2.0 REVIEW OF LITERATURE

Microwave ovens, have become increasingly popular throughout the world since the last decade. The modern tempo of life as well as increasing number of working women require simplified food preparation, convenience in usage as well as time and energy saving. The use of microwave ovens in industrial, commercial, domestic and other premises has increased substantially over recent years. In many medical practices, patients are being informed that, the microwaved food is practically free of microorganisms and is therefore recommendable.

2.1 Origin of microwave heating

Microwave is a by-product of development of radar during the Second World War. The first radar range became available for use in food service in 1947. After the war, Spencer of Raytheon in 1949 connected the heat generated by Radar Antena with the potential for heating foods, thus lead to the first patent for a microwave oven. The first consumer microwave oven was introduced by Japan in 1955 under the license from Raytheon (Spencer, 1950).

FDA(2000) has specified the frequencies of 915 and 2,450 MHz for food processing at industrial and home-type ovens heating respectively.

2.2 Source of microwave radiation and Mechanism

The source of microwave radiation is a radio frequency oscillator in the order of 300 MHz to 300,000 MHz. Specific bands of microwaves are allowed by law such as
2,450 MHz for domestic purpose; 915 MHz for industrial purpose and 433 MHz for medical purpose (Roussy and Pearce, 1995).

Rao et. al.(1995) reported that microwave are very short waves of electromagnetic energy that travel at the speed of light (186,282 miles per second). A device called magnetron generates microwave usually. The molecules within the food, the polar water molecules, aminoacids, lipids and proteins are forced to align themselves with the rapidly changing electrical field. They oscillate around their axis in response to reversal of the electric field that occurs up to 5 billion times per second. This oscillation creates considerable intermolecular friction to result in generation of heat. Thus, the food is heated from the inside outwards leaving the dishes and the oven itself cold, as the microwaves do not directly heat them.

Microwave ovens have emerged as substitute to thermal ovens for a number of food manufacturing processes and products. Microwave processing results in relatively higher retention of nutritional and sensory values as compared to thermal processes and has potentialities of bringing about radical improvement in food manufacturing industries (Sahni et.al.,1997).

Schiffman (1997) found that uneven heating of microwave foods is due to the lack of standardization of a particular wattage among the microwave ovens existing in the United States.

Microwave heating is a novel method that offers technique of heating requiring neither conduction nor convection. Microwaves generate heat within the food rapidly raising the temperatures to the desired extent. Conventional heating process generate a temperature gradient from the outside surface to the center of the product, whereas, in microwave heating the surface temperature is often lost due to evaporative cooling than those at the center of the product (Lu et.al., 1999).
2.3 Heat generation in microwave oven

According to Hedleson and Doores (1994a), microwaves do not pass heat by conduction but penetrate a product through the coupling of electrical energy from the electromagnetic field in the microwave cavity and its distribution in the respective food product being heated.

Heating with microwave frequency involves primarily 2 mechanisms namely dielectric and ionic. Water in the food is often the primary component responsible for dielectric heating. Due to dipolar nature, water molecules try to follow the electric field associated with electromagnetic radiation resulting in very high frequency oscillations to produce heat. The second major mechanism of heating with microwaves is through the oscillatory migration of ions in the food to generate heat under the influence of the oscillating electric field (Metaxas, 1996).

Landgraf and Tassinari (1997) explained theoretically that heat is generated quickly and uniformly by microwave process, but numerous experiments have proven this theory wrong as more research has been done to improve the quality and safety of microwave foods. The centralized magnetron that emits the microwaves is unable to distribute them evenly. Waves leave the source and bounce around the microwave cavity until they are absorbed by the food. This is not a very exacting process.

2.4 Heating pattern of microwave

A time of equilibration would allow hot and cold spots to more evenly disperse heat trapped within the food. This works particularly well when liquid foods such as soups are addressed (Sawyer, 1985). Hot and cold spots were noticed in the microwave treated food (Ryynanen 1995). The penetration of microwaves was noticed to the center of a food almost immediately depending on the depth of the food (Goedeken et al. 1997)

2.5 Heating Rate
Microwave heating is rapid but more complicated. A study conducted by Sawyer (1985) revealed that a post processing temperature rise (PPTR) was likely in most microwaved foods and should be accounted for in heating instructions.

The food may be heated in a shorter amount of time, but obvious hot and cold spots exist in the food due to uneven electrical wave distribution in the product during cooking. As a result of these shorter cooking times and the uneven heating of a product, it is a concern that microbial survival may occur (Aktas and Ozilgen, 1992).

Heddleson and Doores (1994a) found that the food is not heated for as long to reach the desired internal temperature. Discussion arose on whether or not the food has reached suitable temperatures to kill all microorganisms in all areas of the food. Studies have been conducted that established the minimum temperature between 70°C and 75°C should be attained for microbiological safety of microwave prepared food.

A post cook holding time, would be in order to ensure all areas of a food reach the minimum temperature requirements mentioned above. Such findings signal that changes in microwave food product development and package instruction are necessary because many currently recommended times have resulted in inadequate temperatures for pathogen destruction when tested by scientists (Landgraf and Tassinari, 1997).

Goedeken et al., (1997) worked on the microwave tempering of food product which accelerated thawing and decreased thawing times. Microwave tempering differs in microwave heating in the fact that tempering microwave penetration depth is relatively deep and the temperature more uniform. Such practices also help deter “runaway heating” from occurring, a fairly common occurrence when microwave treating frozen foods certain areas of the food thaw quickly and cooks rapidly while other areas of the food are still frozen. Burned edges or interior accompanied by cold regions result when this happens giving lower product quality and satisfaction. As well as lower quality food, the remaining cold spots leave areas for pathogens to find refuge during the microwave heating cycle.

Post processing temperature rise is likely in most microwaved foods and should be accounted for in heating instructions. With this in mind, a post cook holding time would be in order to ensure all areas of a food reach the minimum temperature requirements mentioned above. Such findings signal that changes in microwave food product development and package instruction are necessary because many currently recommended times have resulted in inadequate temperatures for pathogen destruction when tested by scientists (Landgraf & Tassinari, 1997).
Zhang et al. (1999) studied that as food is heated in microwave oven, its microwave absorption capability typically increases, which increases the rate of temperature rise and therefore further increase in the rate of microwave absorption. 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.

2.6 Heating Uniformity

Conventional methods heat a food from the outside in, layer by layer through the medium of heat transfer; therefore, ensuring uniform cooking through each stage of the foods heating. Microwave cooking, on the other hand, heats by a dielectric means, using high frequency waves to align and realign the dipolar molecules (especially water or fat) within the food causing friction (Schiffman, 1993; Hill, 1994).

Scientists (Heddleson and Doores, 1994a; Anantheswaran and Liu, 1994a). experimented on the distribution of microwave in foods and found non uniform heating because of several individual parameters such as concentration and direction of microwaves in all areas of the oven; presence and amount of fat, protein and salt; the size and shape of the food piece, the physical state of water in the food, food composition and the wattage of the microwave.

2.7 Effect of relevant food characteristics on microwave heating

As with microwave heating and other thermal processes, the primary factors that determine safety are temperature and time. A number of critical process factors affect time-temperature history. Some of these critical process factors are moisture, ionic content, microwave frequency, product parameters and the temperature achieved. The thermal conductivity of the food is increased when the amount of free water is increased
and is only countered by the presence of other dielectric elements such as salt, fats and proteins (Fakhouri and Ramaswamy, 1993b).

2.7.1 Shape and size

Hill (1994) studied the inability of microwaves to effectively permeate food pieces if thick, as the penetration of most microwaves is usually only about 10 – 15 mm from each side. It is also important to note that a food of a spherical or cylindrical form with a diameter of 20 – 60 mm will heat unevenly, the center heating more swiftly than the surface. This phenomenon is known as the concentration effect (i.e. heat is focused toward the center of the round). It is important to note that the geometry of the food as well as the shape of the container must be considered before treating food in a microwave oven. It has been observed that food shapes with corners tend to display localized heating in those areas of the sample because of the multidirectional distribution of the electrical energy from the microwave. Conversely, cylindrical or spherical foods reduce edge and corner heating but are subject to center heating or focusing effects. This causes heating of the product to be mainly at its center. Therefore, in regard to the shape of the heating container, it is important to avoid sharp edges and corners as this may also affect the foods reheating pattern.

The size of the portion being heated is another factor that affects the rate at which a food cooks or reheats. The larger a food portion, the less efficiently it heats in the microwave atmosphere and vice versa because of a phenomenon known as “coupling,” larger objects are able to absorb more energy than smaller foods. Ironically, these foods tend to heat slower though due to the fact that more time is required to allow the temperature gradient of the food to equilibrate and decrease due to low penetration ability. Thus very thin foods may receive too much heat while thicker foods are cooked only on the outside layers (Heddleson and Doores, 1994a).

2.7.2 Orientation

The orientation of the product in the oven affects its heating rate because of oven pattern variations. As the microwave source emits radio waves, they bounce off the walls of the microwave until striking and being absorbed into the food. The presence of “hot and cold spots” were noticed in the oven where food consistently heats less efficiently these result from uneven distribution of the waves inside the oven cavity. These temperature variations can be lessened by a wave stirrer but may still present a problem, especially for small products (Calzada et. al., 1995).
2.7.3 Food components

Hill (1994) opined that the sodium and lipid content decreased in wave penetration depth in microwave foods. Sodium and lipid ions reflect the electric waves that are responsible for microwave heating causing ionic polarization.

Milk was used as the low sodium solution and beef broth as the high sodium liquid food to study the effect of microwave on them. Results showed that sodium content and other dielectric components of the system did indeed play a primary role in affecting the thermodurability of bacteria present within the food systems as well as the temperatures that could be achieved in each food. Sodium content proved to decrease the microwave penetration depth of each liquid in turn increasing surface heating rates and giving lower temperatures at greater depths (Heddleson and Doores, 1994b).

Anantheswaran and Liu (1994a) experimented that before reflecting these waves from penetrating deeper into the food, sodium ions are heated and reflect this heat into the organic material directly surrounding themselves. The areas directly in contact with these ions then heat quickly. Yet, by reflecting the electrical waves, sodium lowers the effectiveness of microwave heating and allows the possibility of greater pathogen survival rates.

Fat is a dielectric component of a food system, so it can both absorb heat and reflect it. If this fat is distributed evenly and the food sample is not too thick, the sample will cook faster because it will absorb the microwaves, be heated by dipolar rotation and transfer that heat to the food directly surrounding it (Heddleson and Doores, 1994a).

Goedeken et al. (1997) revealed that microwave tempering differs in microwave heating in the fact that tempering microwave penetration depth is relatively deep and the temperature more uniform. Such practices also help deter “runaway heating” from occurring, a fairly common occurrence when microwave treating frozen foods. Successful microwave heating of a food depends on many characteristics such as moisture content, free ionic salt content, fat, protein and solids content. Because water makes up 50 to 90 percent of most foods, understanding its effect on dielectric activity is vital. Higher moisture content usually lowers the dielectric loss factor of a food; thus, causing it to heat more efficiently. Still, other components such as high salt content may lower a microwave’s wave penetration depth.
2.7.4 Molecular State of Water

Schiffman (1993) cited the perfect example of this dipolar rotation as being water. The more water in a food, the more polar its composition and the more effectively the food is heated in a microwave oven. Because water is a prevalent ingredient in most food systems and present in high concentrations, it tends to dominate the frictional heating of a food that takes place in a microwave.

The thermal conductivity of the food is increased when the amount of free water is increased and is only countered by the presence of other dielectric elements such as salt, as fats and proteins (Fakhouri and Ramaswamy, 1993b).

Goedeken et. al. (1997) stated that water in its liquid state has a much higher dielectric constant than that of ice. Therefore, in dealing with a frozen product, any thawed areas in the product will heat much more quickly than the still frozen sections of the product, resulting in the thawed areas being overcooked and frozen areas being undercooked.

Molecular state of water in a food is an important point of calculation because it determines how the food will absorb the microwaves. The more ionic components (i.e., free water, fat, salt, protein) in the food, the quicker it will heat. Liquid water more readily absorbs microwaves than ice; therefore, wide temperature gradients are able to occur within a frozen product causing a phenomenon known as run-away heating. “Run-away” heating causes quality defects in a frozen or partially frozen product such as thermal degradation and excessive water loss (Hotchkiss and Potter, 1997).

2.8 Proper Package Heating Instruction

Since questions about the safety of microwave heated food first arose, scientists have been testing both food products currently on the market and other potentially marketable foods as well (Heddleson and Doores, 1994a).

Han (1996) stated in the frequency of 2450MHz, microwaves have the property that they are not absorbed by the majority of plastics, glass and pottery.
One such study by Landgraf and Tassinari (1997) employed the modeling of microbial inactivation kinetics and heat transfer of microwave reheating three food types inoculated with $10^4$ cfu/g *S. typhimurium* for destruction during microwave heating. Mashed potato and beef stroganoff samples were heated for 75 seconds as opposed to 50 seconds for baby food and demonstrated a survival rate of 20%, 40% and 83.3%, respectively. All samples were also stirred after treatment to further ensure uniform temperature of the product from interior to surface.

### 2.9 Cooking and reheating

The main reason the people heat their food is to improve its palatability. Heating the food will also inactivate any microorganisms present within the food (Jay, 1996).

Schiffman (1997) commented that with the new technology and increasing availability of microwave ovens for the home, conventional heating methods have become less popular because of increased heating time and less consumer convenience.

When microwave foods were first introduced to the public, it was not a common practice to do such testing with most product development projects dealing with microwave foods.

Many companies simply released their regular product to the public with microwave directions and waited for complaints fixing them as they arose. This was a bad policy considering food safety, product quality and public perception. Now companies do a more thorough job of testing their products before releasing them. Standards of testing need to be stated to further ensure the safety and restore public confidence in these microwave products. Additionally, it would be helpful to aid consumers in understanding the purpose for a hold time when it is included on a product’s direction panel because this step helps alleviate temperature gradients that would otherwise exist in the heated food. Many consumers do not realize that the food temperature and quality characteristics could still go through even more change directly after the heating process has finished. Process completion needs to be emphasized to the consumer in this situation to ensure better, safer results of the cooking process (Landgraf and Tassinari, 1997).

Schiffman (1997) opined that with the rise in popularity of the microwave oven, the safety of this cooking method will continue to be questioned by government, industry and consumers. Since microwave
heating times are much shorter than that of the conventional oven and its non uniform heating results in obvious cold spots in most foods, it is difficult to pinpoint where to start fixing the problem. Some food companies are attempting to correct some of the problems by product formulation while others feel that a government-mandated program for rating of microwave ovens should be in place as in the United Kingdom.

Storage abuse can occur at many points during a food's production and shipment. If packers and warehouse employees do not handle food in a careful manner, food packaging can become ruptured before it leaves the processing facility. Again, this may allow microbial contamination or interfere with any modified atmosphere packaging methods used in the product's containment (Lau et al., 1998).

2.10 Characterization of spoilage and pathogenic bacteria in milk and milk products

Numerous studies address the effect of microwave heating on pathogenic microorganisms in foods. Non-uniform heating by microwaves may lead to survival of food borne pathogens, including many salmonella and L. monocytogenes, in certain locations of foods heated at selected internal locations to endpoint temperatures that would normally be lethal. There do not appear to be any obvious "microwave-resistant" food borne pathogens (Schnepf and Barbeau, 1989).

Gracia et al (1989) showed that 67% of isolates accounted for pseudomonas, as dominant microflora and majority were fluorescent group isolated from raw milk.

The incidence of Bacillus spp. was at 25% in 35 raw milk samples as reported by Netten et al. (1990).

Suarez and Ferreiros (1991) isolated Bacillus spp. and reported out from 95 samples of farm bulk tank milk.
*Pseudomonas* spp were most commonly isolated types after the milk was stored at $4^{\circ}$ or $7^{\circ}$C (Craven and Macauley, 1992).

Critical contamination sites of *Pseudomonas* (30%), members of Enterbacteriaceae (9%) and spore forming bacterium *Bacillus* spp (60%) were observed in packed pasteurized milk samples (Eneroth *et al.*, 1998).

Whitefield *et al.* (2000) isolated *Pseudomonas* and they were recovered from the tainted milk on the agar containing cetrimide. The isolates were identified as *Pseudomonas putida*.

The isolates obtained from raw milk samples collected from pre-chiller, post-chiller and silos were identified as *E.coli* (5), *B.subtilis* (6), *S.aureus* (5), Shigella (6) (Sharma and Anand, 2001).

### 2.11 Mechanism of Inactivation of microorganisms

Number of studies have proven that the thermal effect is the essential contributor to the destruction of microorganisms. Certain studies also doubt about the mechanism of microbial inactivation due to non thermal effect.

#### 2.11.1 Thermal effect

Khalil & Villota (1986) revealed that at a molecular level, microwaves could interfere with the mechanism of bonding of dipicolinic acid to calcium and cause reorientation in the thermostable protein content of the DNA of microbes.

The microwave exposure of microorganisms led to increased rate of phosphoanhydride bond hydrolysis in RNA causing denaturation. The energy absorption from microwaves can rise the temperature of the food high enough to inactivate microorganisms (Sun *et al.*, 1988).

According to Khalil & Villota, (1989), heat injured cells of *S.aureus* released ninhydrin, purine, pyrimidine and ribonucleotides into the heating medium that can be read at 260 nm, detecting the leakage of cell contents due to thermal effect on cells. Microwaves also generate instantaneous thermal energy to heat sensitive subcellular components. Sublethal heat injury hindered oxygen uptake during recovery.

The microscopic size of bacteria resulted in surface area to volume ratios extremely high, helped in rapid heat loss to the surrounding environment. Thus microorganisms possess extremely high dielectric loss factors to maintain even small temperature differences (Sastry and Palaniappan, 1991).
Heddleson & Doores (1994a) found that the thermal effect on microorganisms lead to potentially irreversible heat denaturation of bacterial enzymes, proteins, nucleic acids and metabolites and co-factors crucial to cellular function may leak through membranes damaged by heat.

The microbial lethality is now understood as the result from the penetration of electromagnetic waves into a biological wet material, heating up the intra and extracellular fluids by the transfer of energy from polar water molecules and dissolved ions. This results in the generation of heat within the material itself due to molecular activity (Han, 1996).

Woo et al. (2000) exposed E. coli wild-type strain K-12 in Luria broth and B. subtilis KM107 in nutrient broth with $10^9$ to $10^{10}$ cfu/ml to microwave radiation in microwave oven (2,450-MHz; LG Electronics) that resulted in a dramatic reduction of the viable counts as well as increases in the amounts of DNA and protein released from the cells according to the increase of the final temperature of the cell suspensions. However, no significant reduction of cell density was observed in either cell suspension. It is believed that this is due to the fact that most of the bacterial cells inactivated by microwave radiation remained unlysed. Scanning electron microscopy of the microwave-heated cells revealed severe damage on the surface of most E. coli cells, yet there was no significant change observed in the B. subtilis cells. Microwave-injured E. coli cells were easily lysed in the presence of sodium dodecyl sulfate (SDS), yet B. subtilis cells were resistant to SDS.

Various concentration of sodium chloride (0.5, 1.5, 3%) had no significant effect on the destruction of Listeria monocytogenes by microwave heating. At pH 4.5, the destruction effect of the microwave radiation was significantly greater than those at pH5.5 and 6.5. The destruction effect of the microwave radiation was not affected by acidulant type at pH 4.5 (Shekarforoush and Firozi, 2003).

### 2.11.2 Non-thermal effect

Kozempel et al. (1998) studied another mechanism for inactivation by microwaves involving non-thermal effects like, electroporation, cell membrane rupture, and magnetic field coupling. Electroporation is caused when pores form in the membrane of the microorganisms due to electrical potential across the membrane, resulting in leakage. The critical electric field strength for L. brevis is 13 kV/cm and for E. coli it is 16 kV/cm (human being can withstand 25V). Cell membrane rupture is related in that the voltage drop
across the membrane causes it to rupture. Cell lysis may also occur due to coupling of electromagnetic energy with critical molecules within the cells, disrupting internal components of the cell.

A new system capable of separating the thermal from non-thermal effects of microwaves was designed. The system was a double tube that allowed input of microwave energy but removed thermal energy with cooling water. With this system they did not find any inactivation of the inoculum at sub-lethal temperatures for *Ent. aerogenes, E.coli, Listeria* and *Pediococcus*, or for a yeast like suspension of various liquids which included water, skim milk, egg white, whole eggs, tomato juice and beer. They concluded that, when other stress factors such as pH or heat are not present, the energy from microwaves do not deactivate microorganisms, leading them to suggest that microwave energy can complement or amplify the thermal effects (Kozempel et. al., 2000).

Ramaswamy *et.al* (2000) 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). *Ps. aeruginosa* a resistant psychrotroph when exposed to mw 2450MHz at different time intervals showed 25 sec. to reduce in their number.

### 2.12 Effect of microwave on the bacterial counts on milk and milk products

Microwave has thermal effect on microorganisms and reduced their numbers in milk and milk products.

HTST and LTLT pasteurization carried out using microwave radiation (59s at 700W yielded 71.7°C/15sec; 5 min. at 550 W- 62.8°C) inactivated the inoculated cells of *Salmonella typhimurium, E.coli, Ps. fluorescence* at 10⁴/ml concentration (Knutson *et.al.*, 1988).

Fujikawa *et al.* (1992) studied that the absorption of microwave energy can increase the temperature of foods rapidly, deactivating microorganisms and performing pasteurization or sterilization. The study has shown that the thermal effect is essential in destroying microorganisms.

The pasteurization of goat milk using microwave, reduced the contaminating microflora significantly (Calvo and Olano, 1992).
Rosenberg & Sinell (1994) indicated that the effect of microwave energy on viability of spores of Clostridium sporogenes was indistinguishable from the effect of conventional heating.

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%. Such a difference is attributed to the presence 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 low destruction (Heddleson et.al., 1994).

Heddleson and Doores (1994a) reported the inactivation of Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, Escherichia coli, Enterococcus spp., Listeria monocytogenes, Staph. aureus, and Salmonella by microwave treatment.

A study on the survival of Salmonella species in milk and beef broth was conducted with respect to microwave exposure. At 68°C or higher, no viable cells were detected in milk, but at 66°C, viable salmonellae were recovered. For the beef broth, a temperature of 70°C was discovered to totally eliminate the presence of the salmonellae (Heddleson and Doores, 1994b).

When milk was heated in microwave oven for 47s, the reduction in numbers of S.aureus in the various food systems varied from 1.74 to 2.5 log units, but was not influenced by the suspending menstruum. The amount of destruction of Salmonella spp. varied from 0.44 log log to 3.17 cfu/ml of UHT milk (Heedleson et.al., 1996).

Kindle et.al. (1996) investigated the effect of microwave (2450MHz frequency and 600W power) on P.aeruginosa, E.coli, Enterobacter sakazakii, K.pnuemoniae, S.aureus, Mycobacterium terrae, poliomyelitis vaccine virus and Candida albicans suspended (100cfu/ml) in 5 infant formula preparations(150 ml). Each sample was exposed to microwaves until the 1st signs of boiling were detected (85-100s depending on infant formula type; 82 - 93°C). Colony counts of all the microorganisms were significantly decreased by microwave heating. P.aeruginosa, Mycobacterium terraei and poliomyelitis vaccine virus were completely destroyed. Others showed reduction in viable count by 5000 folds.

The potential pathogens Ps.aeruginosa, E.coli, Ent. sakazakii, K.pnuemoniae, S.aureus, M. terrae, poliomyelitis vaccine virus and Candida albican suspended (100cfu/ml) in 5 infant formula preparations
(150 ml) when exposed to domestic microwave oven can be destroyed or considerably reduced in numbers (Gorge, 1997).

Villamiel et al. (1997) pasteurized goat milk in continuous microwave heating system using 2450 MHz or in a plate heat exchangers at 72.5 and 80.1°C for 15s and studied the destruction of total bacterial count. The degree of microbial destruction was similar for microwave and plate heat exchangers.

2.13 Effect of microwave on the injury and recovery of bacterial cells

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. For thermal processes, the length of incubation may be important in recovering viable cells or spores, because thermally treated cells or spores generally grow slow than non-treated ones. As with other process studies, experimentation will be necessary to determine the optimum conditions and methods for microbial enumeration.

A series of studies by Khalil and Villota (1986; 1988) suggested non-thermal effects of microwave heating. They showed lower D100°C values of spores of *B. stearothermophilus* in 2450 MHz microwaves compared to heating water bath. They also heated cells of *S. aureus* FRI-100 at a sublethal temperature of 50°C and maintained microwave temperature using recirculated cooled kerosene. Microwave heating caused a greater amount of cellular injury of *Staph. aureus* FRI-100, as determined by plating on trypticase soy agar plus 7% sodium chloride increased loss of ultraviolet-absorbing cellular material and extended time for enterotoxin production. They speculated that the microwaves catalyzed oxidative reactions, possibly in membrane lipids, decreasing recovery of exposed cells.

The same authors (1989) exposed the cells of *Staph. aureus* FRI-100 to a sublethal temperature of 50°C for 30min in 0.1M phosphate buffer using either microwave energy or conventional heating source. Following thermal stress, cells were
allowed to recover. Injury was monitored as the difference between cell counts when an inoculum from the recovering cells was plated on trypticase soya agar (TSA) and trypticase soy agar with 7% sodium chloride (TSAS). Total viable population following either heat treatment was $10^6$ cells/ml as indicated by TSA counts. When the same suspensions were plated on TSAS, a viable count of $1.7 \times 10^3$ cells/ml resulted from conventional heating compared to $5.6 \times 10^3$ cells/ml. When the effect of conventional and microwave heating on S. aureus was compared, conventional heating reduced 3 log cycles compared to 4 log cycle reduction in microwave heating this was due to release of more intracellular contents because of faster leakage in microwave heating.

The rapid heating characteristics of microwaves and shorter exposure to heat may cause sublethal injury and thermal stress, allowing microorganisms, to survive a microwave process. Importance of sublethally injured microorganisms and main considerations in the development and implementation of microwave process has to be taken care (Vasavada, 1990).

Aktas & Ozilgen (1992) studied the injury and death of E. coli by dispersing 5% w/v in skim milk medium into microwave in a tubular pasteurization flow reactor using nutrient and violet red bile agars. Cells injured during pasteurization may escape detection and are still capable of repair and producing toxins, therefore they might be potentially more hazardous than uninjured cells. When an inoculum with $10^5$ cfu/ml was pasteurized, the viable microbial concentration reduced to 1% of the initial population.

Rocelle et al. (1995) used sorbitol MacConkey agar that performed poorly in supporting growth of stressed E. coli O157:H7 cells in heat treated trypticase soy broth Upto a 2-log difference was observed between counts on sorbitol MacConkey agar and tryptone soy agar.

### 2.14 Important food pathogens in microwavable foods

Three food pathogens tend to stand out as a concern to public health in prior studies dealing with microwavable foods. Listeria monocytogenes, several Salmonella species and E. coli O157:H7 are
becoming more and more recognized because of their prevalence as sources of causing human illness and further concern for food companies, government agencies, consumer activist groups and consumers themselves (Heddleson and Doores, 1994a). Therefore researchers have often concentrated their efforts in these areas.

2.14.1 Escherichia coli O157:H7

Escherichia coli O157:H7 is a threat to almost everyone, but especially the old, young and immuno compromised. Any small amount of it can be a threat to such consumers. For instance, if a patty of ground beef is cooked or reheated in a microwave but never reaches the proper internal temperature throughout, *E. coli* will most likely survive and infect the person who eats the patty – unless they are of good health. If good health is the case, this person may never know they ate a burger contaminated with viable *E. coli*, but a person with a weaker immune system would in all probability become very ill, become dehydrated from continual diarrhea and possibly be afflicted with hemolytic uremic syndrome (HUS). Considerations such as this make *E. coli* O157:H7 a concern in every operation that it might be introduced (Flores, 1994).
2.14.2 Listeria

monocytogenes

Problems with *L. monocytogenes* are not uncommon in the food processing industry. It is a hardy organism that can survive process temperatures that eliminate many other microbes (Jay, 1996). The survival of *L. monocytogenes* resulting from inadequate processing or post process contamination of pre-cooked refrigerated foods; uneven heating and reheating throughout a food (Landgraf and Tassinari, 1997).

2.14.3 *Salmonella* species

*Salmonella* species contaminate the surface of several common foods, poultry and fresh vegetables being the most notable. Poultry naturally have *Salmonella* spp. as part of their normal gut microflora, which is often transferred to the chicken carcass from feces or during the kill process, so it is always important to thoroughly cook such meat to a full degree of doneness throughout. Otherwise, areas that did not receive full heat may harbor injured cells that may prove to be more harmful than the original live cells. The reason for this is that no one is expecting *Salmonella* spp. to be present and might store the product improperly or eat the product after those cells have had time to repair without cooking it (Cherry-Merritt *et. al.*, 1997; Landgraf and Tassinari, 1997).

Studies on *Salmonella* spp. have served to verify recommended USDA processing schedules, ensure the safety of manufacture’s reheating instructions, and to compare the difference in bacterial destruction between microwave, convection microwave and conventional electric ovens (Dawson, 1999).

2.15 Comparison of conventional and microwave heating
Microwave heating is faster than conventional heating. The packaging material or vessels do not get heated up in microwave heating when compared to conventional heating method.

Galuska et al. (1988) showed that in case of 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. Microwave heating of *L. monocytogenes* in milk for 15 sec at 71.7°C led to 4.5 log cycle reduction compared to conventional heating. It was found that microwave heating was more effective than conventional heating.

Khalil & Villota (1988) compared heating of conventional and microwave heating on *S. aureus*. The conventional heating reduced *S. aureus* of 3 log cycles compared to 4 log cycle reduction in microwave heating. This was due to release of more intracellular contents because of faster leakage in microwave heating.

The conventional and microwave heating both destroyed the 16S subunit of RNA in 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 (Khalil and Villota, 1989).
Microwave 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. Microwave heating may be relatively more uniform than conventional heating, depending on the particular heating situation however, heating uniformity is hard to predict (Datta and Hu, 1992).

Fujikawa et.al.(1992) found no major differences in inactivation kinetics of *E. coli* in phosphate buffer between microwaves and conventional heating.

Welt et.al. (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.

When considering post processing temperature rise (PPTR), the contrast of microwave heating to conventional heating becomes important – the lack of uniformity of microwave preparation. Conventional methods heat a food from the outside in, layer by layer through the medium of heat transfer; therefore, ensuring uniform cooking through each stage of the foods heating (Schiffman, 1993).

Microwaves do not pass heat by conduction but penetrate a product through the coupling of electrical energy from the electromagnetic field in the microwave cavity and its distribution in the respective food product being heated. Theoretically heat is generated quickly and uniformly by this
process, but numerous experiments have proven this theory wrong as more research has been done to improve the quality and safety of microwave foods (Landgraf and Tassinari, 1997).

Datta (2000) compared conventional heating with microwave exposure on food. 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. 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 the heating processes the time/temp relationship is not comparable for microwaves and conventional processes. The changes in material properties as the temperature goes up are more pronounced with microwaves. As the temperature of the material increases, its ability to absorb microwaves also goes up, increasing the rate of temperature increase. This coupled effect can lead to uncontrolled heating (Zhang and Datta, 2000).

FDA (2000) stated that for pasteurization and sterilization, microwave heating is preferred over conventional heating because it is faster and thus requires less time to reach the required process temperature. Other advantages of microwave heating are that it permits turning on and off and that the product can be pasteurized after packaging. The processes utilizing microwaves can also be more energy efficient.

2.16 Shelf life studies of milk and milk products

Food preservation methods increase the shelf life. Microwave heating of milk and milk product is one of the preservation methods of recent origin.

Decareau (1986) used both standard pasteurization at 85°C and microwave heating and observed prolongation in the keeping quality of milk and yogurt.

The shelf life of microwave pasteurized (80.1°C/15s) cow milk was estimated by enumeration of total bacterial count. The microwave treated milk showed 15 days of storage compared to 10 days of storage of conventional heating at 4.5±0.5°C (Villamiel et al., 1997).
Uprit and Mishra (2004) conducted a study to determine the effect of microwave heating on the spoilage microorganisms and shelf life of soy fortified paneer, a heat-acid coagulated dairy product. Soya fortified paneer was prepared from buffalo milk (3.2% fat) and soy milk at the ratio of 85:15. Samples were packed in high density polypropylene containers and exposed to microwave heat at 120W for 60 s. The treated samples were stored at $8\pm2^0C$ and microwave heating improved the shelf life of stored soy fortified paneer by 2 folds from 9 to 18 days at $8\pm2^0C$.

2.17 Application of Microwave processing in dairy and food industry

2.17.1 Pasteurization of Milk

Microwaves can be used in dairy or food industry for applications to inactivate microorganisms, enzymes and insects.

For HTST pasteurization of milk in microwave it is calculated that 1KW of power is required to pasteurize 270 liters of milk/hour (Sale, 1976).

Mudget (1980) modeled milk pasteurization using microwaves and stated that the quality of such milk would be higher than that of conventionally treated milk due to the rapid and uniform heating.
A small microwave applicator was used to pasteurize milk both continuously and in small batches. Substantial reduction in bacterial count was recorded for batch sample but results were variable for continuously pasteurized milk, probably due to poor temperature control (Rosenberg and Bogi, 1987).

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 (Harlfinger, 1992).

Industrial microwave pasteurization and sterilization systems have been reported on and off for over 30 years. Packaging material is also a critical process factor in microwave heating. The ability of microwaves to readily penetrate nonmetallic packaging materials allow this method to be used in heat treating fluid milk after packaging. Normally used packaging materials for microwave are laminates, pouches made of with polyethylene, polyester (mylar), polyethylene terephthalate (PETP) for in-package pasteurization or sterilization, packaging materials need to be microwave transparent and have a high melting point. Also, because metal reflect microwaves, packages with some metal component can considerably change the food temperatures (Tops, 2000).

2.17.2 Microwave exposure for milk products

Cooking of cheese before pressing and after pressing is done for Hallum type of cheese and showed similar organoleptic analysis as for conventional cooking (Hussain et.al., 1986).

Microwave heating of packaged yogurt extended the shelf life of yogurt (Decareau, 1986).
Naresh (2003) carried out experiments in which khoa and peda was exposed to microwave and found that there was 1 log count reduction both in total count as well as coliform when the khoa and peda was exposed to 30 sec.

Paneer sample was microwave treated and stored at 7±1°C. The samples stayed well upto 42 days at 7±1°C and log counts slowly increased. On 14th day of storage the counts of TBC and yeast and mold count were 1.37 and 0.98 log, the counts reached 2.11(TBC) and 1.29 (yeast and molds) log on 42nd day of storage. The untreated sample showed spoilage after 7 days of storage (Karthikeyan, 2005).

Arunkumar (2006), treated paneer spreads with microwave and stored at 7±1°C. The samples showed total bacterial count of 4.95 log, coliform count of 1.77 log and yeast and mold count of 2.32 log upto 56 days of storage at 7±1°C and the samples showed spoilage on 70th day of storage while the untreated sample showed spoilage on 21st day itself with more log counts in TBC, coliform and yeast and mold.

2.17.3 Other foods

Table foods, refrigerated foods, soups & desserts can be conveniently heated in microwave faster compared to conventional method. Microwave cooking being faster in addition to economical in production of instant powders like coffee, tea and cocoa by microwave processing yielded good quality products without any change in colour. Coffee seeds, cocoa and peanut roasting was faster in microwave and helped to reduce mold growth that might have led to decrease in aflatoxin production. (Rosenberg and Bogi, 1987).

Schiffman (1988) reported that, microwave is also used in blanching, roasting, canning and baking
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.

2.17.4 Future processes

Many techniques have been tried to improve the uniformity of heating. These include rotating and oscillating the food package, providing an absorbing medium (such as hot water) surrounding the product, equilibrating after heating, 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 (Lau et.al., 1998).

Wig et.al.(1999) stated that, use of the 915 MHz to improve uniformity of heating may have potential for the future. 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 of food contact surface.

3.0 MATERIALS AND METHODS

Microwave is one of the rapid preservation methods of treating the food samples to improve their keeping quality by reducing the microbial load. In this study spoilage bacteria normally present in milk were isolated and these included *E. coli, Salmonella sp.*, *Staph. aureus, Ps. aeruginosa* and *B. subtilis*. These organisms were exposed to microwave in various media such as broth, milks, paneer, khoa etc. and their survivability was monitored. Further microwave treated samples were stored and their shelf life monitored. The materials required and methods adopted for all the experiments are as follows:

3.1 BACTERIAL CULTURES

In this study, bacterial cultures such as *E. coli, Salmonella sp, Staph. aureus, Ps. aeruginosa* and *B. subtilis* were isolated from raw milk and used for the study as they are frequently found responsible for the reduction in the shelf life of milk & milk products.

3.2 BACTERIOLOGICAL MEDIA

3.2.1 Cetrimide Agar  (Harrigan, 1998)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>1.4 g</td>
</tr>
<tr>
<td>Potassium sulphate</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Cetrimide</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Agar-agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 litre</td>
</tr>
<tr>
<td>pH</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>
Suspended all the weighed ingredients into 1000 ml distilled water, boiled to dissolve completely, cooled and pH adjusted to 7.2 and sterilized by autoclaving at 121°C for 15 min.

3.2.2 Endo Agar (Harrigan, 1998)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>3.5 g</td>
</tr>
<tr>
<td>Sodium sulphite</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Basic fuchsin</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Agar-Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 litre</td>
</tr>
<tr>
<td>pH</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

All ingredients except lactose and basic fuchsin were dissolved in distilled water and pH adjusted to 7.4. The lactose & basic fuchsin were dissolved by boiling, mixed with the other ingredients and sterilized by autoclaving at 121°C for 15 min.

3.2.3 Glucose phosphate peptone water (Harrigan, 1998)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5.0 g</td>
</tr>
<tr>
<td>D-glucose</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate (K$_2$HPO$_4$)</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 liter</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Both peptone & K$_2$HPO$_4$ were dissolved in distilled water, pH adjusted to 7.5, in it dextrose was dissolved and autoclaved at 121°C for 15 min.

3.2.4 Hugh and Leifson’s medium, modified (Harrigan, 1998)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Mannitol</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Bromocresol purple (1% solution)</td>
<td>4 ml.</td>
</tr>
<tr>
<td>Agar- agar</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 litre</td>
</tr>
</tbody>
</table>
pH 7.0

All ingredients except mannitol and bromocresol purple were dissolved in distilled water and pH adjusted to appropriately. To it previously weighed mannitol and prepared bromocresol purple solution were added, transferred to test tubes and autoclaved at 121°C for 15 min.

3.2.5 Mannitol Salt Agar (Harrigan, 1998)

Proteose peptone 5.0 g
Beef extract 1.0 g
D-Mannitol 10.0 g
Sodium chloride 75.0 g
Phenol red 0.025 g
Agar-agar 15.0 g
Distilled water 1 litre
pH 7.4±0.2

All the ingredients except mannitol and phenol red were dissolved in distilled water and pH adjusted to 7.4. Added mannitol & phenol red and dissolved by boiling. Sterilized by autoclaving at 121°C for 15 min.

3.2.6 Modified Yeast Glucose Broth (Pradeep Kumar Reddy, 1991)

Peptone 15.0 g
Yeast extract 10.0 g
Sodium chloride 1.0 g
D-glucose 5.0 g
Beef extract 5.0 g
Distilled water 1 litre
pH 7.0

All ingredients except dextrose were dissolved in distilled water and pH
adjusted to 7.0. Then dextrose was dissolved in it and autoclaved at 121°C for 15 min.

3.2.7 Modified Yeast Glucose Agar (Pradeep Kumar Reddy, 1991)

Modified Yeast Glucose broth 1000 ml  
Agar-agar 15.0 g  
pH 7.0

All ingredients except dextrose were dissolved in Modified Yeast Glucose broth and pH adjusted to 7.0. Then Agar was dissolved in it and autoclaved at 121°C for 15 min.

3.2.8 Modified Yeast Glucose Agar with 2% salt (Pradeep Kumar Reddy, 1991)

Modified Yeast Glucose broth 1000 ml  
Sodium chloride 20.0 g
All ingredients except dextrose were dissolved in Modified Yeast Glucose broth and pH adjusted to 7.0. Then Agar was dissolved in it and autoclaved at 121°C for 15 min.

All ingredients except dextrose were dissolved in distilled water and pH adjusted to 7.0. Then dextrose was dissolved in it.
and autoclaved at $121^0C$ for 15 min.

3.2.9 Nutrient Agar with 2 % agar (Harrigan, 1998)

Peptone 10.0 g  
Beef extract 10.0 g  
Sodium chloride 5.0 g  
Agar-agar 20.0 g  
Distilled water 1 litre  
pH 7.2

The above ingredients except agar were dissolved in distilled water, pH adjusted to 7.0, agar was added and autoclaved at $121^0C$ for 15 min.

3.2.10 Peptone water (Harrigan, 1998)

Tryptone 10.0 g  
Sodium chloride 5.0 g  
Distilled water 1 litre  
pH 7.1

Both tryptone and sodium chloride were dissolved in distilled water, pH adjusted to 7.1, distributed into test tubes and autoclaved at $121^0C$ for 15 min.

3.2.11 Standard plate count broth (Harrigan, 1998)

Tryptone 5.0 g  
Yeast extracts 2.50 g  
D-glucose 10.0 g  
Distilled water 1 litre  
pH 7.0
All ingredients were dissolved in distilled water, pH adjusted to 7.0, distributed into test tubes and autoclaved at 121°C for 15 min.

### 3.2.12 Standard plate count Agar (Harrigan, 1998)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Yeast extracts</td>
<td>2.50 g</td>
</tr>
<tr>
<td>D-glucose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 litre</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
</tr>
</tbody>
</table>

All ingredients were dissolved in distilled water, pH adjusted to 7.0, distributed into test tubes and autoclaved at 121°C for 15 min.

### 3.2.13 Salmonella Shigella Agar (Harrigan, 1998)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Bile salt</td>
<td>8.5 g</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>8.5 g</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Brilliant green</td>
<td>0.00033 g</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.025 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 litre</td>
</tr>
<tr>
<td>Agar-agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>pH</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

All ingredients except lactose, brilliant green &
neutral red were dissolved in distilled water, pH adjusted to 7.0, then lactose, brilliant green & neutral red were dissolved in it and autoclaved at 121\(^0\)C for 15 min.

3.2.14 Simmon's Citrate Agar (citrate utilization) (Harrigan, 1998)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Magnesium chloride, heptahydrate</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Ammonium dihydrogen phosphate</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Bromothymol blue (1% aqueous solution)</td>
<td>8 ml</td>
</tr>
<tr>
<td>Agar-agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>990 ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
</tr>
</tbody>
</table>

All ingredients except Bromothymol blue were dissolved in distilled water and pH adjusted to 7.0. Then added Bromothymol blue, dissolved, transferred to test tubes and autoclaved at 121\(^0\)C for 15 min.

3.2.15 Triple Sugar Iron Agar (Harrigan, 1998)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>D-glucose</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
</tbody>
</table>
Sodium thiosulphate 0.2 g
Diammonium Iron sulphate 0.2 g
Phenol red (1% aqueous solution) 2.5 ml
Agar-agar 15.0 g
Distilled water 1 litre
pH 7.4

All ingredients except lactose, sucrose, D-glucose & phenol red were dissolved in distilled water and pH adjusted to 7.4. Then dissolved lactose, sucrose, D-glucose & phenol red, distributed into test tubes and autoclaved at 121°C for 15 min.

3.2.16 Violet Red Bile Agar (Harrigan, 1998)

Peptone 7.0 g
Yeast extract 3.0 g
Bile salts 1.5 g
Sodium chloride 5.0 g
Lactose 10.0 g
Neutral Red (1% aqueous solution) 3 ml
Crystal Violet (0.05% aqueous solution) 4 ml
Agar-agar 15.0 g
Distilled Water 1 litre
pH 7.4

Peptone, yeast extract, bile salts and sodium chloride were dissolved in water by steaming. Cooled to 50°C adjusted the pH to 7.4. Lactose, agar, neutral red and crystal violet were added and boiled well until the ingredients dissolved. The medium was sterilized by autoclaving at 121°C for 15 min.

3.2.17 Yeast Glucose Broth (Harrigan, 1998)

Peptone 10.0 g
Meat extract 10.0 g
Sodium chloride 5.0 g
Dextrose 5.0 g
Yeast extract 3.0 g
Distilled water 1 litre
pH 7.0

All ingredients except dextrose were dissolved in distilled water and pH adjusted to 7.0. Then dextrose was dissolved and autoclaved at 121°C for 15 min.

3.3 DILUENT USED

3.3.1 Sodium citrate 2% (Harrigan, 1998)
Trisodium citrate (20g) was dissolved in 1 litre of distilled water, distributed in 99ml & 9ml aliquots into dilution bottle & test tube respectively and autoclaved at 121°C for 15 min.

3.3.2 Physiological saline (Harrigan, 1998)
Sodium chloride (8.5 g) was dissolved in 1 litre of distilled water, distributed in 99ml & 9ml aliquots into dilution bottle & test tube respectively and autoclaved at 121°C for 15 min.

3.4 STAINS USED

3.4.1 Crystal violet stain
Crystal violet 0.5 g
Distilled water 100 ml

Crystal violet was weighed and triturated in a clean pestle and mortar. Distilled water was added, mixed thoroughly, filter and stored in a brown bottle.

3.4.2 Gram’s iodine solution
Iodine 1.0 g
Potassium iodide 2.0 g
Distilled water 100 ml

Iodine and potassium iodide were weighed and triturated in a clean pestle and mortar. Distilled water was added, mixed thoroughly, filter and stored in a brown bottle.

3.4.3 Malachite green solution
Malachite green 0.01 g
Distilled water 100 ml
Malachite green was weighed and tritculated in a clean pestle and mortar. Distilled water was added, mixed thoroughly, filter and stored in a brown bottle.

3.8.4 Safranin solution
Safranin 0.25 g
Distilled water 100 ml
Safranin was weighed and tritculated in a clean pestle and mortar. Distilled water was added, mixed thoroughly, filter and stored in a brown bottle.

3.9 REAGENTS USED IN BIOCHEMICAL TESTS
3.5.1 Kovac’s indole reagent
Amyl alcohol 150 ml
p-Dimethylaminobenzaldehyde 10.0 ml
Concentrated hydrochloric acid 50.0 ml
The aldehyde was dissolved in alcohol, the slowly added the acid and stored in the refrigerator in a brown reagent bottle.

3.5.2 Methyl red solution
Methyl red 0.1 g
Ethanol (95%) 300 ml
Distilled water to 500 ml
The methyl red was dissolved in the ethanol and made up to 500 ml with distilled water.

3.5.3 Voges-Proskauer test reagent
a) Potassium hydroxide 16 g
   Distilled water 100 ml
Potassium hydroxide was dissolved in the ethanol and made up to 500 ml with distilled water.

b) α - Naphthol 6%
   Ethanol (95%) 100 ml
α-Naphthol was dissolved in the ethanol and stored in brown bottle.

3.6 MILK AND MILK PRODUCTS
3.6.1 Skim milk: Reconstituted skim milk was prepared by dissolving 9 g of skim milk powder in 100 ml. of distilled water. The powder manufactured by Mandya district co-operative milk producers societies union limited, Product Dairy, Gejjalagere-571428 marketed by KMF, Bangalore was procured locally.

3.6.2 Whole milk: Freshly drawn whole milk from University Dairy farm, Bangalore-24 with 3.5% fat and 8.5% SNF was used in this study.

3.6.3 Khoa and Paneer: Khoa and Paneer manufactured and marketed by Nandini Milk Products Pvt. Limited Bangalore were used in the present study.

3.7 REAGENTS REQUIRED FOR DNA ESTIMATION (Davis et.al. 1986)

3.7.1 TRIS Buffer of 10mM (pH 8.0)
Solution A - 0.2M solution (Dissolved 24.2g of Hydroxy methyl amine in 100ml. of distilled water)
Solution B – 0.2M HCl
Mixed 50ml of solution A with 26.8ml of solution B to obtain 0.2M solution of pH8.0 TRIS buffer. Diluted this solution to obtain 10mM TRIS buffer.

3.7.2 SODIUM CHLORIDE 10mM solution
Prepared 1M solution by dissolving 58.5g of sodium chloride in 1000ml of distilled water and diluted to obtain 10mM

3.7.3 EDTA 10mM solution
Prepared 1M solution by dissolving 404.5g in 1000ml of distilled water and diluted to obtain 10mM

3.8 DETAILS ABOUT DOMESTIC MICROWAVE
3.9 CHARACTERIZATION OF BACTERIAL CULTURES

In this study, raw milk was used for the isolation of various types of bacteria required for the investigation.

3.9.1 Isolation of bacterial cultures

Raw milk collected from University Dairy farm was serially diluted and plated on different selective media such as Endo agar for *E.coli*, Salmonella Shigella Agar (SSA) for *Salmonella sp.*, Cetrimide Agar (CA) for *Ps.aeruginosa* and Mannitol Salt Agar (MSA) for *Staph.aureus*. To isolate *B.subtilis*, the milk sample was first heat treated to 80°C for 10 min, to kill all vegetative cells cooled to room temperature and then plated on 2% Nutrient agar to obtain spore count. The plates were incubated at appropriate temperatures and types of colonies formed were noticed. Dark red with metallic sheen colonies on Endo agar, black nucleated colonies on SSA, blue green colonies form on CA, yellow coloured colonies from MSA and all colonies formed on 2% Nutrient Agar were selected and carried out Gram’s staining. After confirming for Gram’s reaction, purification of the isolate was done by streaking on to Standard Plate Count Agar for three times. The purified isolates were maintained on SPCA slants. The stock cultures of the isolates were subcultured once in 15 days onto the slants and for working culture in SPC broth.

3.9.2 Identification of bacterial cultures
The young cultures of purified isolates were subjected to preliminary tests such as Gram’s reaction, spore staining, catalase and oxidase test (Harrigan, 1998). Then the isolates were further subjected to a battery of biochemical tests and growth studies for identification as shown in Keys 1-5 (Krieg and Holt, 1984 and Sneath et al., 1986)

**KEY 1: ISOLATION & IDENTIFICATION OF E.coli**

1. **RAW MILK**
2. PLATED ON ENDO AGAR
3. GRAM STAINING
4. GRAM NEGATIVE RODS
5. OXIDASE TEST
6. NEGATIVE
7. CATALASE TEST
8. POSITIVE
9. TRIPLE SUGAR IRON HYDROGEN SULPHIDE AGAR (TSI H₂S TEST)
   - ACID SLANT, ACID BUTT, NO H₂S & GAS PRODUCTION
   - INDOLE TEST - Positive; METHYL RED TEST - Positive
   - VOGES PROSKAUER TEST - Negative; CITRATE - Negative

**IDENTITY - E.coli**

**KEY 2: ISOLATION & IDENTIFICATION OF Salmonella Sp.**

1. **RAW MILK**
2. PLATED ON SS AGAR
3. GRAM STAINING
4. GRAM NEGATIVE RODS
5. OXIDASE TEST
6. NEGATIVE
7. CATALASE TEST
POSITIVE

TRIPLE SUGAR IRON HYDROGEN SULPHIDE AGAR
(TSI H₂S TEST)

ACID SLANT
ACID BUTT
H₂S PRODUCED

↓

NO

GAS PRODUCTION

INDOLE TEST - Negative, METHYL RED TEST - Negative
VOGES PROSKAUER TEST - Negative CITRATE - Negative

IDENTITY - *Salmonella sp.*

KEY 3: ISOLATION & IDENTIFICATION OF *Ps.aeruginosa*

CHILLED RAW MILK

↓

PLATED ON CETRIMIDE AGAR

↓

CREAM COLOURED COLONIES

↓

GRAM STAINING

↓

NEGATIVE RODS
OXIDASE TEST

POSITIVE

CATALASE TEST

POSITIVE

HUGH AND LEIFSON’S OXIDATIVE FERMENTATIVE TEST

O$^+$F$^-$

IDENTITY – *Ps aeruginosa*

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KEY 4: ISOLATION & IDENTIFICATION OF *Staph. aureus*

RAW MILK

PLATED ON MANNITOL SALT AGAR

GRAM STAINING

POSITIVE

CATALASE TEST

POSITIVE

OXIDASE

POSITIVE
HUGH LEIFSON’S O/F TEST

O+F*

IDENTITY – Staph. aureus

KEY 5: ISOLATION & IDENTIFICATION OF B. subtilis

RAW MILK

HEAT TREATED TO 80°C / 10 Min

PLATED ON 2% NUTRIENT AGAR

GRAM’S STAINING

GRAM POSITIVE RODS

SPORE STAINING

SPORE FORMERS

OXIDASE TEST

POSITIVE
3.9.2.1 Gram’s staining technique

Young culture of bacterial isolate was made into thin smear on a clean grease free slide and heat fixed. The smear was treated with crystal violet solution for 1 minute and washed with water. Then smear was flooded with Gram’s iodine and left for 1 min. The smear was decolorized with alcohol; finally counter stained with safranine; washed with water, air dried and observed under 100X using cedar wood oil. Purple coloured cell as are Gram positive cells while pink coloured cells were Gram negative cells.

3.9.2.2 Spore staining technique

Young culture of bacterial isolate was made into thin smear on a clean grease free slide and heat fixed. The smear was treated with saturated solution of malachite green and heated over the mantle till the stain boils. The smear was then washed with water, stained with safranine for 30 seconds. The stain was poured off and again washed with water; air dried and observed under 100X using cedar wood oil. Green coloured cell were the bacterial spores while pink coloured cells were vegetative bacterial cells.

3.9.2.3 Catalase test

A loopfull of 18 hour broth culture was placed on a clean slide and a drop of hydrogen peroxide(3%) was mixed with the culture. This presence of catalase was indicated by the liberation of free oxygen as gas bubbles.

3.9.2.4 Oxidase test

A drop of 1% aqueous solution of tetramethyl-p-phenylene diamine dihydrochloride was added to a strip of filter paper in a sterile petriplate. With Pasteur pipette some young solid culture of bacteria was smeared on to the impregnated filter paper. The oxidase positive reaction was indicated by development of purple colour within 60 seconds.

3.9.2.5 Indole test
Inoculations were made in glucose phosphate peptone water from young agar slants and incubated at 37°C for 2-7 days. After incubation, 0.5 ml of Kovac’s reagent was added to 2 ml of the culture and mixed well. A deep red colour developed in the presence of indole that separated out in the alcohol layer was considered as positive.

3.9.2.6 Methyl red test

Inoculation were made in glucose phosphate peptone water from young agar slants and incubated at 37°C for 2-7 days. After incubation for 2 days, five drops of methyl red reagent was added to 5 ml of the culture. The positive cultures showed red colouration in the medium. Yellow colouration was recorded as negative.

3.9.2.7 Voges Proskauer test

Inoculation were made in glucose phosphate peptone water from young agar slants and incubated at 37°C for 2-7 days. After incubation for 2 days, five drops of methyl red reagent was transferred to 5 ml of culture. The positive cultures showed red colouration in the medium. Yellow colouration was recorded as negative.

3.9.2.8 Citrate utilization test

Simmon Citrate agar slants were inoculated from young slant cultures by streaking over the surface. The tubes were incubated at 37°C/2-7 days. The utilization of citrate and growth on the citrate agar resulted in an alkaline reaction, changing the bromothymol blue indicator in the medium from green to Persian blue. When no growth occurred and citrate was not utilized, the colour of the medium remained unchanged.

3.9.2.9 Triple sugar iron agar test

The inoculation of young isolates were made by stabbing into the butt and streaking on the T.S.I. hydrogen sulphide agar slants from young agar slants. The tubes were incubated at 37°C/2-7 days. Fermentation of sugars that led to acid production changed the colour of the medium from wine red to yellow if produced in butt or in the slant portion and read as acid butt and acid slant. The gas production was noticed as breaks in the agar, gas packets or movement of butt from below. Blackening of the medium after incubation for 24 hours was considered to be positive for hydrogen sulphide production.

3.9.2.10 Growth at 65°C
Sterile standard plate count broth were inoculated with broth cultures of isolates and incubated at 65°C for 2-7 days. Development of turbidity in test tube was taken as positive evidence of growth of the culture.

3.9.2.11 Oxidative and fermentative test

Two butts of sterile Hugh Leifsons semisolid medium were stab inoculated with young culture of bacteria. One of the inoculated tube was sealed with sterile liquid paraffin about 2 cm. length. Both the tubes were incubated at 37°C/5-7 days, the colour change from purple to yellow indicate positive reaction.

3.9.2.12 Observation of pigmentation

Poured plates of standard plate count agar were streaked with young culture and incubated at 37°C/2 days. Red colouration at the back of colony should be observed.

3.9.2.13 Maintenance of bacterial isolates

The stock bacterial isolates were maintained in MYGA slants and subcultured once in a month. Between subculturing the isolates were stored in refrigerator.

The working bacterial isolates were subcultured once in a week, and maintained in the MYG broth. The purity was tested by Gram’s staining and stored in a refrigerator.

3.10 RECORDING OF TEMPERATURES OF DIFFERENT MENSTRA EXPOSED TO MICROWAVE TREATMENT

Microwave oven of domestic type used in the study was operated at 2450 frequency. In order to record the temperature raise on microwave treatment, 50 ml quantity of modified yeast glucose broth, skim milk or whole milk in different sterile conical flask were kept in microwave oven, and exposed to different periods for upto 50 sec. At intervals samples were drawn and tested for the temperature raise in the samples. Khoa & Paneer samples taken in glass dishes were also exposed to microwave for 60 sec and at intervals the samples were drawn and examined for the temperature raise in the samples. As the temperature recorded in the samples at different places showed different temperature readings, correct recording of temperature was not established and hence discontinued.

3.11 SURVIVABILITY OF BACTERIAL CULTURES IN DIFFERENT
MENSTRA EXPOSED TO MICROWAVE

All the bacterial cultures were individually added to MYG broth, skim milk, whole milk, khoa and paneer exposed to microwaves for different periods.

3.11.1 Survivability of bacterial cultures in Modified Yeast Glucose (MYG) broth:

All the experimental cultures were grown individually in MYG broth for 24 hrs. at 37°C, appropriate quantity of the broth culture, so as to have a known number of these cultures was added to MYG broth taken in conical flask, and exposed to microwave treatment in a microwave oven for up to 30 secs. At intervals, the broth samples were drawn cooled to room temperature by using chilled water, plated on to selective media and MYGA and incubated at appropriate temperature to determine the number of survivors.

3.11.2 Survivability of bacterial cultures in skim milk and Whole milk

Skim milk/whole milk taken in flask was inoculated with different cultures individually and the procedure mentioned above was followed.

3.11.3 Survivability of bacterial cultures in khoa and paneer

A known quantity of khoa/paneer (50g) was taken into a sterile mortar-pestle and to it known quantity of overnight culture was added. After thorough mixing, the samples were transferred aseptically into conical flasks and exposed to microwave as explained above. The number of survivors was determined using selective media and MYGA.

3.12 ENUMERATION OF HEAT INJURED CELLS OF TEST CULTURES EXPOSED TO MICROWAVE IN DIFFERENT MENSTRA

Although there are no well established procedures to determine the number of injured and stressed cells of different types of microbes, a study has been reported that stressed and heat injured cells of certain cultures fail to form colonies when plated on agar medium containing 2% sodium chloride, as salt would prevent the injured cells to get their damage repaired and become healthy cells. In this study an attempt was made to assess the number of salt sensitive cells upon exposure to microwaves. This was carried out by adding test cultures individually to broth, skim milk, whole milk, paneer and khoa and these were exposed to microwave for a period of upto 60 sec and at intervals, the samples drawn were plated on MYGA to get both healthy and injured cells and MYGA plus 2% salt to get only healthy cells and incubated at 37°C. The numbers of colonies formed were enumerated and the difference between the counts of two agars was considered as injured cells.

3.13 ESTIMATION OF DNA AND PROTEIN RELEASED DURING MICROWAVE EXPOSURE OF BACTERIAL CULTURES

Exposure of bacterial cells to microwave leads to release of DNA and protein from the cells due to weakening & disintegration of cell wall and cell membrane. In this study all the bacterial cultures were grown in MYG broth at 37°C for 18 hrs. After incubation, the cultures were centrifuged at 8000 rpm for 15
min and cell pellets were suspended in 100 ml TRIS buffer to have a cell count of 10⁷/ml. The cell suspension was distributed in 50 ml quantity in two flasks and one of the flasks was subjected to heating at 80°C/10min and the other flask was exposed to microwave for 30 sec. The cell suspension was then cooled in chilled water and centrifuged at 8000 rpm for 15min. The supernatant collected was read for DNA at 260 nm and protein at 280 nm in a UV based spectrophotometer. The DNA concentration was calculated using the following formula (Devis et al, 1986):

\[ \text{DNA concentration} = (\text{OD}_{260}) \times (\text{dilution factor}) \times (50 \, \mu g \, DNA/1 \, \text{OD}_{260} \, \text{unit}) \]

and the protein concentration was calculated using the formula (Lowery et al., 1995):

\[ \text{Protein concentration (mg/ml)} = 1.45 \, D_{280} - 0.74 \, D_{260} \]

3.14 SHELF LIFE STUDIES OF MICROWAVE TREATED SKIM MILK AND WHOLE MILK

Skim milk and whole milk were inoculated with 10⁵ per ml of test bacteria individually and subjected to microwave treatment in duplicate upto 50 sec, cooled and stored at ambient temperature/refrigeration temperature (5°C). On every third day ambient temperature stored samples were drawn while on every fifth day the refrigerated samples were drawn and examined for physico-chemical tests such as COB, TA, alcohol alizarin test & curdling. At these intervals the samples were examined for the number of survivors until spoilage of milk occurred.

3.15 SHELF LIFE STUDIES OF KHOA AND PANEER

Paneer and Khoa samples were weighed at 50 gm. each into 150 ml sterile glass flasks and sterilized at 121°C/15min. These samples were cooled and inoculated with selected bacteria at 10⁵ cells/ml separately in duplicates. Then the inoculated samples were exposed to microwave for upto 60 sec, cooled and one set was stored at ambient temperature and the other at refrigeration temperature. On every third day, ambient temperature stored samples were recorded for their physico-chemical changes while on every fifth day the refrigerated samples were examined for physico-chemical changes. At these intervals treated samples were examined for survivors through serial dilution technique using MYGA.

3.16 EFFECT OF POLYETHYLENETHERAPHTALATE (PET) PACKAGING ON
THE SHELF LIFE OF KHOA AND PANEER

Paneer and Khoa samples were weighed at 50 gm. each into sterile glass flasks and sterilized at 121°C/15min. The samples were cooled and inoculated with selected bacteria at 10^5 cells/ml separately in duplicates and transferred to polyethylenterephthalate (PET) sachet. Then the samples were exposed to microwave for 60 sec after making a pin hole by piercing the sachet with a sterile pin in order to allow escape of expanded air from the sachet, during microwave exposure, cooled and one set stored at ambient temperature and the other at refrigeration temperature. On every third day, ambient temperature stored samples were recorded for their physico-chemical changes while on every fifth day the refrigerated samples were examined for physico-chemical changes. At these intervals, treated samples were examined for both survivors through serial dilution technique using MYGA.

3.16 POWER DENSITY

In this study a sample size of 50 gm. was used in a microwave oven which had a wattage of 800 & the power density was calculated as 800/50=16 w/gm.

3.17 STATISTICAL ANALYSIS

Statistical analysis has been carried out using ANOVA technique (Zar, 2003)