Synergistic Effects

Microwave processes are sometimes combined with conventional heating. Synergistic effects, where the total effect of the combined process on the microorganism is more than the sum of individual effects of microwave and the other process, have not been reported.

1.6. Current Limitations/Status

As mentioned earlier, only 2 companies (Tops 2000; Akiyama 2000) could be located worldwide that are currently using microwave technology for pasteurization/sterilization of foods. Other systems may be operational, but details were not available (Bassani 1999; Anonymous 1999). Some reasons given for the lack of success in commercial operation are complexity, expense, non-uniformity of heating, inability to ensure sterilization of the entire package, lack of suitable packaging materials, and unfavorable economics when compared to prepared frozen foods in the United States. Current research at several universities (Washington State University, Cornell University) and a government agency (U.S. Army Laboratories at Natick, MA) is aimed at further commercial use of microwave sterilization, particularly in the context of providing improved quality rations for soldiers.

2. Pathogens of Public Health Concern Most Resistant to the Technology

2.1. Identification of Pathogens Resistant to Microwaves

Numerous studies address the effect of microwave heating on pathogenic microorganisms in foods. Bacteria reported to be inactivated by microwave heating include *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, pathogenic *Escherichia coli*, *Enterococcus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* (Heddleson and others 1994; Rosenberg and Bogl 1987; Knutson, and others 1987; Chipley 1980). The nematode *Trichinella spiralis*, the organism that causes trichinosis, may also be inactivated (Zimmerman 1983). Foodborne pathogens have been shown to be inactivated by microwave heating in various poultry, beef, fish, and pork products, milk, and eggs; however, Heddleson and Doores (1994), reported that "in-home pasteurization" of milk was "problematic" and "potentially dangerous" due to non-uniform heating and lack of standardization of home microwave ovens.

It is very difficult to precisely compare the effectiveness of microwave heating to conventional heating based on the literature, because of the different techniques employed or the lack of detail in the methods or materials used, especially in relation to temperature monitoring (Heddleson and Doores 1994). A recurring conclusion in the literature is that non-uniform heating by microwaves may lead to survival of foodborne pathogens, including Salmonella and L. monocytogenes, in certain locations of foods heated at selected internal locations to endpoint temperatures that would normally be lethal (Schnepf and Barbeau 1989; Harrison and Carpenter 1989). For example, several studies have demonstrated that the measured internal temperature of poultry does not indicate the extent of inactivation of surface-inoculated Salmonella on poultry due to lower temperatures at the product surface (Schnepf and Barbeau 1989).

There do not appear to be any obvious "microwave-resistant" foodborne pathogens. Various studies have shown increased resistance of *S. aureus*, *C. perfringens*, or *Enterococcus faecalis* but not necessarily to the point that these could be labeled as resistant. As with conventional heating, bacteria are more resistant to thermal inactivation by microwave heating than yeasts and molds and bacterial spores are more resistant than vegetative cells.

2.2. Effects of Critical Process Factors on Inactivation

As with other thermal processes, the primary factors that determine safety are temperature and time (that is, integrated time-temperature history). A number of critical process factors affect time-temperature history. These are discussed in detail in Section 4.1. Some of these critical process factors are moisture, ionic content, microwave frequency, product parameters (including mass, density, geometry), specific heat, and the temperature achieved. It is important to note that in the context of microwave processing, these critical process factors do not change the rate of inactivation per se. Rather, these factors change the spatial distribution of microwave absorption and, therefore, the spatially distinct heating rate and time-temperature history. The spatial distribution of time-temperature history, in turn, changes the distribution of the extent of inactivation within the food, thus generally changing the total inactivated population within a given food sample. For example, the population of cells heated for 47 s at 700 W in a microwave oven in phosphate buffer were reduced by 99.8%, while those in 1% sodium chloride were reduced only by 62.4% (Heddleson and others 1994). Such a difference is attributed to the effect of salt in decreasing the penetration of microwaves. Less microwave penetration leads to a lower internal temperature and a lesser destruction in the interior regions, resulting in an overall lower destruction.

2.3. Shape of Inactivation Curves

The shapes of the inactivation curves are expected to be similar to those for conventional heating.

3. Mechanisms of Inactivation

3.1. Pathogen Culture Maintenance

As stated above, microwave or radio frequency processing causes microbial inactivation predominantly through thermal effects. In reviewing the literature, no pathogen is identified as uniquely resistant to these processing methods. Therefore, maintenance of cultures (pathogen, surrogate vegetative cells, or spore crops) for evaluating the process or processing unit effectiveness should follow generally accepted culturing procedures for thermal process evaluation. Conditions used for preparing, culturing, or storing vegetative cells or spores should be such that they produce the most resistant cell or spore. Appropriate conditions may be determined by consulting thermal resistance literature (see Section 4.3.). Generally, specific conditions for the growth of the particular test microorganism should be defined. Cells incubated to stationary phase usually demonstrate maximum resistance. As suggested in the Overarching Principles Section 2, sublethal stress conditions also need to be evaluated, as they may increase resistance. As a rule, one should ensure that the test microorganism has a heat resistance equivalent to that generally recognized for the particular genus, species, and strain used.

3.2. Microbial Enumeration Conditions and Methods

Once the vegetative cell or spore is treated with microwaves, it must be enumerated to determine if it is still viable. The objective of the recovery process is to provide optimum conditions for treated cells or spores to grow to obtain a measure of the maximum number of non-injured and injured survivors (Overarching Principles Section 2). For thermal processes, the length of incubation may be important in recovering viable cells or spores, because thermally treated cells or spores generally grow slower than non-treated ones. As with other process studies, experimentation will be necessary to determine the optimum conditions and methods for microbial enumeration.

3.3. Detailed Analysis of Inactivation Mechanisms

Two mechanisms are proposed for inactivation of microorganisms by microwaves. The first states that microwaves inactivate microorganisms entirely by heat through mechanisms comparable to other biophysical processes induced by heat, such as denaturation of enzymes, proteins, nucleic acids, or other vital components, as well as disruption of membranes (Heddleson and Doores 1994). There is no question as to the validity of this mechanism. A second proposed mechanism for inactivation by microwaves involves non-thermal effects. Four predominant theories have been used to explain non-thermal inactivation by microwaves or "cold pasteurization": selective heating, electroporation, cell membrane rupture, and magnetic field coupling (Kozempel and others 1998). The selective heating theory states that solid microorganisms are heated more effectively by microwaves than the surrounding medium and are thus killed more readily. Electroporation is caused when pores form in the membrane of the microorganisms due to electrical potential across the membrane, resulting in leakage. Cell membrane rupture is related in that the voltage drop across the membrane causes it to rupture. In the fourth theory, cell lysis occurs due to coupling of electromagnetic energy with critical molecules within the cells, disrupting internal components of the cell.

These mechanisms have been studied extensively since the 1970s by a number of researchers. Culkin and Fung (1975) reported earlier studies that suggested microwave heating at 2450 MHz caused greater destruction of *Aspergillus*, *Penicillium*, *Rhizopus*, aerobic microorganisms, *Salmonella* and *Proteus* in foods than heating alone. Culkin and Fung (1975) exposed *E. coli* and *Salmonella* Typhimurium in soups to 915 MHz microwaves and then determined survivors in the top, middle, and bottom regions of the product. The temperatures were measured using temperature-sensitive strips. They found that the greatest survival in the soups was in the top layer, which was also shown to have the lowest temperature. A series of studies by Khalil and Villota (1988; 1989a;b) suggested non-thermal effects of microwave heating. They first determined that *Bacillus stearothermophilus* spores in various media (water, NaCl, sucrose, phthalate, or phosphate buffers) had lower $D_{100\,°C}$ values when 2450 MHz microwaves were used compared to using a heated water bath. The experiment appears to have involved 6 tubes at a single temperature with no replication. In addition, the come-up times, although a small part of the overall heating times (microwaves: 58 - 83 s out of 90 - 190 min, conventional heat: 100 - 135 s out of 113 - 240 min), were not considered. Heddleson and Doores (1994) disputed the above conclusions due to inaccuracies in temperature measurement. Khalil and Villota (1988) further studied the effect of microwaves (2450 MHz assumed) on injury of *S. aureus* FRI-100. They heated cells at a sublethal temperature of 50 °C and maintained microwave temperature using recirculated cooled kerosene. Microwave heating caused a greater amount of cellular injury as determined by plating on trypticase soy agar plus 7% sodium chloride, increased loss of ultravioletabsorbing cellular material, and extended time for enterotoxin production. Their findings also showed that microwave-injured cells recovered better when microwave heating was carried out anaerobically. This effect was not seen with conventional heating. They speculated that the microwaves catalyzed oxidative reactions, possibly in membrane lipids, decreasing recovery of exposed cells. In another study, Khalil and Villota (1989b) demonstrated that while both conventional and microwave heating destroyed the 16S subunit of RNA of sublethally-heated *S. aureus* FRI-100, only microwave heating affected the integral structure of the 23S subunit. Moreover, when cells were allowed to recover following injury, it took longer for the microwave treated cells to restore their 23S RNA. Heddleson and Doores (1994) again concluded that these studies suffered from the lack of proper method of temperature measurement due to the unavailability of fiber optic thermometry.

Kozempel and others (1998) designed a system in which various fluids were exposed to microwave energy (5.0 - 5.4 kW) and then cooled so as to maintain temperatures of the fluids at 40 °C. The fluids were inoculated with a bacterium reported to be *Pediococcus* strain NRRL B-2354 prior to exposure. The greatest kill took place in apple juice (up to 4.6-logs in ± pass), with moderate lethality (up to 0.7-logs in 1 pass), occurring in water and 10% glucose. The amount of kill with multiple passes through the system was not constant. In some products the kill rate was reduced following the first pass. Little or no lethality was demonstrated with skim milk, pineapple juice, tomato juice, apple cider, or beer. In addition to the influence of the product itself, the medium used to grow the *Pediococcus* strain also appeared to affect cells counts; however, none of the product characteristics such as insoluble solids, pH, and conductivity could fully explain the variation. The authors concluded that they had demonstrated "significant microorganism kills in some fluids using microwave energy at sublethal temperatures." Kozempel and others (2000) subsequently designed a new system that was capable of isolating thermal and non-thermal effects of microwave energy. The system was a double tube that allowed input of microwave energy but removed thermal energy with cooling water. With this system, the researchers found no inactivation of *Enterobacter aerogenes*, *E. coli*, *Listeria innocua*, *Pediococcus*, or a yeast in various fluids including water, egg white, whole egg, tomato juice or beer at sublethal temperatures. They concluded that, in the absence of other stresses such as pH or heat, microwave energy did not inactivate microorganisms; however, they did suggest that microwave energy may complement or magnify thermal effects. In tests with *Saccharomyces cerevisiae* and *Lactobacillus plantarum* in apple juice, Ramaswamy and others (2000) also found that the non-thermal effect of microwave energy at sublethal temperatures is insignificant. However, they determined that, at equivalent heat treatments, microwaves enhanced inactivation. They demonstrated in a continuous flow system that *E. coli* K - 12 had significantly lower D-values (12.98 s at 55 °C, 6.31 s at 60 °C, 0.78 s at 65 °C) using microwave energy than equivalent heat treatments with hot water (44.7 s at 55 °C, 26.8 s at 60 °C, 2.00 s at 65 °C) or steam (72.71 s at 55 °C, 15.61 s at 60 °C, 2.98 s at 65 °C). They concluded that, while there was no non-thermal effect of microwaves, there was a significant enhancement of thermal treatments.

Apart from the described studies, most research has concluded that there is little or no non-thermal effect of microwaves on microorganisms (Rosenberg and Bögl 1987; Knutson, and others 1987) and that inactivation of microorganisms is due only to heat. Goldblith and Wang (1967) heated suspensions of *E. coli* and *Bacillus subtilis* under conventional heating and with microwaves at 2450 MHz. The degree of inactivation of both microorganisms was identical with conventional and microwave heating. Vela and Wu (1979) heated various bacteria, fungi, and bacteriophages in 2450 MHz microwaves in water and as lyophilized cultures. There was no inactivation of dry cultures even after extended exposure. Similarly, Jeng and others (1987) found no difference in inactivation of *B. subtilis* spores under conventional or microwave (2450 MHz) heating in automated computer-controlled temperature monitoring systems. Kazbekov and Vyacheslavov (1978) found that thymidine and thymine uptake, leakage of potassium and hydrogen ions, and uptake of DNA by cells of *E. coli* or *B. subtilis* under low power microwaves were typical of that shown for heating. Fujikawa and others (1992) found no major differences in inactivation kinetics of *E. coli* in phosphate buffer between microwaves and conventional heating. Welt and others (1993a) demonstrated no difference between conventional and microwave inactivation of *Clostridium sporogenes* PA3679 at 90, 100 and 110 °C. A suspension of spores that was exposed to microwaves, but continuously cooled in silicone tubing demonstrated no detectable inactivation.

While there is some controversy as to the additional inactivation of microorganisms over the thermal effect of microwaves, this additional inactivation is small and inconsistent. In many studies comparing microwave heating to conventional heating, microwave heating appears less effective due to nonuniform heating effects from unpredictability of cold spots and changing product parameters, such as specific heat. Therefore, when developing methods for describing the inactivation kinetics of microwave heating, it is recommended that only thermal effects be included in the model.

Under the assumption of only thermal effects, the kinetic parameters presented in Table 1A of the Overarching Principles are recommended for use in design of processes involving the microwave treatment of foods. The kinetic parameters used to design thermal processes have been presented and defined in an introductory chapter of the main document. The pathogens of concern will be the ones defined for thermal processing and described in the Overarching Principles Section 3 of this report.

4. Validation/Critical Process Factors

4.1. Identification and Description of Critical Process Factors

Time-temperature history at the coldest point determines the microbiological safety of the process, as in other thermal processing. Once temperature is known at the coldest point as a function of time, accumulated lethality can be calculated following the well-known equation

$$F_0 = \int_0^{tT} 10^{(T-250)/z} \, dt$$

where *T* is the cold point temperature at any time *t*, *z* is the z-value in 0F and t_f is the total duration of heating. There are, however, major differences between conventional and microwave heating in terms of the location of the cold point and how time-temperature history of the cold point is affected by a number of critical process factors. *Note that the effect of the factors are discussed in a simplistic way in order to illustrate the concepts--the actual influence of the factors can be quite complex and are only known from detailed experiment or mathematical modeling.* Such effects are discussed in detail in books such as Datta and Anantheswaran (2000), Buffler (1993) and Decareau (1985) or review papers such as Saltiel and Datta (1998).

Time-temperature history at the coldest point for a conventional thermal process is generally quite predictable for a food that is all solid or all fluid. For example, for a conduction-heated (solid) food, it is usually the geometric center. In microwave heating, even for a solid food, the coldest point is less straightforward to predict and can change during the heating process (Fig. 3), depending on a number of food and oven factors (Fleischman 1996; Zhang and others 1999). Accordingly, relatively sophisticated modeling based on measured properties of the foods needs to be used and subsequently validated to ascertain the location of the point of lowest integrated time-temperature history. Well-developed but simple procedures, such as the Ball formula (Ball and Olson 1957) would be much harder to achieve for microwave heating. Changes in properties during heating have a more pronounced effect in microwave heating as compared to conventional heating. As the food heats, its microwave absorption capability typically increases, which increases the rate of temperature rise and therefore further increases the rate of microwave absorption. Such coupling could lead to runaway heating (Zhang and others 1999; Zhang and Datta 1999). Figure 3 also illustrates the coupling effect. Initially, at lower temperatures, microwave absorption is lower, so the waves are able to penetrate a lot further into the material. As the material heats up, it absorbs microwaves more readily and the waves are not able to penetrate as far. Especially in foods with high ionic concentrations, the surface at higher temperatures can act as a shield.

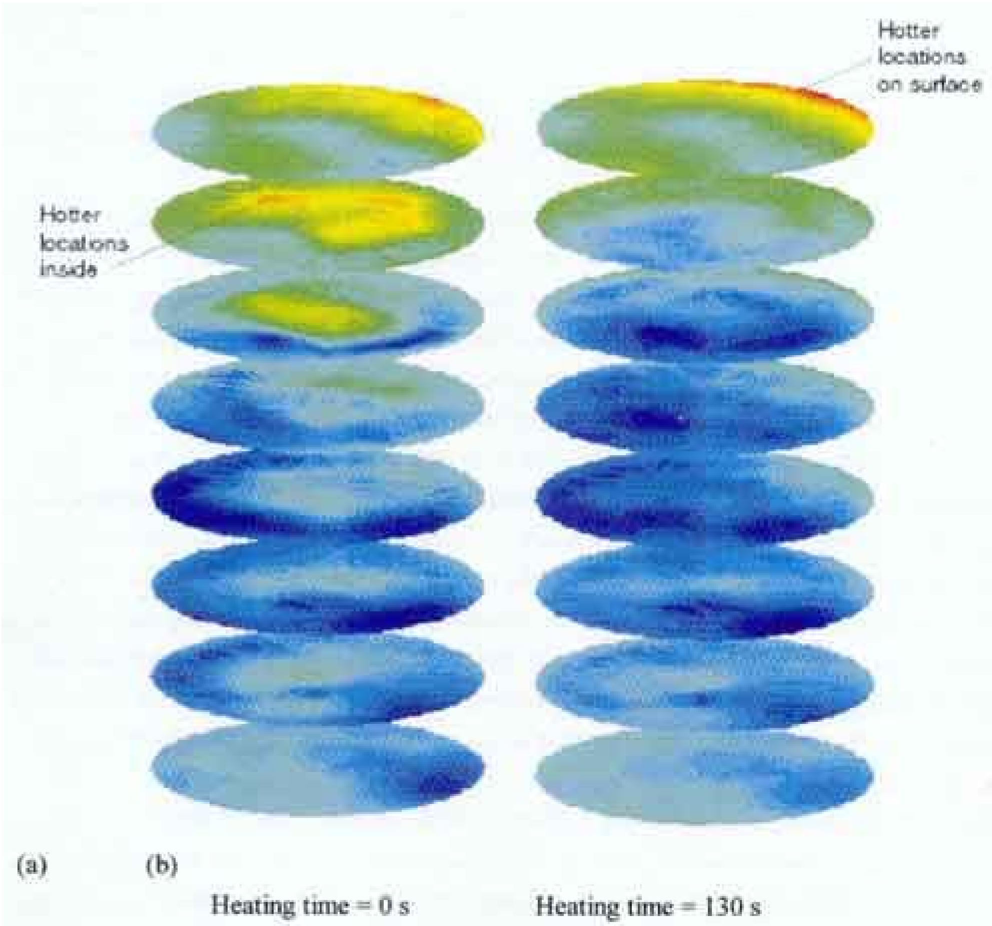

Figure 3. Microwave power absortion (Q in equation 1) patterns in a sterilization process can change dramatically during heating, as shown by the migration of hottest locations (in red) from interior (a) to surface (b). Shown are computed results for a ham cylinder (0.7% salt) heated in a microwave oven similar to domestic microwave ovens (Zhang and others 1999).

Since heat is constantly generated everywhere in the food, but at different rates, the difference between the temperature at the coldest and the warmest points in the food keeps increasing with time. This is unlike conventional heating where the coldest point approaches the warmest temperature of the system (typically the heating medium temperature) with time. In conventional heating, the surface is at the highest temperature, corresponding to the temperature of the heating medium. In microwave heating, the food heats up while the surrounding air stays cold (Datta 2000). The cold air keeps the surface temperature lower than locations near the surface of food. Surface evaporation, especially when heating an unpackaged food, can further decrease the surface temperature. In some heating applications, such as with frozen foods that are spherical, the surface could be the coldest location.

In conventional heating, the maximum temperature is limited by the heating medium temperature, such as steam in a retort. Since microwave absorption continuously generates heat, temperature keeps increasing in the microwave heating process. To keep the temperature within reasonable limits, microwaves need to be turned on and off (cycled) once the target temperature has been reached.

One of the advantages of microwave heating is that the come-up time is short. It is this shorter come-up time that helps retain the organoleptic qualities and that is the basis for preferring microwave processing to conventional thermoprocessing. In calculating the process time, the come-up time in microwave heating cannot be given nearly as much importance as in conventional heating (see Fig. 1).

4.1.1.Factors related to product and package

Food shape, volume, surface area, and composition are critical factors in microwave heating (Zhang and others 1999). These factors can affect the amount and spatial pattern of absorbed energy, leading to effects such as corner and edge overheating, focusing, and resonance. For example, a curved shape can focus microwaves and produce a higher internal rate of heating than near the surface (Ohlsson and Risman 1978). Such heating patterns can also change with time, as illustrated in Fig. 3. The effect of food volume on total amount of energy absorbed by the food for a given setting of power level is typically as shown in Fig. 4. Since the total energy absorbed lags the increase in volume, average temperature rise drops (however, food as a whole heats slower).

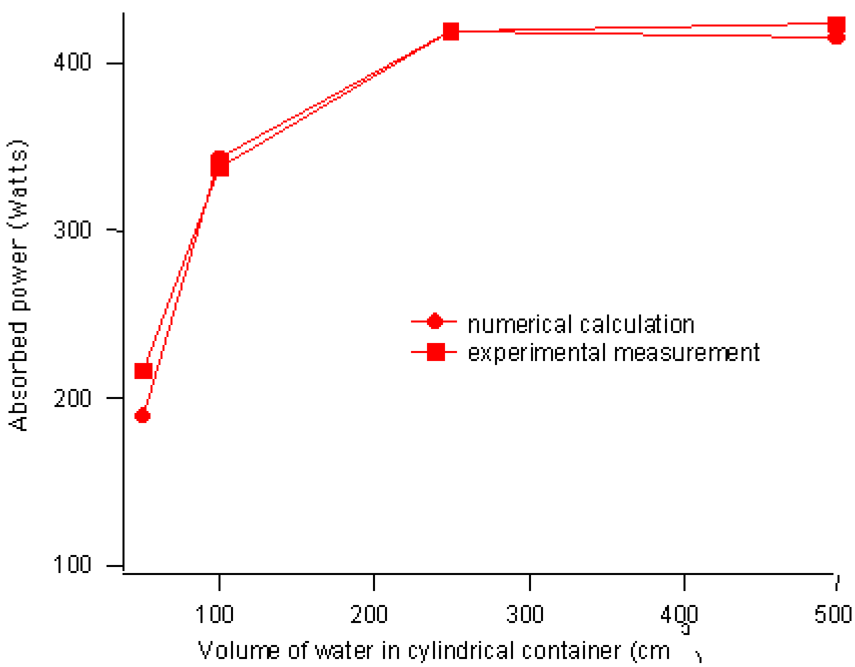

Figure 4. Magnitude of total absorbed power as a function of volume of food, obtained from experiments and electromagnetic simulations (Zhang and others 1999).

Composition, in particular moisture and salt percentages, has a much greater influence on microwave processing than in conventional processing, due to its influence on dielectric properties. High salt and moisture content increases the efficiency of microwave absorption, thereby decreasing the depth of penetration. Thus, interior locations generally get heated less in foods with high salt or moisture content, reducing microbial destruction. Composition can also change the thermal properties such as specific heat, density, and thermal conductivity and, thereby, change the magnitude and uniformity of the temperature rise. For example, the temperature of a low specific heat oil increases at a much faster rate than that of water when compared at the same level of absorbed power.

The different components of mixed food products, such as multi-compartment frozen dinners, will heat differently (Ryynanen and Ohlsson 1996; Zhang and others 1999). Packaging material is also a critical process factor. In contrast to commercial canning, where metal containers offer minimum thermal resistance and are not a critical process factor, metallic components present in a package, such as aluminum foil and susceptors, can dramatically influence the heating rates of the packaged food.

4.1.2.Factors related to process and equipment

Several process and equipment factors are critical in microwave heating (Zhang and others 1999). Design (size, geometry, and so on) of the microwave oven can significantly affect the magnitude and/or spatial variation of the power absorption in the product. In addition, presence or absence of devices added to improve uniformity, such as mode stirrers and turntables, are major factors affecting temperature distribution.

The placement of the food inside the oven can also have a significant influence on the magnitude and uniformity of power absorption (Zhang and others 1999). Other factors related to the equipment are the temperature of the medium surrounding the product and the level of food surface evaporation (especially significant for unpackaged food), both affecting food surface temperature. Cooling effects due to surface evaporation have been shown to lead to survival of *Trichina* larvae in pork (personal communication with J. Gerling cited in Datta 1991).

Heating of containerized liquids with microwaves without agitation causes flow and thermal stratification inside the container. Warmer liquid moves to the top, much like in conventional heating. Due to variations in product characteristics (such as viscosity), in package components (such as metal in aluminium foil), and in equipment factors, the patterns of temperature distribution in heated liquids (static or flowing) can be quite complex, and the slowest heating location needs to be determined for distinctly different situations (Prosetya and Datta 1991; Anantheswaran and Liu 1994a 1994b; Datta and Hu 1992; Tajchakavit and Ramaswamy 1995).

In addition to the aspects discussed above, power level and cycling of the microwave input are critical process factors in microwave heating. Also, power output by the magnetron (the component in an oven generating the microwaves) changes as the magnetron heats up over time. Thus, equipment specific "wait time" may be necessary before the power output becomes stable. Due to differences in penetrating ability, the frequency of the microwaves can dramatically affect the heating rates and their spatial distribution. In a simplified view, a lower frequency of 915 MHz has a higher depth of penetration than the 2450 MHz used for home microwaves. At this lower frequency, uniformity of heating can improve with reduced edge heating (Lau and others 1998).

Equilibration of the product following heating can help to level the temperature distribution and improve uniformity. Its important effects have been demonstrated (Fakhouri and Ramaswamy 1993; Ramaswamy and Pilletwill 1992).

A summary of the various product, package, process, and equipment factors discussed above is provided in Table 2. Due to the number of critical factors implicated, none of them alone can be treated as a critical process factor by itself, unless all others are held constant.

Table 2. Critical Process Factors in Microwave Heating

Food	Shape, size, composition (moisture, salt, and so on), multiple components (as in a frozen dinner), liquid against solid
Package	Presence of metallic elements such as aluminum foil, susceptor
Process	Power level, cycling, presence of hot water or air around the food, equilibration time
Equipment	Dimensions, shape and other electromagnetic characteristics of the oven, frequency, agitation of the food, presence of mode stirrers and turntables

4.1.3.Identification of the effect of process factors on cold point using mathematical simulation

Due to the complexity of the system where the heating pattern depends on such a large number of factors, simulation-based design can save significant time and resources in developing microbiologically safe processes. Such simulation-based design can drastically reduce the number of experiments needed to predict the location of cold points and the time-temperature history at these locations for the actual food and equipment combinations. State of the art commercial software simulating the electromagnetic and heat transfer properties has been used for microwave food process design (Dibben 2000; Zhang and Datta 2000). Such software can provide a comprehensive insight into the heating process by showing interior power absorption (that is, heating rates) in a 3D object (Fig. 3), difficult using experimentation. Simulation-based design can allow the process and equipment designers to judiciously choose proper combinations of food and process parameters in an efficient manner, reducing some of the time and expenses in prototype building. Location of coldest point (the critical process factor) and its time-temperature history can be predicted this way. As the user friendliness, accuracy, and linkages with other software improve, more food processors are expected to use these programs routinely.

4.2. Description of Methods to Measure/Monitor Critical Process Factors

Monitoring the temperature of microwave processed food poses a challenge. Thermocouples and other metallic probes used to record temperatures for conventional processing in static systems are generally unsatisfactory for precision temperature measurements in microwave ovens for several reasons. Firstly, metallic probes reflect and absorb the energy of incident microwaves, and require special grounding and installation to withstand microwave operations. Secondly, electromagnetic field disturbances caused by the presence of metallic probes create localized changes in heating patterns that can produce variability in overall heating patterns.

Valuable alternatives to metallic probes, however, are fiber-optic temperature probes. These are on the market and have been used to monitor temperatures during microwave heating. They are inert to the electric and magnetic fields of the microwaves or radio frequencies. Additional advantages are their accuracy (from 0.2 to 0.05 0C) and fast response (milliseconds). Some disadvantages of fiber-optic probes are their current price (although it is dropping) and somewhat fragile nature.

4.3. Description of Microbial or Chemical Surrogates/Indicators

For determining the kinetics and efficiency of microwave inactivation of microorganisms, surrogate/indicator microorganisms could be selected from those traditionally used in thermal processing studies. No microorganisms with unique resistance to microwave processing have been reported in the literature, suggesting that classical surrogates (vegetative cells or spores) would be appropriate for process determination and validation.

Since microwave or radio frequency processing is primarily a heat process, microbiological validation tests should be designed using procedures that parallel those used for thermal processing. Important considerations for an inoculated pack study of a new thermal process include selection of a surrogate microorganism, preparation and handling of the test microorganism, size and volume of inoculum, method of inoculation, processing levels and conditions, number of containers, product data collection, statistical techniques and methods for determining survivors (for example, incubation, microbiological recovery). A more detailed discussion of these procedural considerations is available in the *Laboratory Manual for Food Canners and Processors* (National Canner's Association 1968). The principles and methods are similar, whether the objective is pasteurization, pathogen reduction, or commercial sterilization.

One problem that needs to be carefully considered is the method of inoculation. Since microwave heating can be nonuniform and the cold spot is not easy to identify, the inoculum should be distributed throughout the food product. This could be a particular problem with solid products, unless made homogeneous by grinding or placed in locations where significant spatial variation in heating rates is expected.

The use of history indicators or time-temperature integrators (see reviews by Hendrickx and others 1995; Van Loey and others 1996), either biological (microbiological or enzymatic) or chemical, could be a way to monitor the process impact and could be helpful in identifying critical process parameters. As in thermal processing, proper calibration of the kinetic parameters of the surrogates/indicators is required. The same principles as for thermal processing apply and extra care should be given to critical factors associated with the microwave/radio frequency heating process in that the presence of the indicator should not influence the heating process. An example is the validation of sterilization patterns by correlating thermally induced chemical changes in the food (a history indicator) to bacterial destruction. Intrinsic chemical markers (Kim and Taub 1993; Prakash and others 1997, Zhang and others 1999) whose extent of formation is a function of time-temperature history have been used recently in several processing situations, including microwave sterilization (Lau and others 1999a; Zhang and others 1999; Wig and others 1999). This approach can provide information on spatial distribution of the integrated time-temperature history within a packaged system and on any variation among packages in a continuous process operation.

5. Process Deviations

Process deviations in microwave processing present some special issues and challenges. Temperatures are generally more difficult to monitor and measurement of power output from microwave generators may not accurately reflect product temperature, unless the sensitivity of the heating process to changes in food composition, size, shape, placement in the oven and other factors discussed earlier are taken into consideration. Due to the complex nature of the process, adjustments such as extending the time or increasing the power level will not be simple. It is generally believed that complete reprocessing would be the most reliable way to handle underprocessed material.

5.1. Basic Detection Methods for Process Deviations

Process parameters under direct control of the operator are the power level (including cycling), spatial distribution of power (number and positioning when multiple microwave generators are used) and time of exposure. As mentioned above, once a deviation has taken place, adjustments could be complex.

In one implementation (Tops 2000), the exact placement of a product in the tunnel is known and power levels of multiple microwave generators are programmed precisely to provide the custom-tailored heating profile for that tray and product. For example, the center of the tray is provided with higher microwave power. The detection system consists of a variety of monitors. For example, broken generators and insufficient power level delivered by a generator is automatically recorded. Infrared surface measurement of each tray can be made while they are being transported to the hold system. Swelling of the top surface of individual packages due to internal steam generation during heating is monitored using a distance tracer--adequate heating produces enough steam for the package to swell sufficiently. Visual control is also made by placing maxi-thermometers (that measure maximum temperatures) at precise locations in the package. An endoscope is also used to observe the heating process inside the microwave tunnel and therefore to monitor it manually.

In another implementation (Harlfinger 1992), power settings for individual magnetrons are stored over time. If the power delivered varies from the set values, an alarm warns the operator. An additional warning signal comes from any blockage of the product feeding system that may lead to unintended cooling of the preheated products. This publication also reported the use of a DataTrace metallic temperature data-logger inside the package by ± company to monitor the time-temperature history. Use of such metallic data-loggers requires careful considerations and interpretations.

5.2 Methods to Assess and Correct Deviations

See general discussions under Section 5.1. above. Details about process deviations are hard to obtain. Only ± company was willing to share information about current production of microwave pasteurized and sterilized food (Tops 2000). In this company's implementation, a product is rejected based on an automated control system. Rejection is done at the end of the cooling system based on infrared surface temperature measurement, detection of broken microwave generators, and other means described in Section 5.1. Further loading of food into that tunnel is automatically stopped following a rejection. The temperature control system for each microwave tunnel can also be adjusted, if necessary, following a deviation. The control system is also programmed for each individual product.

In general, extensive experimentation would be needed to validate the effectiveness and reliability of the methods to assess and correct deviations.

6. Research Needs

Research needs have been identified in the following areas:

- Effects of food formulation on heating patterns.
- Effects of equipment design factors, including frequency (for example, 915 MHz is sometimes proposed instead of the commonly used 2450 MHz for better uniformity of heating).
- Development of variable frequency ovens (although currently more expensive for food applications) for improved uniformity of heating.
- Understanding factors affecting heating patterns, including qualitative changes occurring with frequency changes.
- Monitoring and real-time adjusting for process deviations in microwave and radio frequency processing.

Glossary

A complete list of definitions regarding all the technologies is located at the end of this document.

Conventional heating. Heating of a substance by transfer of thermal energy from a heating medium at higher temperature to a low temperature product.

Focussing. Concentration of electromagnetic waves inside a food due to its curved surface, much like a lens focussing light waves. It leads to enhanced heating at the interior.

Internal energy generation. Heat generation within a material and throughout its volume due to the presence of an energy source that is dissipated throughout the volume (see volumetric heating).

Liquid crystals. Materials with properties that are useful for thermal sensing. Liquid crystals typically change color with temperature.

Magnetron. The physical component of a microwave system that generates the microwaves.

Microwaves. Electromagnetic waves at frequencies 915, 2450, 5800, and 24225 MHz.

Non-thermal effects. Effects due to the exposure to a process that are not of thermal origin, that is, cannot be explained by measured temperature changes.

Penetration depth. The distance the electromagnetic waves (of a certain frequency) travel in a material before it loses 63% of its energy.

Power cycling. The process of the microwave source turning on and off .

Radio frequency. Electromagnetic waves at frequencies of 13.56, 27.12 and 40.68 MHz.

Runaway heating. A cycle of increasing temperature in food causing an increasing rate of energy (microwave/ohmic) absorption that further increases the rate of temperature rise. It is more prominent in foods undergoing phase change from ice to water and in foods containing significant salt and other ions.

Specific heat. The ability of a material to store heat. Technically as the amount of energy required to raise the temperature of unit mass of an object by a unit increment in temperature.

Variable frequency. Sweeping over a range of frequencies during the microwave heating process to improve uniformity.

Volumetric heating. Heating by internal energy generation throughout the volume of a material (see also internal energy generation).

Waveguide. The physical component of a microwave system that guides the microwaves from magnetron to the cavity where the food is heated. When applied in the form of pulses, it reverses the charge for each pulse and pulse intensity gradually decreases.

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