Effect of Processing Parameters and Composition of Food on the Bactericidal Efficiency of Microwave Heating

By
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MASTER'S RESEARCH PROJECT
Submitted in partial satisfaction of the requirement for
Master degree of Food Biotechnology
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Abstract

Due to which kind of microorganisms that used to cause foodborne disease and inactivated microbial of microwave exposure in composition of foods, this research aimed to study the effects of time, power of microwave and composition of foods (pH, salt and fat) on the bactericidal activities. Three strains of bacteria *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* were investigated in this experiment. The suspension containing 10^8 $CFUmL^{-1}$ of each strain was exposed in microwave at different treatment times (1, 2, 3 and 5 min) and powers (50, 75 and 100%). Simultaneously, effects of different pH (4.3, 7.0 and 8.5), salt concentrations (1, 2 and 3%) and presented of fat (skim milk and full fat milk) were investigated.

For pH, either high or low pH, which was out of the optimal range for growth, increased the destruction efficiency of microwave heating when low power was used. At full power of microwave, the microorganisms were totally destroyed, especially at longer exposure time. *B. cereus* showed the highest resistant to the microwave heating, with the D-value of 0.8, 0.7 and 0.4 min for 50, 75 and 100% microwave power operating at pH 7, respectively. The presence of salt had also influence on the survival of microorganisms when they were exposed to the microwave heating at different heating powers and times. Increasing of salt concentration leaded to increase destruction effect. *E. coli* was the most sensitive bacteria to the microwave heating, while the spore forming bacteria *B. cereus* was resisted to the microwave heating. Longer heating time or increasing in microwave power was required for complete destruction of *B. cereus* in the presence of salt. The presence of fat in food system provided the protective effect to the microorganisms subjected to microwave heating, especially spore forming bacteria like *B. cereus* that required longer time of microwave heating to destroy this organism.

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List of Abbreviations

E. coli: Escherichia coli

B. cereus: Bacillus cereus

S. aureus: Staphylococcus aureus

SDS: sodium dodecyl sulfate

cfu/g: Colony- Forming Unit per gram

A_w: Water Activity

LAB: Lactic acid bacteria

D-values: decimal reduction time (the time required at a certain temperature to kill 90% of the organisms being studied)

FDA: the Food and Drug Administration

NA: nutrient agar

NB: nutrient broth

$logCFU.mL^{-1}$: the logarithm of Colony- Forming Unit per milliliter

Chapter 1
Introduction

The use of microwave radiation has increased not only for household food cooking processes, but also in the food industry for thawing, drying and baking foods (Rosenberg and Bogl, 1987; Woo et al., 2000), as well as for the microbial inactivation in foods (Kakita et al., 1995; Rosenberg and Bogl, 1987a; 1987b). Hence, it is important to understand the effects of microwave heating on microorganisms in foods. The bactericidal effect of microwave on contaminated foods has been studied (Conder and Williams, 1983; Goksoy et al., 2000). Rosenberg and Bogl (1987a) reported that microwave heating provided the great potential for pasteurization of foods, as well as, sterilization of food products (Chipley, 1980; Fung and Cunningham, 1980; Cross and Fung, 1982; Rosenberg and Bögl, 1987). It also provided less destruction to food due to short heating and exposure time (Fujikawa et al., 1992).

Many studies have reported the possible effect of microwave on microorganisms especially bacteria, such as *Escherichia coli*, *Streptococcus faecalis*, *Staphyloccocus aureus*, *Bacillus subtilis* spores, *Salmonella* sp., *Lactobacillus plantarum*, *Listeria* spp., *Clostridium perfringens* and yeast such as *Saccharomyces cerevisiae* (Heddleson et al., 1994; Welt et al., 1994; Pothakamury et al., 1995; Atmaca et al., 1996; Shin and Pyun, 1997; Farber et al., 1998; Metaxas and Meredith, 1988; Fujikawa et al., 1992; Vela and Wu, 1979; Woo et al., 2000). It was not only bacteria and mold spores, but the bacteriophage PL-1, which is specific to *L. casei*, was also sensitive to microwave heating (Ishitani et al., 1981; Kakita et al., 1995; Khalil and Villota, 1985). In addition, the use of microwave to reduce the number of microorganisms in various foods, including turkey, beef, corn-soy milk, chicken, frozen foods, and potatoes have been studied (Aleixoet al., 1985; Bookwalter et al., 1982; Craven and Lillard, 1974; Dahl et al., 1980; Farber et al., 1998; Lin and Sawyer, 1988; Spite, 1984.). Although many studies reported the lethal effects of microwave on bacteria, the effects of processing parameters and composition of foods have not been reported elsewhere. Therefore, this research aimed to study the effects of processing parameters (time and power of microwave) and composition of foods (pH, salt and fat) on the bactericidal effect of microwave heating.

1.1 Objectives

1. To study the effect of pH at various powers and times of heating on the bactericidal effect of microwave.

2. To study the effect of salt at various powers and times of heating on the bactericidal effect of microwave.

3. To study the effect of fat at various powers and times of heating on the bactericidal effect of microwave using milk as a model.

Chapter 2

Literature Review

2.1 Microwave

2.1.1 Principle of microwave heating

Microwave is a form of non-ionizing electromagnetic radiation that has frequency as ultra-high frequency (UHF) (0.3-3 GHz), super high frequency (SHF) (3-30 GHz) and extremely high frequency (EHF) (30-300 GHz). Electromagnetic radiation in the 1 mm to 1 m wavelength range (300 MHz to 300 Ghz) is referred to as microwave radiation (Figure 2.1 and Table 2.1), and is part of what is known as radiofrequency (RF) radiation (Cleveland and Ulcek, 2009).

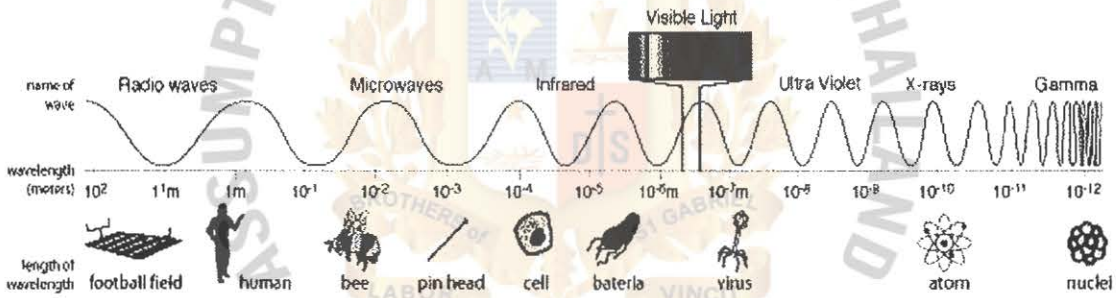

Figure 2.1: The electromagnetic spectrum (Nasa, 2011)

To prevent interference issues, only four microwave frequencies (915±13, 2,450±50, 5,800±75 and 24,150±125 MHz) are permitted by Federal Communications Commission (FCC) for industrial, scientific and medical applications (FDA 47 CFR 18.301). In food industry, food processors commonly use the 915 MHz frequency, while microwave ovens for home used is the 2450 MHz frequency. The U.S. Food and Drug Administration (FDA) currently allow only 915 and 2450 MHz frequencies for commercial and home ovens (FDA, 2009).

Table 2.1 Regions of the Electromagnetic Spectrum (Nasa, 2004)

	Wavelength (m)	Frequency (Hz)	Energy (J)
Radio	$> 1 \times 10^{-1}$	$< 3 \times 10^{9}$	$< 2 \times 10^{-24}$
Microwave	1×10^{-3} - 1×10^{-1}	3×10^{9} - 3×10^{11}	2×10^{-24} - 2×10^{-22}
Infrared	7×10^{-7} - 1×10^{-3}	3×10^{11} - 4×10^{14}	2×10^{-22} - 3×10^{-19}
Optical	4×10^{-7} - 7×10^{-7}	4×10^{14} - 7.5×10^{14}	3×10^{-19} - 5×10^{-19}
UV	1×10^{-8} - 4×10^{-7}	7.5×10^{14} - 3×10^{16}	5×10^{-19} - 2×10^{-17}
X-ray	1×10^{-11} - 1×10^{-8}	3×10^{16} - 3×10^{19}	2×10^{-17} - 2×10^{-14}
Gamma-ray	$< 1 \times 10^{-11}$	$> 3 \times 10^{19}$	$> 2 \times 10^{-14}$

2.1.2 Component of microwave heating

Although, magnetrons are classified as pulsed or continuous-wave, the continuous-wave magnetron is used predominately for industrial heating applications (Püschner, 2005).

Heating with microwave involves primarily two mechanisms. The first is dielectric heating. Water in the food is often the primary component responsible. Due to their dipolar nature, water molecules try to follow the electric field associated with electromagnetic radiation as it oscillates at the microwave frequencies. Such oscillations of the water molecules produce heat. The second major mechanism of heating with microwaves is through the oscillatory migration of ions in the food that generates heat under the influence of the oscillating electric field (FDA, 2009).

Regardless of the continuous flow or batch heating systems type, these systems are currently comprised of three main parts: the microwave source, waveguide and applicator (Figure 2.2). In most systems, the microwave source is attached to a waveguide, which guides propagating waves to the applicator (Cubides, 2011).

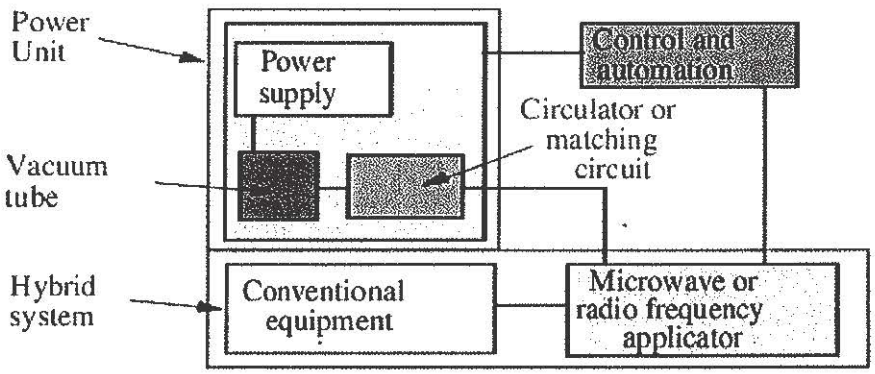

Figure 2.2: Typical microwave heating set up (adapted from IEE, 1991)

2.1.2.1 Waveguide

Waveguides are hollow metal conductors used to convey electromagnetic energy from the magnetron to the food source to be heated or the applicator. Because they are made of metal, they do not absorb electromagnetic energy but rather, they reflect it. Their shape can be circular or rectangular and their size defines a minimum frequency at which wave can propagate. The most common waveguide is rectangular with a width equal to double its height (Reiger and Schubert, 2005).

2.1.2.2 The generation of wave

The source of radiation is the magnetron tube, which converts 60 Hz power line electric current to electromagnetic radiation of 2450 MHz. The high voltage (typically 3,000 to 4,000 volts) powers from the magnetron tube is produced by a step-up transformer rectifier and filter, which converts the 120V AC (alternating current or 60 Hz line voltage) to 4 kV DC (direct current). Microwave generates internal heat by dielectric and ionic mechanisms. When the water molecules in the food vibrate (at a rate of 2,450,000,000 times per second), the food absorbs the microwave radiation; and the movements of the molecules produce friction, which causes heat (Datta et al., 2001).

The magnitude and uniformity of heating in microwave was affected by three factors:

1. Strength and distribution of electromagnetic fields where the food is placed.

2. Reflection of electromagnetic waves from the food, as characterized by its property and geometry.

3. Propagation of the waves inside the foods also characterized by the food properties and geometry.

2.1.2.3 Thermal effect

This effect comes from irradiated materials (energy from water, fats and sugar cause the dielectric heating). Parts of the electromagnetic spectrum with a specific frequency of 915 or 2450 MHz have the capacity to penetrate the food and create heat by friction, resulting from the oscillation of dipole molecules of water, which will try to orient and align with the field. This is the macroscopic thermal effect of increasing temperature within the material (Zhang and Datta, 2001).

Destruction of microorganisms (cells and spores) is accomplished by the heat mainly through denaturation of proteins and nucleic acids. Different kinds of food will absorb different energy and heat at different rates. Heating rates of various components are different due to electromagnetic as opposed to thermal effect. The thermal effects come from two sources: the dielectric and the heat from interaction between the components of placement and the composition of food (Datta et al., 2001).

2.1.2.4 Non-thermal effect

This is a direct effect of the radio frequency (dielectric). One of the effects of such quantum energy is breakage of chemical bonds to produce one ion pair. Electromagnetic radiation above 2500 x 10^6 MHz, which possesses such a capability, is mostly referred to as ionizing radiation (example X-rays, gamma-rays, etc.). As the wavelength increases and frequency decreases, not enough energy is available to break chemical bonds (Datta et al., 2001).

Ultraviolet, visible and possibly infrared rays have energy to break weak hydrogen bonds, but microwaves do not have sufficient energy to break any chemical bonds and therefore belong to the group of non-ionizing forms of radiation.

In this case, one more distinction is recognized. The effects are considered non-thermal if they are independent of sample temperature, and if they existed but also depended on temperature, they are considered as enhanced thermal effects.

Some scientists believe that microwave cooking may not be good for health because of the molecular damage to the food through, causing substantial damage to the surrounding molecules. Recent research shows that microwave oven cooked food

suffers severe molecular damage. When eaten, it causes abnormal changes in human blood and immune systems (Datta et al., 2001).

2.1.3 Microwave in the food industry

Microwave heating has more advantages than conventional method, includes speed of heating, energy saving, precise process control, and faster start up and shut down. Moreover, the others are higher quality product in term of taste, texture and nutritional contents. The shorter drying time in microwave process not only helped to heat product in seal package even in an insulator and to reduce bacteria counted in pasta, but also increased product quality and reduced nitrosamine formation with bacon cooking. Except thawing, tempering, reheating, drying, cooking, baking and blanching with more convenient and economical than conventional method, microwave process also can apply in sterilization and pasteurization. The food application of microwave is presented in Table 2.2.

Table 2.2 Microwave food processing applications (IFT, 1989)

Application	Frequency (MHz)	Power (kW)	Products
Tempering, batch or continuous	915	30-70	Meat, fish, poultry
Drying, vacuum, of freeze-drying	915 or 2450	30-50	Pasta, onions, snack foods, fruit juices
Precooking	915	50-240	Bacon poultry, sausages, meat patties, sardines
Pasteurization/ sterilization	2450	10-30	Fresh pasta, milk, semisolid foods, pouch- packaged foods
Baking	915	2-10	Bread, doughnut proofing

The dielectric properties are very important factor in describing the way foods are heated by microwaves. Electric field intensity or power density distribution surrounding an object (load) during microwave heating is necessary to create the model to predict heating patterns in microwave-heated foods and for computer

simulation of food processing. E-field probe based on a fiber-optic temperature sensor was developed to measures the temperature of a resistive element (Wickersheim. and Sun., 1987; Randa., 1990; Wickersheim et al., 1990). However, very few studies to measure the electric field inside a food sample heated in a microwave oven with using this probe are reported (Fu, 2004). Di-electric can be changed by the effect of composition of food (Kent, 1987).

Food shape, volume, surface area and composition are critical factors affected on microwave heating of food. Moisture and salt percentages are the most effect factors of food composition, which influent on dielectric property in microwave. The following effect are caused by the corner and edge overheating, focusing, and resonance, surface cooling, interior burning and steam distillation of volatiles. Moreover, short cooking time alter the extent of interactions (Hui and Smith, 2004).

Because of microwave energy rapidly heats food materials leading to improve nutrient retention, the convenient operation and the potential for energy saving have leaded microwave ovens to be used for thawing frozen food, reheating of re-cooked food in household. Base on the penetration, thermal inactivation and injured or sub-lethal stress organism, microwave ovens was also used in pasteurization or even sterilization of food in the shorter time than conventional heating (Vasavada, 1986). Although there are two systems of microwave heating, batch heating and continuous-flow heating, the continuous flow microwave heating of foods is an emerging technology (Fu, 2004).

Sterilization refers to the complete destruction of microbial organisms to reduce all pathogenic, toxic-producing organisms and spoilage organisms to safe levels. Microwave sterilization operates in the temperature range of 110-130°C. Pasteurization is gentle heat treatment usually at temperatures between 60-82°C. The main advantage of microwave sterilization or pasteurization is the effective reduction in the time required for the heat to penetrate to the food center. So, this function can apply in milk at precise time without denaturation (Ahmed and Ramaswamy, 2004).

Commercial systems performing microwave pasteurization and/or sterilization of foods are currently available in Europe; however, the use of microwaves in USA to produce shelf stable low acid (pH >4.6) foods requires FDA acceptance. The first industrial implementation of continuous flow microwave sterilization for low acid products has been implemented by Yamco in Snow Hill, NC. Moreover, a sweet

potato puree product sterilized using continuous flow microwave processing and aseptic packaging was recently granted by the FDA acceptance.

Although microwave processes in the food industry such as microwave pasteurization or microwave drying are already in use, most of their optimization is still based on trial-and-error. With batch and continuous operation modes, microwave sterilization system is investigated to provide fast and relatively uniform heating (Meda et al, 2007).

2.1.4 Bactericidal effect of microwave heating

Destruction of microorganism has been affected by lethal agent or process in a specific time, metabolic state, gram type, and spore formation. Microwave exposure with heat generated can inactivate bacteria. The decrease of microbial quantity depends on the power and heating time has illustrated in more study from 1960s until now. Moreover, microwave's short heating and exposure time is less destructive to food than longer conventional heating (Vasavada, 1986) and microbial destruction by microwave radiation has great potential in the pasteurization of foods (Ahmed and Ramaswamy, 2007). In recent years, more study has investigated in sterilization characteristic of microwave frequency including thermal and "athermal" effects.

When the reduction of microbial load is the time-temperature relationship, the temperature in microwave heating attains the same level with conventional heating, so the exposure time becomes the most important thing in destruction process. Landgraf (2007) reported that *Salmonella typhimurium* was destroyed approximately 47.8% using conventional microwave and 93.3% using preset control microwave from 50s to 75s in mash and baby food. The result illustrated that microwave heating was not sufficient to destroy *S. typhimurium* in order to assure food safety.

The microwaves produced by a "home-type" microwave oven (2.45 GHz) completely inactivate bacterial cultures, mycobacteria, viruses, and *Bacillus stearothermophilus* spores within 60 s to 5 min, depending on the challenge organisms (Latimer et al., 1977; Sanborn et al., 1982; Rosaspina et al., 1994).

Another study confirmed these results, but also found that higher power microwaves in the presence of water may be needed for sterilization (Najdovski et al., 1991). Complete destruction of *Mycobacterium bovis* was obtained with 4 min of microwave exposure (600W, 2450 MHz) (Rosaspina et al., 1994). The effectiveness of microwave ovens for different sterilization and disinfection purposes should be

tested and demonstrated as test conditions affecting the results (e.g., presence of water, microwave power).

In the case of *E. coli* and *B. subtilis* cell suspensions in microwave, the correlation between the increased final temperature and reduction of viable counts as well as released DNA and protein from the cell were illustrated. However, no significant reduction of cell density was observed in either cell suspension. Most of *E. coli* cells had been severe damage on the surface but no significant change observed in the *B. subtilis* cells. *E. coli* cells were also easily lysed in the presence of sodium dodecyl sulfate (SDS), yet *B. subtilis* cells were resistant to SDS (Woo et al., 2000). This illustrated that *E. coli* is more sensitive to heat and saline.

Some studies had examined the kinetics of food-borne pathogens, heating variables, such as adjustment of power levels and temperature-distribution measurements (Heddleson et al., 1994), whereas others focus on the food variables because of composition differences, dielectric properties, structure and specific heat (Jamshidi et al, 2007; Tewari and Juneja, 2007). The food composition does affect the heat tolerance, with some components providing a protective effect for bacteria (Meda et al., 2005). Unfortunately, many of the studies do not provide as specific as to the power level or means of temperature measurement. Sterilization of metal instruments can be accomplished but requires certain precautions of concern (Rohrer and Bulard, 1985). Home-type microwave ovens may not have even distribution of microwave energy over the entire dry device (there may be hot and cold spots on solid medical devices); hence there may be areas that are not sterilized or disinfected. There is suggestion for disinfect intermittent-use catheters by using of microwave ovens. Researchers found that test bacteria (e.g., *E. coli, Klebsiella pneumonia and Candida albicans*) were eliminated from red rubber catheters within 5 min. However, microwaves used for sterilization of medical devices have not been FDA cleared (Mervine, 1997). Sterilization of metal instruments can be accomplished but requires certain precautions (Rohrer and Bulard, 1985). So, researchers in the field have found inconsistent outcomes. A pasteurization process for orange juice by microwave heating was investigated at 70°C at 15 s at 600W for continuous flow. Nikdel et al. (1993) illustrated that microwave heating was sufficient to reduce ~10^8 CFU.mL^{-1} of *Lactobacillus plantarum* by 6 logs, but an increase in temperature to 80°C at the same time was necessary to reduce the population to non-detectable levels.

Tajchakavit et al. (2007) compared thermal resistance values between the bath heat treatment and continuous flow at 700W of microwave process for the inactivation of *S. cerevisiae* and *L. plantarum* in apple juice. The result showed a faster microbial destruction rate with microwave heating.

Crespo et al. (2009) examined the dynamics of thermal destruction of naturally occurring bacteria by comparing the conventional oven heating with microwave heating. At internal heating of 34, 61 and 75°C temperature (from 149°C to 232°C external heating), number of microbial load in hamburger was ~0.18, 1.48 and 3.61 $\log CFU.g^{-1}$, respectively, reflecting very little destruction of microorganisms. This might be due to ineffective temperature distribution in microwave-heated hamburger and lack of time at killing temperatures. Moreover, the direct effects may be heat sensitivity of the individual organisms and the location of the load within the oven.

In frozen ready to eat meals, pasteurization using microwave heating has extended their self life recognized for many years (Burfoot et al., 1988). Moreover, Ryynanen and Ohlsson (1996) studied the importance of chemical and physical modifications in four-component chilled ready meals during uniform microwave heating. Food component and interaction effect has a significant effect in microwave heating of multi-component food systems. In contrast, chemical modifications, such as saltiness, did not notably affect the heating uniformity.

2.2 Bacteria used as bactericidal effect indicators

Due to which kind of microorganisms that used to appear and cause foodborne disease and the condition, three strains of bacteria were chosen for this experiment; name as *Escherichia coli, Bacillus cereus, and Staphylococcus aureus*. All of these turn in representative of gram negative facultative rods, gram positive spore forming aerobes and non-spore forming cocci, respectively. These microorganisms are also affected by intrinsic and extrinsic factors. In general, logarithmic phase cells are more susceptible to adverse situations than stationary cells, gram-negative bacteria are more sensitive than gram-positive cells; and vegetative cells are more vulnerable than sporulated cells.

2.2.1 *Bacillus cereus*

Bacilus spp. is the cause of number cases of food-borne illness. This genus contains about 80 species. The specie that causes food poisoning is *B. cereus*. This pathogen can cause two types of food-borne illness as the diarrheal type and the emetic or vomiting type.

B. cereus is a gram-positive, facultative aerobic spore-former with large vegetative cells, typically 1.0 μm by 3.0-5.0 μm in chains. It grows over the temperature range from 8-55°C, optimally around 28-35°C, and does not have any marked tolerance for low pH (min. 5.0-6.0, depending on acidulant) or water activity (min ~ 0.95). *B. cereus* cells are large rods and whose spore is ellipsoidal in shape and do not cause swelling in the sporangium. As the spore former, *B. cereus* can widely distribute in the environment and also can be found in the human gut. The spores show a variable heat resistance; recorded D-values at 95°C in phosphate buffer range between around 1 min up to 36 min. Furthermore, *B. cereus* can produce heat resistant toxin. If cooked food is allowed to cool slowly, the spores can germinate. If growth occurs then the toxin can form under certain conditions. Reheating or lightly cooking the food will not destroy this toxin. A wide variety of foods including meats, milk, vegetables and fish have been associated with the diarrhea type food poisoning. The largest risk is cross-contamination, where cooked material comes into contact with raw produces or contaminated materials (cutting boards). Although this bacterium can grow and produce toxin at refrigeration temperatures, it does so much slower than that of room temperature. Precooked food should not be stored in the refrigerator for more than 2-3 days (Adams and Moss, 2008).

2.2.2 *Escherichia coli*

Escherichia, which was first isolated from children feces, is the type genus of the Enterobacteriaceae family. *E. coli* is the type species of the genus and indicator of fecal contamination. *E. coli* is a gram negative, non-spore forming rod. It may or may not be mobile. Some rods are flagellated and some are not. The organism is a facultative anaerobe and ferments simple sugars such as glucose to form lactic, acetic, and formic acids. It is a catalase-positive and oxidase-negative. Water can also be a source of these bacteria (Adams and Moss, 2008).

The optimal conditions for growth are a temperature of 98.6°F (37°C), with a range of 45 to 114°F (7.2-45.5°C). The following table (Table 2.3) indicates the generation times for *E. coli* O157:H7.

Table 2.3: Growth of *E. coli* O157: H7 in trypticase soy broth at different temperatures

Temperature (°F)	Generation Time (minutes)
36	No growth
77	87.6
86	34.8
99	30.0
104	38.0
111	65.0
113	72.6
114	No growth

Source: Adapted from Doyle and Schoeni (1984)

The optimum pH for growth is 6.0 to 8.0. However, growth can occur as low as pH 4.3 and as high as pH 9 to 10 (Banwart, 1983; Mitscherlich and Marth, 1984). *E. coli* O157:H7 can survive in ground beef at -4°F for several months without changes in numbers (Doyle and Schoeni, 1984). Dupont et al. (1971) determined on the basis of a human study that ingestion of 10^6 to 10^8 cells of some pathogenic strains of *E. coli* causes diarrheal illness in a healthy person.

Raw foods, particularly those of animal origin, are frequently contaminated with *E. coli.* Raw milk can contain *E. coli.* Carcasses are often contaminated with fecal material of infected animals or from other contaminated carcasses or equipment. Prepared foods can become contaminated with *E. coli* from equipment that has not been cleaned and sanitized after it was used to prepare raw food products and from infected food handlers (FDA, 2009).

The FDA developed destruction standards for *E. coli* O157:H7 in ground beef in 1993 based on the data of Line et al. (1991). The D-values were used to extrapolate mathematically to the destruction values at 140°F, 145°F, 150°F, and 155°F. The z-value was approximately 8.3°F.

Using these data from these sources, the times needed to destroy 99.999% (5 decimal reductions or 5 D-value) of *E. coli* O157:H7 in ground beef at 140°F, 145°F, 150°F and 155°F are 8.7 min, 2.7 min, 52 s and 15 s, respectively (Line et al., 1991).

2.2.3 *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive coccus forming spherical with the size about 1 mm in diameter. It appears in pairs, short chains, or bunched, grape-like clusters. Some strains are capable of producing a highly heat-stable protein toxin that causes illness in humans (FDA, 2009).

Staphylococci are catalase-positive, oxidase-negative, facultative anaerobes. Their ability to ferment glucose can be used to distinguish them from the strictly respiratory genus Micrococcus, although there are species in both genera where this distinction is not clear cut due to low acid production by some staphylococci. Animals and poultry also carry this bacterium on their bodies and all raw meat and poultry products should be handled as though they are contaminated. Raw milk can also be a source of this bacterium. *S. aureus* appears on the skin and in the nasopharnyx area of humans and animals. Often, *S. aureus* will get into food from food handlers, from animal skin, or from dirty food preparation surfaces. *S. aureus* cannot grow at refrigeration temperatures and is a relatively poor competitor with other food microflora. *S. aureus* growth usually occurs during temperature abuse. Temperature abuse occurs when food is kept in the temperature danger zone of 4.4 to 60°C (40 to 140°F) for prolonged periods of time (Adams and Moss, 2008).

The optimal temperature for *S. aureus* growth is between 18 to 40°C (64 to 104°F). According to poor growth of *S. aureus* in the presence of other food microflora, these organisms tend to grow better in cooked or processed foods. In addition, *S. aureus* can grow at lower a_w compared to most other bacteria (as low as a_w 0.85). Therefore, *S. aureus* may grow under a reduced a_w or on high salt foods that will inhibit the growth of most pathogens as long as the temperature permits growth. Metabolically, *S. aureus* can utilize mannitol, which is not seen with other staphylococcal species, such as *S. epidermidis* (FDA, 2009).

The production of the enzyme coagulase by a majority of *S. aureus* strains can also be used to differentiate this species from other staphylococci. Some strains of *S. aureus* have the ability to produce staphylococcal enterotoxins (SEs) while growing in foods. The main symptoms of SE intoxication are vomiting and diarrhea (without

fever) 4h to 12h after consumption. Foods commonly associated with staphylococcal food poisoning are deli meats (especially ham), deli salads (ham, chicken, potato) and cream puffs. Note that these foods all undergo preparation steps involving human handling after processing. In addition, these products may potentially undergo temperature abuse, either before or after sale to the consumer (FDA, 2009).

Large numbers of enterotoxigenic *S. aureus* (greater than 10^6 CFU.g^{-1}) are needed to produce enough staphylococcal enterotoxin to cause this type of food intoxication. Although it is desirable to have no *S. aureus* in food products, small numbers of *S. aureus* (less than 10^3 CFU.g^{-1}) are often present. Due to large numbers are required to produce detectable quantities of staphylococcal enterotoxin. *S. aureus* is often enumerated in food products as an indication of temperature abuse and the potential for *S. aureus* to cause foodborne disease (Adams and Moss, 2008).

Chapter 3

Effect of pH on Bactericidal Efficiency of Microwave Heating

3.1 Introduction

Microorganisms require water, nutrients, appropriate temperature and pH level for growth. As the same way pH affects all living thing, pH also has the efficiency effect on the growth of bacteria. Prabhat (2003) has showed the dielectric properties in continuous flow microwave heating systems give the better affect on the low-acid multiphase food product than the conventional heating. Thibault (2011) also illustrated when treating diced Roma tomatoes in a continuous flow microwave system, changing pH (3.9 and 4.3) combine with salt has been assessed the sterility by microbial assay. Generally, pH of food affects the time and temperature required for sterilization and pasteurization (Rahman, 2007).

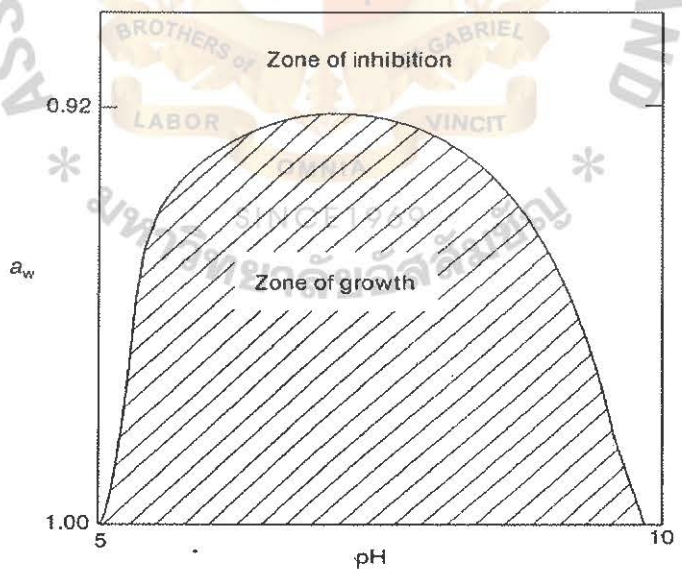

Figure 3.1 Effects of pH and water activity on growth of bacteria (Rahman, 2007)

Bacteria prefer a certain pH balance like all living organisms. Extreme changes in the pH balance of the local environment for bacteria tend to kill them. Moreover, effect on bacterial growth depends on the bacterium which is an

acidophile, neutralophile or alkaliphile. The optimum cytoplasmic pH in the range 4.5–6.0 is acidophiles, neutrophiles is 7.5–8.0, and alkalophiles is 8.4–4.9. Minimum pH level depends on the types of food. Below pH 4.2, most other food poisoning microorganisms are well controlled (Rahman, 2007). However, bacteria are hardly to grow in highly acid or too much alkaline (Figure 3.1) (Adams and Moss, 2008). To mention the range in which bacteria could not be damaged before treatment time, the number of extreme pH was adjusted in this experiment at pH 4.3 and pH 8.5.

3.2 Materials and methods

3.2.1 Preparation of bacterial cultures

A slant of each culture, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*, was transferred into 100 mL nutrient broth (NB) and then incubated at 37°C for 24 h to obtain the number of microorganisms as high as 10^8 CFU mL^{-1}.

The extent of the effect of pH on microorganism in food may be modified by the other component such as the synergist additive, strain, a_w, treating temperature. For the destruction of spoilage microorganisms to ensure food safety, pH 4.4 is the maximum desirable for safety.

3.2.2 Microwave heating

After incubation, pH of the cell suspensions was adjusted to 4.3 and 8.5 using 0.1 M HCl and 0.1 M NaOH, respectively, whereas the non-pH adjusted culture flask were used as the control (pH 7.0). The cell suspensions were heated by using microwave Midea brand; model MM820AAU (with 1250 W rated input power and 800W rated output power) at 50, 75 and 100% microwave output power for 1, 2, 3 and 5 min. The flasks were then cooled down immediately for further analysis.

3.2.3 Microbiological analysis

The cell suspensions after cooling were 10-fold serial diluted by using 0.1% peptone solution, which was sterile at 121°C for 15 min. The numbers of survival bacteria were determined by using spread plate technique and nutrient agar (NA). The petri dishes were incubated at 37°C for 24 h and the numbers of survival cells were reported as log CFU mL^{-1}.

3.2.4 Statistical analysis

A randomized block and 3x4x3 factorial designs with 3 replications were used in this experiment. The mean differences were analyzed by using Duncan's multiple range tests.

3.3 Results and discussions

After treatment at 50, 75 and 100% power of microwave, the survival colonies of *E. coli*, *B. cereus*, and *S. aureus* were counted. The affects of microwave power combine with pH and exposure time of *E. coli*, *B. cereus* and *S. aureus* has showed in Tables 3.1, 3.2 and 3.3, respectively.

Effect of pH, microwave power and exposure time on E. coli

The effect of pH, microwave power and exposure time on the survival of *E. coli* is shown in Table 3.1.

It was recognized the destruction of this microorganism by microwave heating was pH independent, while the microwave power and exposure time had significantly affected ($p<0.05$) on the survival of this organism with the interaction of each other. As 25% power increased, the number of cells reduced by approximately 50%.

E. coli appeared to be more tolerant to high pH rather than low pH. The bactericidal effect was reduced when the pH 8.5 and 50% power were used. This might be caused by alkaline adaptation of *E. coli* (Erdogrul and Erbilir, 2005).

Moreover, the prolong time under the effects of heat and dielectric of microwave make *E. coli* cannot stable. At 5 min with all powers of microwave exposure, the survival of *E. coli* was not found and at every treatment time with full power of treatment process (100%) also gave the same result. It was recognized that the changes of pH did not significantly affect ($p>0.05$) to the survival of this organism during microwave heating. This microorganism can grow in the wide range of pH as pH 2.71 to 8.45 (Presser et al., 1997). In the absence of lactic acid, E. coli grew at pH 4.0 but not at pH 3.7. *E. coli* was significantly ($p<0.05$) sensitive to the heat intensity generated by microwave in term of time and microwave power.

Table 3.1 Number of survival cells of *E. coli* (logCFU.mL^{-1}) after exposure to microwave radiation at different powers, times and pH

pH	Power (%)	Treatment Time (min)				D-Value (min)
		1	2	3	5	
4.3	50	7.3 ± 0.1	4.3 ± 0.1	< 1	< 1	0.35[b]
	75	5.9 ± 0.2	2.9 ± 0.2	< 1	< 1	0.30[b]
	100	< 1	< 1	< 1	< 1	NA**
7.0	50	7.2 ± 0.1	4.8 ± 0.2	< 1	< 1	0.45[a]*
	75	4.6 ± 0.3	2.4 ± 0.2	< 1	< 1	0.38[b]
	100	< 1	< 1	< 1	< 1	NA
8.5	50	7.3 ± 0.1	4.0 ± 0.1	3.0 ± 0.0	< 1	0.48[a]
	75	5.3 ± 0.0	3.0 ± 0.1	< 1	< 1	0.45[a]
	100	< 1	< 1	< 1	< 1	NA

* The same letters mean there were no significant different ($p>0.05$).

** NA is not applicable.

The interaction between treatment time and microwave power was also significantly observed. Similar to the other heat treatment, higher power of microwave required shorter time of exposure. The result of this studied showed that the number of viable cells decreased according to the exposure time and the power used, which was parallel to the studied of Fujikawa et al. (1992). The result also found no remarkable difference between the destruction profiles for microwave exposure and conventional heating. This may be due to the enhancement of surface temperature during microwave heating, which can be higher than 70°C when the full power of domestic microwave was used (Jamshidi et al., 2010).

Similarly, Park et al. (2006) also found a 2-minute exposure to microwave radiation resulted in only 30-40% inactivation of *E. coli*. No survival organisms were detected after a 4-minute exposure.

Effect of pH, microwave power and exposure time on B. cereus

The *B. cereus* was treated with different microwave powers and exposure times at different pH. It was noticed that there were interaction among these three

factors. This also showed that the most effect factors on *B. cereus* were treatment time and microwave power, including their interaction. Table 3.2 showed that although there was no significant different ($p>0.05$) between too high or too low pH values used, lowering of pH increased the bactericidal effect of microwave heating when at least 75% power was used. *B. cereus* is a food-borne pathogen that pH plays a fundamental role in controlling the growth. The combination of acid (pH 4.3) and microwave heating (75% and 100% power) destroyed *B. cereus* within less than 2 min. Moreover, increasing in microwave power and exposure time reduced the number of survival cells. Barkhudarov et al. (2007) also demonstrated the bacterial destruction of this organism using microwave.

Table 3.2 Number of survival cells of *B. cereus* (logcfu.mL^{-1}) after exposure to microwave radiation at different powers, times and pH

pH	Power (%)	Treatment Time-B. cereus (Mean No. of log CFU/ml)				D-Value
		1	2	3	5	
4.3	50	5.4± 0.2	3.2± 0.2	1.7± 0.1	< 1	0.52[b]
	75	4.2± 0.1	< 1	< 1	< 1	0.20[c]
	100	0.6± 0.0	< 1	< 1	< 1	NA**
7.0	50	6.2± 0.2	4.4± 0.1	3.6± 0.1	< 1	0.80[a*]
	75	4.7± 0.5	2.4± 0.2	< 1	< 1	0.70[a]
	100	0.9± 0.1	< 1	< 1	< 1	0.40[b]
8.5	50	6.7± 0.5	4.5± 0.2	3.4± 0.2	< 1	0.70[a]
	75	4.8± 0.2	2.7± 0.1	< 1	< 1	0.45[b]
	100	3.7± 0.2	0.4± 0.0	< 1	< 1	0.27[c]

* The same letters mean there were no significant different ($p>0.05$).

** NA is not applicable.

Conversely, Aksen et al. (2004) reported no destruction of this organism after microwave exposure for 5 min but it was completely destroyed when 10 min was applied. Reagen et al. (1982) also found that *B. cereus* was the most tolerant microorganisms of microwave radiation. Inactivation of this organism was only 4.6%

and 3.4% after 7-min and 12-min exposure, respectively. The surface temperature ranges after 7 min and 12 min were 86.4-105.2°C and 107.2-123.3°C, respectively. Park et al. (2006) and Wu (1996) also demonstrated that inactivation of *B. cereus* was observed when 20 min exposure was used.

Effect of pH, microwave power and exposure time on S. aureus

Effect of pH, microwave power and exposure time on the inactivation of *S. aureus* was found to have interaction to each factor and each factor had significantly ($p<0.05$) influence on the destruction by microwave heating (Table 3.3).

Table 3.3 Number of survival cells of *S. cereus* (log cfu mL^{-1}) after exposure to microwave radiation at different powers, times and pH

pH	Power (%)	Treatment Time- *S. aureus* (Mean No. of log CFU/ml)				D-Value
		1	2	3	5	
4.3	50	7.0 ± 0.5	4.7 ± 0.1	< 1	< 1	0.45[b]
	75	4.6 ± 0.5	2.4 ± 0.1	< 1	< 1	0.40[b]
	100	1.8 ± 0.3	< 1	< 1	< 1	0.23[c]
7.0	50	5.9 ± 0.2	4.8 ± 0.2	3.0 ± 0.2	0.4 ± 0.1	0.65[a]*
	75	2.9 ± 1.0	1.0 ± 0	< 1	< 1	0.45[b]
	100	< 1	< 1	< 1	< 1	NA**
8.5	50	7.4 ± 0.2	3.7 ± 0.0	< 1	< 1	0.45[b]
	75	3.7 ± 0.1	< 1	< 1	< 1	0.30[b]
	100	2.7 ± 0.2	< 1	< 1	< 1	0.26[c]

* The same letters mean there were no significant different ($p>0.05$)

** NA is not applicable.

It was also recognized that at pH 7, 50% microwave power and 5-min exposure there was no destruction of this microorganism, while pH either increase or decrease provided higher efficiency of destruction when 50% power was used. On the other hand, at 75% power more destruction was observed only at increased pH. At pH 7 and 100% power, this organism was completely destroyed within 1 min, which was

similar to the studies of Aksen et al. (2004) and Tanaka et al. (1998), who reported that *S. aureus* was completely inactivated within 1 min when home microwave was used. Yeo et al. (2003) also found that cell viability of *S. aureus* was reduced as the exposure time increased, with complete bacterial inactivation at 110s. This might be caused that microwave-stressed cells of *S. aureus* had shown greater metabolic imbalance than conventionally heated cells (Barnabas et al., 2010). Conversely, Ronald et al. (1996) reported that the pH of food did not affect survival of thermally stressed *S. aureus*.

Dreyfuss and Chipley (1980) attempted to characterize some of the effects of sublethal microwave irradiation in cells of *S. aureus*. Upon exposure to microwave irradiation the activities of various metabolic enzymes such as Glucose-6-phosphate dehydrogenase, membrane ATPases, alkaline phosphatase, malate dehydrogenase, lactate dehydrogenase, cytochrome oxidase adenosine triphosphatase, were affected; but the irradiation affected *S. aureus* in a manner quite different from conventionally heated cells. This thus indicates the athermal behavior of microwave irradiation.

On the other hand, Silva et al. (2006) reported the microwave resistance of *S. aureus* when it exposed to 6-min of microwave heating.

3.4 Conclusion

Efficiency of microwave heating for microbial destruction were influenced by pH of food, microwave power and exposure time. Either high or low pH increased the destruction efficiency of microwave heating when low power was used. At full power of microwave, the microorganisms were totally destroyed, especially at longer exposure time. Among these three tested microorganisms, *B. cereus* showed the highest resistance to the microwave heating, with the D-value of 0.8, 0.7 and 0.4 min for 50, 75 and 100% microwave power operating at pH 7, respectively.

Chapter 4

Effect of Salt on Bactericidal Efficiency of Microwave

4.1 Introduction

As a natural way to control the presence of microorganisms in food industry, salt was used to prevent the microbial growth because of water loss from the cells by osmosis action. So, high salt concentration can inhibit bacterial food spoilage, except some facultative halophiles which can grow in high concentration of salts. Two percent of salt is a level that would inhibit the growth of other bacteria. For bacteria with salt tolerance, growth and salt concentration have a direct correlation. As the amount of salt in the growth medium increases, bacterial growth decreases. On the other hand, a bell curve of growth was observed for bacteria that required salt (Ray, 2004).

Salts are often used to adjust water activity of the solution in which microorganism live. Reducing water activity is one way to reduce the growth rate of pathogen. The following table indicates to effect of NaCl on a reduction of water activity (Table 4.1).

Table 4.1 The correlation between NaCl and water activity

NaCl (g)	Water (g)	% NaCl	A_w
0.9	99.1	0.9	0.995
1.7	98.3	1.7	0.990
3.5	96.5	3.5	0.980
7.0	93.0	7.0	0.960
10.0	90.0	10.0	0.940
13.0	87.0	13.0	0.920
16.0	84.0	16.0	0.900
22.0	78.0	22.0	0.860

Source: USFDA- The Bad Bug Book, 2008

Most foods have a water activity above 0.95 and that will provide sufficient moisture to support the growth of bacteria, yeasts, and mold. The amount of available moisture can be reduced to a point which will inhibit the growth of the organisms (Adam and Moss, 2008).

When food is heated by microwave, food absorbs energy and converts it to heat. The higher the water content of a food, the faster it will heat. Solute like salt does not influence only the rate of heating by microwave, but also penetration depth. Diluted salt water significantly more affected than pure water because of the field-induced motion of salt ions especially that of large-size ions, by the microwave electric field and energy transfer to water molecules by collisions.

4.2 Materials and methods

4.2.1 Preparation of cultures

A slant of each culture, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*, was transferred into 100 mL nutrient broth (NB) and then incubated at 37°C for 24 h to obtain the number of microorganisms as high as 10^8 CFU.mL^{-1}.

4.2.2 Microwave heating

After incubation, the cell suspensions were added separately into 1%, 2% and 3% of salt solution. The cell suspensions were then heated by using microwave Midea brand, model MM820AAU (with 1250 W rated input power and 800W rated output power) at 50, 75 and 100% microwave output power for 1, 2, 3 and 5 min. The flasks were then cooled down immediately for further analysis.

4.2.3 Microbiological analysis

The cell suspensions after cooling were 10-fold serial diluted by using 0.1% peptone solution, which was sterile at 121°C for 15 min. The numbers of survival bacteria were determined by using spread plate technique and nutrient agar (NA). The Petri dishes were incubated at 37°C for 24 h and the numbers of cells were reported as log CFU.mL^{-1}.

4.2.4 Statistical analysis

A randomized block and 3x4x3 factorial designs with 3 replications were used in this experiment. The mean differences were analyzed by using Duncan's multiple range tests.

4.3 Results and discussions

The effect of microwave power combined with salt concentration on the survival of *B. cereus* and *S. aureus* has showed in Tables 4.1 and 4.2, respectively.

Effect of salt concentration, microwave power and exposure time on E. coli

The combination of salt concentration, microwave power and exposure time on the survival of *E. coli* demonstrated greatly destruction of *E. coli*, even at 50% power for 1 min. All *E. coli* were completely destroyed in all treatments, indicating highly sensitivity to heat and salt of this microorganism.

E. coli has optimal growth in the absence of salt, but in the presence of salt it will grow with an attenuated rate (Ray, 2004). This research result was in contrast with that of Fujikawa et al. (1992). They reported that the higher the salt concentration was, the fewer the cells that were destroyed by microwave irradiation. On the other hand, Hayashi et al. (1991) illustrated that the more concentration of sodium chloride and core temperature of mashed potato increased, the more microorganism in mash decreased.

Effect of salt concentration, microwave power and exposure time on B. cereus

In this experiment, it was observed that salt concentration, treatment time and microwave power either alone or combination had significantly ($p<0.05$) influence on the survival of this microorganism during microwave heating (Table 4.1). As the concentration of salt increased, the destruction of this organism increased.

B. cereus has salt tolerance ability. This result in Table 4.1 has showed an attenuated rate depending on the increasing power and treating time. This microorganism was completely destroyed when it was exposed to the microwave heating for 5 min for all salt concentrations and microwave power. Conversely,

Hayashi et al. (1991) showed that *B. cereus* could be completely destroyed by 2 min of microwave heating in mashed potato with no added salt.

Table 4.1 Number of survival cells of *B. cereus* (log cfu mL^{-1}) after exposure to microwave radiation at different powers, times and salt concentration

Salt concentration (%)	Power (%)	Treatment Time- *B. cereus* (Mean No. of log CFU/ml)				D-Value
		1	2	3	5	
1	50	6.1± 0.0	4.6± 0.3	2.9± 0.2	< 1	0.70^{a*}
	75	3.9± 1.0	2.7± 0.2	1.2± 0.2	< 1	0.60^{ab}
	100	2.8± 0.1	0.4± 0.0	< 1	< 1	0.42^{b}
2	50	5.8± 0.2	3.5± 0.2	2.2± 0.1	< 1	0.60^{ab}
	75	3.8± 0.1	1.7± 0.1	< 1	< 1	0.50^{ab}
	100	2.4± 0.1	0.2± 0.0	< 1	< 1	0.35^{b}
3	50	5.6± 0.3	3.2± 0.1	1.7± 0.1	< 1	0.42^{b}
	75	3.6± 0.2	0.6± 0.0	0.5± 0.0	< 1	0.33^{b}
	100	1.5± 0.1	< 1	< 1	< 1	0.15^{c}

* The same letters mean there were no significant different ($p>0.05$)

Wang et al. (2003) also illustrated that the spore of *B. cereus* is the common heat- resistant depending on types and containers. They showed the different microwave tolerance ($p<0.05$) even at a power level of 100%. In contrast, Aksen et al. (2004) illustrated that *B. cereus* has been destroyed at 10 min and the appropriated physical or chemical disinfectant agent is very important parameter in term of sterilization.

Effect of salt concentration, microwave power and exposure time on S. aureus

It was noticed that salt was not the significant effect factor in case of *S. aureus* because *S. aureus* is the pathogen most likely to grow on products with reduced a_w. The significant effects of microwave exposure and treatment time including their combination were also found.

It was recognized that at high microwave power and high exposure time (5 min) for all concentrations of salt, this microorganism was completely destroyed. For 50% and 75% microwave power, as the concentration of salt increased, the D-value was significantly ($p>0.05$) decreased (Table 4.2).

Table 4.2 Number of survival cells of *S. aureus* (log cfu mL^{-1}) after exposure to microwave radiation at different powers, times and salt concentration

Salt concentration (%)	Power (%)	Treatment Time- *S. aureus* (Mean No. of log CFU/ml)				D-Value
		1	2	3	5	
1	50	6.2 ± 0.3	3.4 ± 0.2	1.0 ± 0.0	<1	0.32[a*]
	75	3.1 ± 0.1	1.0 ± 0.0	<1	<1	0.27[ab]
	100	<1	<1	<1	<1	NA**
2	50	6.6 ± 0.2	3.3 ± 0.2	<1	<1	0.27[ab]
	75	3.6 ± 0.1	<1	<1	<1	0.25[ab]
	100	<1	<1	<1	<1	NA
3	50	6.1 ± 0.2	2.5 ± 0.0	<1	<1	0.20[ab]
	75	2.6 ± 0.1	0.7 ± 0.0	<1	<1	0.18[ab]
	100	<1	<1	<1	<1	NA

* The same letters mean there were no significant different ($p>0.05$)

** NA is not applicable.

Parfentjev et al. (1964) assessed the tolerance of *S. aureus* to high concentration of sodium chloride in liquid medium. On the other hand, *S. aureus* has a high surface hydrophobicity characteristic (Jonsson and Wadstrom, 1984). That causes the auto-aggregation in saline. The coagulation might protect *S. aureus* in salt solution. Hayashi et al. (1991) indicated that *S. aureus* is more salt resistant at 2 min than *E .coli* and *B. cereus*. Nevertheless, Heddleson et al. (1996) examined the survival of *S. aureus* cells in a 700W microwave oven with the food component and concluded that sodium content was the primary influent of the uniformity of temperature achieved within foods and in turn on the survival of bacteria. This supported the result in this experiment that there was no survival of this organism detected when 75% power- 3 minute exposure and 100% power- 1 minute exposure

were used. Rate of destruction of microorganism decreased when microwave power and exposure time were increased. Zhen et al. (2007) also reported the higher destruction of *S. aureus* when the salt concentration increased, which was similar to the results obtained from Watanebe et al. (2000). Moreover, Morozov and Petin (1998) found that hypertonic solutions (1.0%) of sodium chloride were less effective in protecting cells against heat damage during microwave heating than during thermal heating. Recently, Arizina et al. (2012) reported that *S. aureus* was completely destroyed after 2 min of microwave heating in pizza.

4.4 Conclusion

The presence of salt had influence on the survival of microorganisms when they were exposed to the microwave heating at different heating powers and times. Increasing of salt concentration leaded to an increased destruction effects. *E. coli* was the most sensitive to the microwave heating in the presence of salt, while the spore forming bacteria like *B. cereus* was resist to the microwave heating. Longer heating time or increasing in microwave power was required for complete destruction of *B. cereus* in the presence of salt.

Chapter 5

Effect of fat on the bactericidal efficiency of microwave

5.1 Introduction

For composite or heterogeneous foods, microwave processing becomes more difficult because of the different loss characteristics, specific heats and thermal conductivities. Different compositions affected heat absorption and heat generation at different rates. With essentially different electrical properties, the composition of food can cause overheating in one area or underheating in another (Datta, 2001).

The full fat milk and skim milk were used in this experiment for the study the effect of fat on the bactericidal effect of microwave heating.

Milk composition varies depending on the species (cow, goat, and sheep), breed (Holstein, Jersey), the animal's feed, and the stage of lactation. In general, the composition of cow's milk is water, lactose (carbohydrate), fat, protein, and minerals (referred to as ash) as well as vitamin C. Cow's milk has a pH ranging from 6.4 to 6.8, making it slightly acidic (Nollet and Toldrá, 2010).

5.2 Materials and methods

5.2.1 Preparation of cultures

A slant of each culture, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*, was transferred into 100 mL nutrient broth (NB) and then incubated at 37°C for 25h to obtain the number of microorganisms as high as 10^8 $CFUmL^{-1}$.

5.2.2 Microwave heating

Full fat milk and skim milk were used in this research for the food model of high and low fat content foods. Milk (50 mL) was mixed with the culture of each bacterium (50 mL). The mixtures were then heated in the microwave oven Midea brand; model MM820AAU (with 1250 W rated input power and 800W rated output power) at 50, 75 and 100% microwave power for 1, 2, 3 and 5 min. The flasks were then cooled down immediately for further analysis.

5.2.3 Microbiological analysis

The cell suspensions after cooling were 10-fold serial diluted by using 0.1% peptone solution, which was sterile at 121°C for 15 min. The numbers of survival bacteria were determined by using spread plate technique and nutrient agar (NA). The Petri dishes were incubated at 37°C for 24 h and the number of cells was reported as log CFU mL^{-1}.

5.2.4 Statistical analysis

A randomized block and 2x4x3 factorial designs with 3 replications were used in this experiment. The mean differences were analyzed by using Duncan's multiple range tests.

5.3 Results and discussions

After treatment at 50, 75 and 100% power of microwave, the survival cells of *E. coli*, *B. cereus* and *S. aureus* were counted and showed in Tables 5.1, 5.2 and 5.3, respectively.

Effect of fat, microwave power and exposure time on E.coli

The effect of fat, microwave power and exposure time on the survival of *E. coli* is shown in Table 5.1.

It was recognized that the destruction of this microorganism by microwave heating, time and fat had significantly affected ($p<0.05$) on the survival of this organism either alone or combination of each other. The log reduction of this organism in full fat milk was lower than that of skim milk. For example, at 100% power, the numbers of *E. coli* were below the detectable level for all heating time in skim milk, while some *E. coli* survived after 1-2 min of heating in full fat milk, indicating the protective effect of fat. Similar to the previous result, as time and power increased, the destruction of this microorganism increased.

The present of fat in milk plays as an important hurdle in cell inactivation with microwave. It might probably that fat is a dielectric component of a food system, so it can reflect microwaves from reaching the center of the food (Schiffman, 1993; Heddleson and Doores, 1994). Moreover, the presence of fat in food reduced the

dielectric loss factor and penetration depth of microwave heating, resulting in high number of survival cells (Gunasekaran et al., 2002, Hix, 2000).

Table 5.1 Number of survival cells of *E. coli* (logcfu mL^{-1}) after exposure to microwave radiation at different powers and times in full fat milk and skim milk

Types	Power (%)	Treatment Time- *E. Coli* (Mean No. of log CFU/ml)				D-Value
		1	2	3	5	
Full Fat Milk	50	6.9 ± 0.5	5.4 ± 0.0	2.9 ± 0.2	0.8 ± 0.1	0.65[a*]
	75	4.2 ± 0.1	2.6 ± 0.2	0.4 ± 0.1	0.3 ± 0.0	0.60[a]
	100	2.6 ± 0.2	0.7 ± 0.1	<1	<1	0.55[b]
Skim Milk	50	5.5 ± 0.5	3.0 ± 0.2	1.0 ± 0.0	<1	0.51[b]
	75	3.9 ± 0.3	1.3 ± 0.2	<1	<1	0.42[c]
	100	<1	<1	<1	<1	NA[**]

* The same letters mean there were no significant different ($p>0.05$).

** NA is not applicable.

In addition, Fung and Cunningham (2009) illustrated that microwave heating of food is more "food dependent" than conventional heating and may not destroy high levels of bacteria. It was concluded that the microorganism in food which presented fat has more difficult to destroy than that in the food without fat.

Woo et al. (1999) observed protein and DNA released when final temperature of the cell suspensions increased and the viable counts decreased under the microwave-heated cell. Most *E. coli* cells have been revealed severe damage on the surface by scanning electron microscopy.

Effect of food composition, microwave power and exposure time on B. cereus

There were the significant effects of each factor on the destruction of *B. cereus*, including their interaction of each factor. It was remarkably recognized that the presence of fat in heating provided the protective effect to the cells. In full fat milk, at 100% power for 5 min, the survival cells were detected, simultaneously, none of the survival cells was observed in skim milk. The destruction of this microorganism was similar to the result obtained from Woo et al. (1999), who stated

that microwave heating gave a dramatic reduction of the viable counts of *B. cereus* but less injured *B. cereus*'s cell wall structure. Microwave radiation was found to lead to the release of DNA and proteins but not to cause cell lysis (Woo et al., 2000). Aksen et al. (2004) and Reagan et al. (1982) also reported the high resistance of this microorganism to 5 min of microwave heating. Although longer time of microwave exposure provided higher temperature, the temperature was not enough to destroy this bacterium (approximately 95°C at 3 min of microwave heating) because of its heat resistance property (Arifa et al., 2009).

Table 5.2 Number of survival cells of *B. cereus* (log cfu mL^{-1}) after exposure to microwave radiation at different powers and times in full fat milk and skim milk

Types	Power (%)	Treatment Time- *B. cereus* (Mean No. of log CFU/ml)				D-Value
		1	2	3	5	
Full Fat Milk	50	6.4±0.2	4.8±0.4	3.2±0.2	1.9±0.2	0.75^{a*}
	75	4.7±0.4	2.4±0.1	1.9±0.1	1.2±0.0	0.70^{a*}
	100	3.2±0.6	1.4±0.2	1.3±0.1	0.9±0.0	0.50^{b*}
Skim Milk	50	5.5±0.2	3.7±0.1	< 1	< 1	0.55^{b}
	75	3.7±0.4	1.4±0.0	< 1	< 1	0.40^{c}
	100	1.1±0.2	< 1	< 1	< 1	0.14^{d}

* The same letters mean there were no significant different ($p>0.05$)

Effect of fat, microwave power and exposure time on S. aureus

S. aureus was significant affected by treatment time, the combination of power and treatment time also gave the same result (Table 5.3). An increasing of either time or microwave power can increase the destruction efficiency to this bacterium. In particular, microwave-stressed cells of *S. aureus* exhibited a greater metabolic imbalance than conventionally heated cells (Kozempel et al., 1998). Khalil and Villota (1975) also concluded that thermal effect was the major part of injured *S. aureus* in microwave heating.

Similar to other microorganisms, the presence of fat in the heating medium provided the protective effect to the microorganisms, leading to higher number of

survival after microwave heating at the same microwave exposure time and power. This phenomenon was similar to that of conventional heating that fat acts as insulator to delay the heat penetration to destroy bacteria. Therefore, D-values of this bacterium in full fat milk were higher than those of skim milk.

Table 5.3 Number of survival cells of *S. cereus* (log cfu mL^{-1}) after exposure to microwave radiation at different powers and times in full fat milk and skim milk

Types	Power (%)	Treatment Time- *S. aureaus* (Mean No. of log CFU/ml)				D-Value
		1	2	3	5	
Full Fat Milk	50	5.9 ± 0.7	3.8 ± 0.6	2.3 ± 2.2	< 1	1.15[a]
	75	4.4 ± 0.2	3.5 ± 0.4	1.1 ± 1.0	< 1	0.70[b]
	100	2.8 ± 0.3	< 1	< 1	< 1	0.35[c]
Skim Milk	50	5.4 ± 0.7	3.2 ± 1.3	0.7 ± 0.6	< 1	0.63[b]
	75	3.1 ± 0.1	1.0 ± 0.0	< 1	< 1	0.42[c]
	100	0.7 ± 1.1	< 1	< 1	< 1	0.09[d]

* The same letters mean there were no significant different ($p>0.05$)

Yeo et al. (1999) also reported that cell viability of *S. aureus* was reduced as the exposure time increased, with complete bacterial inactivation at 110s, attaining a temperature of 61.4°C.

Moreover, microwave energy is a non ionizing form of radiation and it has been tested to study the effectiveness in destroying bacteria and extending the shelf life of meat products without affecting the quality, taste or reducing the weight of the product (Aziz et al., 2002; Heddleson and Doores, 1994; Kozempel, 2000). Vitamins A, C, E, and B1 (thiamine) tend to be susceptible to irradiation (Handan-Dincer and Baysal, 2004; Roberts, 1998). So, the food composition may lead different temperature in different position of food by non-uniform microwave heating. Shorter microwave heating time is better in control microbial destruction and reduces damage nutrient in food composition. For microwave effect of microbial destruction in food composition, it necessitates combining the highest power with the shortest exposure time. However, the additional factors which affect heat transfer need to be considered

in microwave destruction process. Following that, the holding time at the end the process may be achieved the equilibration temperature in the food.

5.4 Conclusion

The presence of fat in food system provided the protective effect to the microorganisms subjected to microwave heating. Especially, spore forming bacteria like *B. cereus* that required longer time of microwave heating to destroy this organism due to its higher heat-resistant ability than the others.

Chapter 6

Conclusion and Recommendation

6.1 General conclusion

Efficiency of microwave heating for microbial destruction were influenced by food composition (as pH, salt and fat contents), microwave power and exposure time. For pH, either high or low pH, which was out of the optimal range for growth, increased the destruction efficiency of microwave heating when low power was used. At full power of microwave, the microorganisms were totally destroyed, especially at longer exposure time. Among these three tested microorganisms, *B. cereus* showed the highest resistant to the microwave heating, with the D-value of 0.8, 0.7 and 0.4 min for 50, 75 and 100% microwave power operating at pH 7, respectively. The presence of salt had influence on the survival of microorganisms when they were exposed to the microwave heating at different heating powers and times. Increasing to salt concentration leaded to an increased destruction effects. *E. coli* was the most sensitive to the microwave heating in the presence of salt, while the spore forming bacteria like *B. cereus* was resist to the microwave heating. Longer heating time or increasing in microwave power was required for complete destruction of *B. cereus* in the presence of salt. The presence of fat in food system provided the protective effect to the microorganisms subjected to microwave heating, especially spore forming bacteria like *B. cereus* that required longer time of microwave heating to destroy this organism.

6.2 Recommendations

6.2.1 The effect of pH, salt and fat on the survival of pathogen should be investigated for better understanding of microwave heating efficiency.

6.2.2 The effect of pH and salt in the different food models should be investigated.

6.2.3 The changes in food temperature during microwave heating should be investigated to determine the relation between food and raised temperature.

6.2.4 The suspension studies were an indication of the disinfectant efficacy on a fluid. Assess the destructive effect of three common pathogenic species of bacteria by microwave exposure, the expected effectiveness from the studied showed the tested agents can be recommended for microbial disinfection and need to consider when applied to the real process.

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Appendix

Appendix Table 1: *Escherichia coli*_ pH effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.846E15	37	7.692E13	72.256	.000
Intercept	2.730E14	1	2.730E14	256.412	.000
a	2.888E12	2	1.444E12	1.356	.264
b	8.148E14	3	2.716E14	255.127	.000
c	4.989E14	2	2.494E14	234.312	.000
a * b	8.993E12	6	1.499E12	1.408	.224
b * c	1.489E15	6	2.482E14	233.130	.000
a * c	6.826E12	4	1.706E12	1.603	.183
a * b * c	2.117E13	12	1.764E12	1.657	.096
Rep	3.439E12	2	1.720E12	1.615	.206
Error	7.452E13	70	1.065E12		
Total	3.194E15	108			
Corrected Total	2.921E15	107			

a: pH , b: Treatment time, c: Microwave output power

Appendix Table 2: *Bacillus cereus*_ pH effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.750E14	37	4.730E12	2.715	.000
Intercept	7.744E12	1	7.744E12	4.446	.039
a	7.906E12	2	3.953E12	2.269	.111
b	2.252E13	3	7.507E12	4.310	.008
c	1.461E13	2	7.303E12	4.193	.019
a * b	2.322E13	6	3.870E12	2.222	.051
b * c	4.250E13	6	7.083E12	4.066	.001
a * c	1.546E13	4	3.865E12	2.219	.076
a * b * c	4.538E13	12	3.782E12	2.171	.023
Rep	3.404E12	2	1.702E12	.977	.381
Error	1.219E14	70	1.742E12		
Total	3.047E14	108			
Corrected Total	2.969E14	107			

a: pH, b: Treatment time, c: Microwave output power

Appendix Table 3: *Staphylococcus aureus*_ pH effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.232E15	37	6.032E13	12.992	.000
Intercept	1.282E14	1	1.282E14	27.609	.000
A	6.938E13	2	3.469E13	7.472	.001
B	3.797E14	3	1.266E14	27.260	.000
C	2.549E14	2	1.275E14	27.455	.000
a * b	2.106E14	6	3.510E13	7.559	.000
b * c	7.551E14	6	1.259E14	27.107	.000
a * c	1.387E14	4	3.469E13	7.471	.000
a * b * c	4.211E14	12	3.509E13	7.559	.000
Rep	2.262E12	2	1.131E12	.244	.784
Error	3.250E14	70	4.643E12		
Total	2.685E15	108			
Corrected Total	2.557E15	107			

a: pH, b: Treatment time, c: Microwave output power

Appendix Table 4: *Bacillus Cereus*_ Salt effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.853E12	37	1.852E11	14.369	.000
Intercept	5.963E11	1	5.963E11	46.259	.000
A	1.148E11	2	5.742E10	4.454	.015
B	1.626E12	3	5.418E11	42.033	.000
C	1.101E12	2	5.506E11	42.710	.000
a * b	2.650E11	6	4.417E10	3.427	.005
b * c	3.046E12	6	5.077E11	39.382	.000
a * c	1.910E11	4	4.776E10	3.705	.009
a * b * c	4.566E11	12	3.805E10	2.952	.002
Rep	5.311E10	2	2.655E10	2.060	.135
Error	9.024E11	70	1.289E10		
Total	8.352E12	108			
Corrected Total	7.756E12	107			

a: salt concentration, b: Treatment time, c: Microwave output power

Appendix Table 5: *Staphylococcus aureus*_ Salt effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.332E14	37	3.600E12	3.713	.000
Intercept	9.754E12	1	9.754E12	10.058	.002
a	1.878E12	2	9.390E11	.968	.385
b	2.922E13	3	9.741E12	10.045	.000
c	1.945E13	2	9.726E12	10.029	.000
a * b	5.626E12	6	9.377E11	.967	.454
b * c	5.828E13	6	9.714E12	10.017	.000
a * c	3.744E12	4	9.360E11	.965	.432
a * b * c	1.122E13	12	9.347E11	.964	.491
Rep	3.788E12	2	1.894E12	1.953	.149
Error	6.788E13	70	9.698E11		
Total	2.108E14	108			
Corrected Total	2.011E14	107			

a: salt concentration, b: Treatment time, c: Microwave output power

Appendix Table 6: *Escherichia coli* _ Fat effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.005E14	25	1.202E13	5.657	.000
Intercept	1.473E13	1	1.473E13	6.931	.011
a	1.251E13	1	1.251E13	5.886	.019
b	4.156E13	3	1.385E13	6.519	.001
c	2.920E13	2	1.460E13	6.870	.002
a * b	3.513E13	3	1.171E13	5.510	.003
b * c	8.238E13	6	1.373E13	6.461	.000
a * c	2.501E13	2	1.250E13	5.883	.005
a * b * c	7.024E13	6	1.171E13	5.508	.000
Rep	4.521E12	2	2.260E12	1.064	.354
Error	9.776E13	46	2.125E12		
Total	4.130E14	72			
Corrected Total	3.983E14	71			

a: Present of fat, b: Treatment time, c: Microwave output power

Appendix Table 7: *Bacillus cereus*_ Fat effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.688E13	25	6.751E11	18.722	.000
Intercept	1.091E12	1	1.091E12	30.253	.000
a	6.054E11	1	6.054E11	16.791	.000
b	2.997E12	3	9.992E11	27.710	.000
c	2.009E12	2	1.004E12	27.858	.000
a * b	1.633E12	3	5.442E11	15.094	.000
b * c	5.503E12	6	9.172E11	25.438	.000
a * c	1.105E12	2	5.525E11	15.322	.000
a * b * c	2.967E12	6	4.945E11	13.714	.000
Rep	5.713E10	2	2.856E10	.792	.459
Error	1.659E12	46	3.606E10		
Total	1.963E13	72			
Corrected Total	1.854E13	71			

a: Present of fat, b: Treatment time, c: Microwave output power

Appendix Table 8: *Staphylococcus aureus*_ Fat effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.921E12	25	2.768E11	1.712	.056
Intercept	5.293E11	1	5.293E11	3.272	.077
a	1.276E11	1	1.276E11	.789	.379
b	1.475E12	3	4.916E11	3.039	.038
c	1.003E12	2	5.015E11	3.101	.055
a * b	3.558E11	3	1.186E11	.733	.537
b * c	2.816E12	6	4.693E11	2.902	.017
a * c	2.303E11	2	1.151E11	.712	.496
a * b * c	6.527E11	6	1.088E11	.673	.672
Rep	2.609E11	2	1.304E11	.807	.453
Error	7.440E12	46	1.617E11		
Total	1.489E13	72			
Corrected Total	1.436E13	71			

a: Present of fat, b: Treatment time, c: Microwave output power