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Destruction of *Escherichia coli* and *Salmonella typhimurium* in Microwave-cooked Soups

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ABSTRACT

Single serving (i.e. 200 ml) portions of tomato soup, vegetable soup, and broth inoculated with *Escherichia coli* or *Salmonella typhimurium* at about 10^7 organisms/ml were exposed to 915 MHz microwaves. After various timed exposures the temperature of the top, middle, and bottom regions as indicated by changes in previously positioned assemblies of temperature sensitive paper strips were noted and aliquots were removed from the same regions for standard plate count determination of survivors. For any given exposure time, the temperature of the middle region was warmest; that of the bottom, intermediate; and the temperature of the top, coolest. Despite the relative temperatures of the regions, however, the consistent finding was that, for any exposure time, the closer the sampled organisms were to the top, the lower their level of survival. In terms of temperature, it was noted that organisms in the top had declined to a given level of survival at a temperature lower than the temperature corresponding to the same survival level in the middle or bottom soup regions. These data suggest that the heat generated during the microwave exposure alone is inadequate to fully account for the nature of the lethal effects of microwaves for microorganisms.

One of the more intriguing aspects of the use of microwave energy has been the idea of achieving pasteurization or even sterilization of foods at lower temperatures or in shorter times than those required in conventional methods. This would be achieved only if such radiation had a selective killing effect on microorganisms that was independent of the heating of their surroundings (*2,3*). Even before development of the microwave oven in the 1940's many reports existed concerning the effects of the radio, dielectric, and microwave portions of the electromagnetic spectrum on microorganisms. Since development of electronic ovens even more reports have appeared, especially ones dealing with effects on microorganisms of the frequencies employed in such ovens. However, the consensus of these reports is that much controversy exists concerning the lethal effects of high frequency radiation on microorganisms. Many of the published data seem to indicate a non-thermal radiation effect on microbial survival, but there also exist several reports that attribute the lethal effects of such radiation solely to heat generated by the waves.

Most frequencies used by early investigators in this controversy were not actually within the defined microwave range (30-300,000 MHz); rather, they involved the radio and the dielectric portions of the energy spectrum. During the 1940's actual microwave frequencies were applied to food processing (*14*) and by 1965 consumer ownership of microwave ovens had become economically feasible (*6*). These developments prompted the Federal Communications Commission to set aside two frequencies for use in microwave ovens (*8*). It is with these two frequencies (915 and 2450 MHz) that several of the more recent works have been concerned.

Using a 2450 MHz microwave conveyer assembly, Olsen (*13*) reported a great reduction in viability of common bread mold spores. Bread slices inoculated with spores of *Aspergillus niger, Rhizopus nigricans,* and a *Penicillium* species were exposed for a total of 2 min during which time the highest recorded temperature was 65.6 C. Claiming that the reported thermal death point for *Aspergillus* and *Penicillium* is within the range of 68-71 C for 20 min, Olsen stated that his observed lethal results were probably not due to conventional thermal effects. In an experiment comparing water bath and microwave heating of several eucaryotic and procaryotic organisms, Olsen (*14*) found that, for a given degree of kill, lower temperatures were recorded in the microwave treatments. In the same report he stated that a comparison study revealed that chicken and shrimp cooked by a microwave-plus-steam process had lower total and *Salmonella/Proteus* counts than did samples processed by steam alone. The surface temperatures of cooked foods in both processes, however, did not differ radically.

Goldblith and Wang (*9*) were unable to demonstrate per se microwave effects in their work concerning irradiation (2450 MHz) of *E. coli* and spores of *Bacillus subtilis.* They concluded that microwave inactivation of the two organisms paralleled results that would be expected due to thermal inactivation alone. Lechowich et al. (*12*) also concluded that detrimental effects of 2450 MHz irradiation on *Streptococcus faecalis* and *Saccharomyces cerevisiae* were due to the heat generated by the microwaves and not to any radiation effects.

In a comparison study, Grecz et al. (*10*) claimed that controlled temperature microwave heating of suspensions of *Clostridium sporogenes* PA3679 spores was consistently more lethal than was conventional heating. However, microwave treatment of dried spores did not appreciably affect viability. Delany et al. (*7*) were also unable to show any significant decrease in viability after dried spores of *A. niger* and *B. stearothermophilus* were exposed to 915 or 2450 MHz microwaves. Craven and Lillard (*5*) heated chicken inoculated with *C. perfringens* spores with microwaves and reported that when spores were subsequently heat shocked a significant reduction

of viability occurred as compared to controls which were heat shocked only. They suggested that the microwave irradiation had affected either the heat resistance or germination requirements of the spores.

This investigation was undertaken to study the pattern of destruction of two bacteria, *Escherichia coli* and *Salmonella typhimurium* in microwave cooked soups in relation to the time of microwave exposure and the temperatures achieved during the exposure. An attempt was made to ascertain whether the lethality of the radiation was solely explainable in terms of heat generated in the surroundings of the bacteria by comparing patterns of destruction of the organisms to the pattern that would be expected on the basis of the heating pattern in the soups.

MATERIALS AND METHODS

Organisms

E. coli and *S. typhimurium* obtained from the stock culture collection of the Department of Microbiology, The Pennsylvania State University were used as test organisms. They were grown in Tryptic Soy Broth (Difco) for 18-24 h at 37 C before being inoculated at 1% concentration into the liquid foods (ca. 10^7 organisms/ml in the soups.) Viable cell counts of all cooked and uncooked foods were determined by the Standard Plate Count Method (*1*). Plates were incubated at 37 C for 24 h before counting.

Sample preparations

Liquids used were condensed tomato soup, vegetable soup (with beef stock), and beef broth (all obtained from Campbell Soup Co., Camden, NJ). Cans of soup were aseptically opened and the contents of each can were emptied into an 800 ml beaker. Sufficient distilled water was added, with occasional stirring, to bring the volume of diluted soup up to the 600 ml mark on the beaker. The beaker was then covered with aluminum foil and the contents were mixed with a magnetic stirring device (Thermolyne-Sybron Corp., Dubuque, IA) for 3-5 min. The pH of the diluted soup was measured with a Beckman Zeromatic SS-3 pH meter (Beckman Instruments, Inc., Fullerton, CA) with a Fisher Micro-Combination Electode (Fisher Scientific Co., Fairlawn, NJ). The pH values for vegetable soup, tomato soup, and beef broth were 5.2, 4.3, and 5.6, respectively.

For experiments on bacterial survival in single servings of microwave cooked soups, 200 ml of inoculated soup was decanted into specifically prepared 400 ml beakers. These beakers had had a temperature strip assembly (to be described in a later section) taped to the bottom center and another to the side wall at the 100 ml mark. After the soup was added, a third assembly was submerged about 1/8 inch below the surface of the liquid. For similar experiments involving triple servings (i.e., 600 ml), temperature strip assemblies were taped to the bottom and to the 300-ml mark of an 800-ml beaker. Again the third strip was submerged just below the soup surface. For experiments involving the use of the modified graduated cylinder (to be described in a later section), the temperature strip assemblies were positioned at the levels of the five side-arms and then 375 ml of inoculated soup were added. After soups had been decanted into these containers, 1 ml aliquots were removed for pre-cooking viable cell counts.

Cooking and timing procedures

The microwave oven used in these experiments was a Versatronic range, model Np. J896 (General Electric Co., Louisville, KY). The frequency of the radiation emitted in this oven was 915 MHz. The magnetron tube (source of the waves) was located beneath the bottom of the oven but the waves actually entered the oven via an antenna located in the center of the top oven wall.

The appropriate vessel containing the food was set in the center of the rotating shelf in the oven. Next, the oven door was latched, the ELECTRONIC POWER knob was turned to the "HI" setting and the ELECTRONIC TIMER knob was turned to a time setting which was at lease 1 min above the actual time desired. It had been determined that it was difficult to set this time to match accurately the desired cooking intervals and therefore the intervals were timed with a watch. A 1-min allowance on the timer was necessary so that the oven power did not automatically switch off before the desired cooking interval was completed. Since the magnetron tube required a 1-2 min "warm-up" period, timing did not actually begin until the ELECTRONIC POWER "ON" light had lit. This light signals that the magnetron tube is fully operational. At the end of the interval the ELECTRONIC POWER knob was manually switched to the "OFF" position thus cutting off the microwave source.

For experiments involving survival of bacteria in different regions of liquid foods, the soup portions were exposed for intervals up to 150 sec for single servings, up to 300 sec for soup contained in the modified graduated cylinder, and up to 10 min for triple servings.

Temperature measurements and sample removal

Temperature sensitive paper strips (THERMOPAPER strips; Paper Thermometer Co., Inc., Natick, MA) were used for all the temperature measurements in microwave cooked foods. Each strip was designed to change from a whitish-grey color to black when a certain temperature was achieved in the surroundings of the strip.

For use in this work, two series of graded temperature strips were constructed; one series contained strips for 38, 43, 46, 49, 54, and 60 C; whereas the other series consisted of 66, 71, 77, 82, 88, and 93 C (Fig. 1). Procedures for making the series were as follows: (*a*) individual

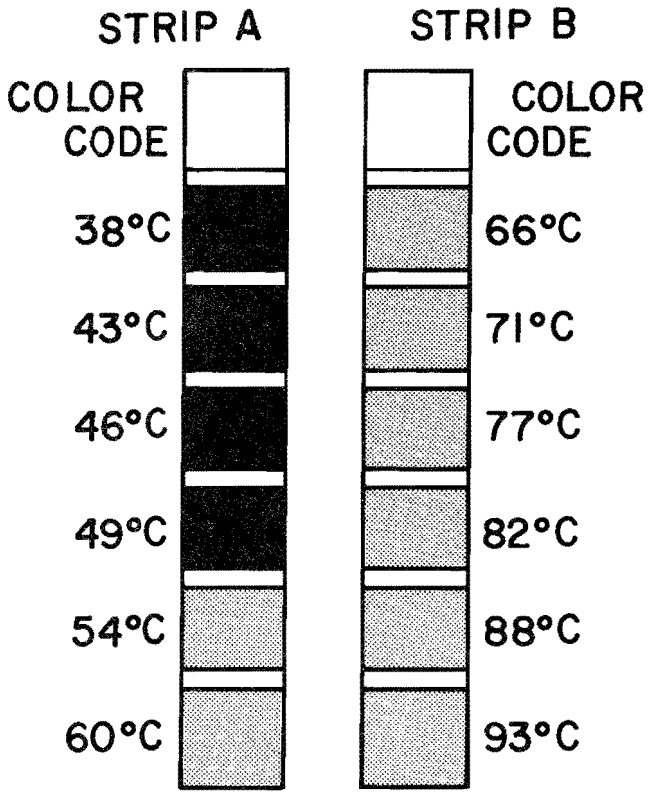

Figure 1. *Temperature sensitive paper strip assembly. The individual squares on the strips were designed to change from grey to black when the indicated temperature was reached in the surroundings of the strip. Visual inspection of Strip A indicates that a temperature of 49 C had been reached.*

strips were split into thirds down the long axes of the strips, (*b*) the 1/6 × 2 inches strips were then positioned longitudinally in orderly sequence on a sheet of achesive-coated paper, (*c*) the assemblage was cut latitudinally at 1/8 to 1/4 inch widths to yield the small temperature strip series, and (*d*) the two series of graded temperature strips with appropriate identifications were then securely "sandwiched" between two pieces of adhesive cellophane tape. The dimensions of this final temperature strip assembly were ca ½ × 1½ inches. The accuracy of

these strips to determine temperatures in heated water was tested against a mercury thermometer and a Mettler TM15 digital thermometer (Mettler Instrument Corp., Princeton, NJ).

In studying bacterial survival in relation to temperature at various positions within soup samples, temperature measurements as indicated by color change of the strips were recorded immediately after the exposure to microwaves. One-milliliter aliquots for determination of remaining viable cells were removed sequentially from bottom to top (unless otherwise noted) with a sterile pipette (samples in beakers), or were removed sequentially from top to bottom with a sterile 2-ml syringe fitted with a 21 gauge needle (samples in the modified graduated cylinder).

In all instances, samples for viable cell counts were removed from the cooked foods as quickly as possible to minimize the aftercooking heat effects on bacterial survival. Approximately 10-15 sec were required to remove samples from bottom, middle, and top regions of the soup in beakers, and 20-25 sec were required to remove the five samples from the modified graduated cylinder.

Modified graduated cylinder

The modified cylinder (Fig. 2) consisted of a 500-ml size graduated

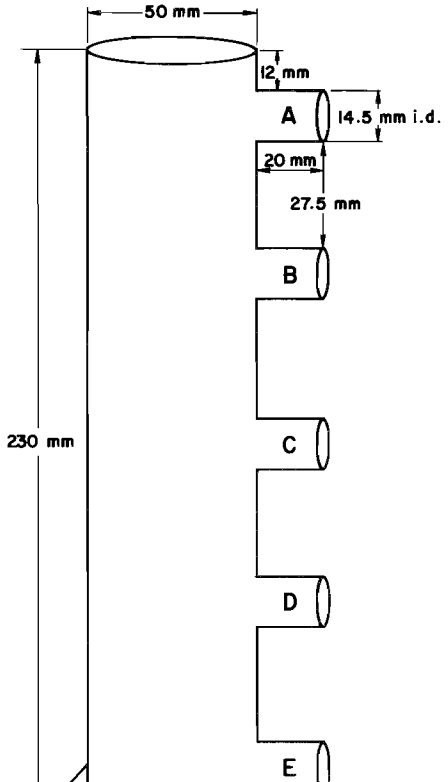

Figure 2. *The Modified Graduated Cylinder. Dimensions of the components are as labelled.*

cylinder with five short glass tubes (side-arms) attached to the cylinder at regular intervals. The open end of the side-arms were sealed with rubber serum caps. The use of the serum caps facilitated repeated samples withdrawal and at the same time minimized the chance of leakage. When filled to about 4 mm from the top, the cylinder accommodated 375 ml of liquid.

RESULTS

Survival patterns of E. coli and S. typhimurium in microwave cooked soups

For both *E. coli* and *S. typhimurium* results of three determinations of survival and temperature in bottom, middle, and top regions during timed microwave exposures of single serving portions (i.e., 200 ml) of soups were averaged. These values were then used to plot % survival versus exposure time as well as % survival versus temperature. Plots of temperature versus exposure time were also compiled for the three regions of the soup.

Tomato and vegetable soups. Results of experiments using tomato and vegetable soups were quite similar and comparable. Graphical data are presented only for tomato soup, but the findings, except was noted, pertain also to the data collected for vegetable soup.

The temperature profile for the three regions of tomato soup (Fig. 3) shows that, at any time, the middle

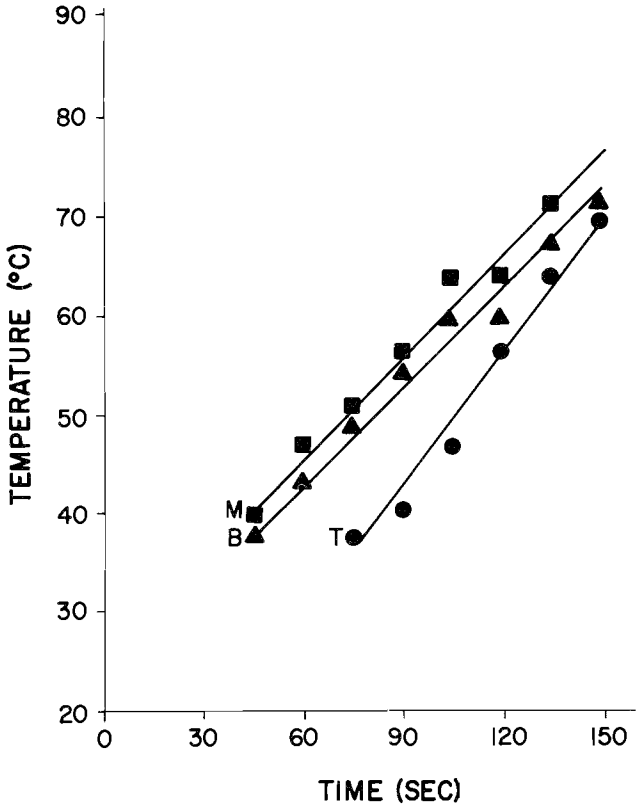

Figure 3. *Temperature profile of single serving of uninoculated tomato soup during microwave exposure. (Profiles obtained from inoculated soup showed consistent results.) Circles indicate Top (T), Squares indicate Middle (M), and Triangles indicated Bottom (B) regions of soup.*

region of this soup had the warmest temperature, while the top region exhibited the coolest temperature. Figure 4 and Figure 5 represent the decrease in survival with increasing exposure time for *E. coli* and *S. typhimurium* respectively in tomato soup. From these graphs it can be seen that, for any given time, organisms in the top region had the lowest percentage survival even though, according to data in Figure 3, this was the region of lowest temperature at that time. The organisms in the middle region (the warmest region from Fig. 3) had an intermediate level of survival and those in the bottom of the soup had the greatest survival over the entire heating period.

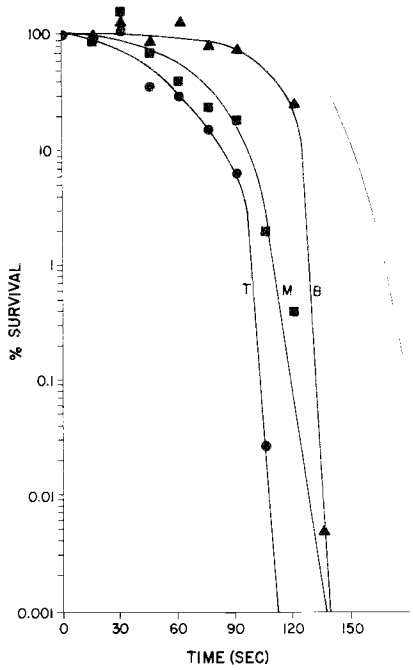

Figure 4. *Decrease in survival of* Escherichia coil *in single serving of tomato soup with respect to microwave exposure time. Circles indicate Top (T), Squares indicate Middle (M), and Triangles indicate Bottom (B) regions of soup.*

Further evidence that organisms in the top region were being inactivated at lower temperatures than organisms in the other regions of the soup is provided in Figures 6 and 7. These graphs present the decrease in survival of *E.*

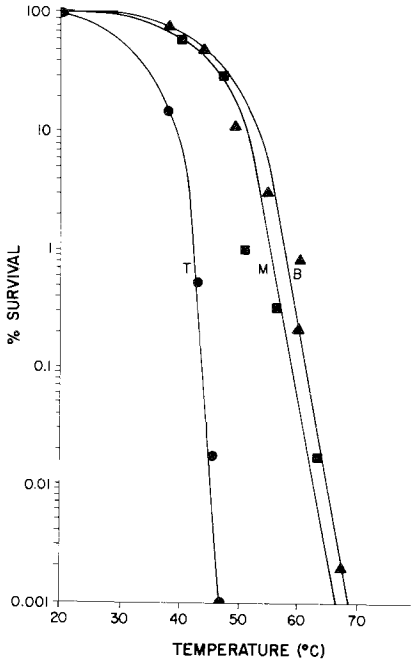

Figure 5. *Decrease in survival of* Salmonella typhimurium *in single serving of tomato soup with respect to microwave exposure time. Circles indicate top (T), Squares indicate Middle (M), and Triangles indicate Bottom (B) regions of soup.*

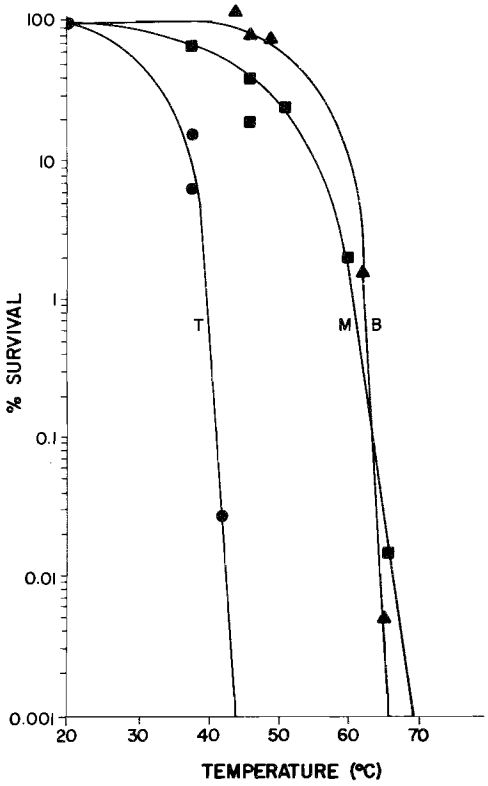

Figure 6. *Decrease in survival of* Escherichia coli *in single serving of tomato soup with respect to temperatures reached during microwave exposure. Circles indicate Top (T), Square indicate Middle (M), and Triangles indicate Bottom (B) regions of soup.*

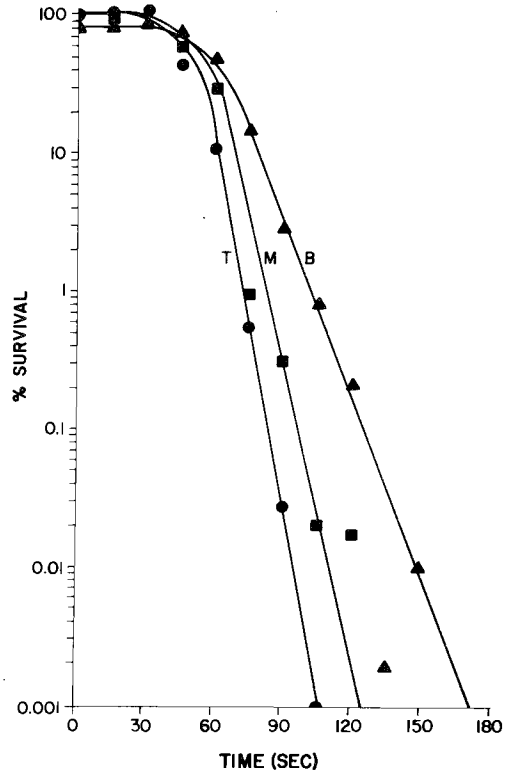

Figure 7. *Decrease in survival of* Salmonella typhimurium *in single serving of tomato soup with respect to temperatures reached during microwave exposure. Circles indicated Top (T), Squares indicate Middle (M), and Triangles indicate Bottom (B) regions of soup.*

coli and *S. typhimurium* respectively in terms of the temperatures achieved in the three tomato soup regions. From these graphs it can be noted that, for any given temperature, organisms at the top of the soup had decreased to the lowest level of survival. Organisms in the middle region generally had the intermediate survival, and organisms in the bottom of the liquid had the greatest percentage survival. Within the range of 30-60 C, the temperature at which a given percentage survival was reached in the top region was up to 22° (25° for vegetable soup) lower than the temperature at which that same percentage survival was reached in the bottom region.

Data collected for this experiment also indicate that viable *E. coli* were not detectable in the top tomato soup region when the temperature had reached ca. 45 C (55 C in vegetable soup). *S. typhimurium* was no longer detectable in the top region when the temperature had reached ca. 48 C (50 C in vegetable soup). In contrast, the temperature had reached 65-70 C before either organism was undetectable in the middle or bottom regions of either soup.

Beef broth. The temperature profile of beef broth (data not shown) indicates that the middle portion of the soup was the warmest region throughout the microwave heating period, as had been found in the other two soups. However, in beef broth, unlike the situation in the other soups, the bottom region was the coolest region during the exposure periods, and the top area had the intermediate temperature. Also, the temperature difference between the three regions at any given time was less in beef broth as compared to the other two soups (see Fig. 3).

The patterns of survival of both organisms in the three regions of beef broth in terms of exposure time or temperature were similar. At any given time organisms in the top region had the lowest survival, those in the middle had intermediate survival, and organisms in the bottom had the highest survival (data not shown). These results were similar to those found in tomato and vegetable soups (Fig. 4 and 5).

With respect to a given temperature, organisms in the top of the beef broth again had the lowest level of survival. Up to ca. 52 C, the middle was the region of intermediate survival and the bottom was the region of greatest survival with respect to temperature. In tomato and vegetable soups the difference in the temperatures which corresponded to given levels of survival in the top and bottom regions ranged up to 22-25°. In beef broth the temperature range was only 5-7°.

In the top region of beef broth, *E. coli* was non-detectable by the time the temperature had reached ca. 55 C; *S. typhimurium,* by the time the temperature had reached ca. 60 C. Organisms in the middle and bottom regions of this soup were non-detectable by the time the temperature had reached ca. 62-67 C.

Relationship of sampling procedures and soup volume to survival of E. coli in microwave cooked tomato soup

In the previous experiments, heated soups had been sampled sequentially from bottom to top (i.e., BMT). It was therefore necessary to determine whether the delay (10-15 sec) between removal of aliquots from the top and bottom regions influenced the pattern of survival of organisms in the three regions. In this experiment, samples from single servings of tomato soup inoculated with *E. coli* were removed sequentially from top to bottom (i.e., TMB) for viable counts of remaining cells. Duplicate determinations were made for each time period and results were averaged.

The results (data not shown) indicated that the sequence of removal of aliquots at the end of heating periods had no effect on the pattern of survival in the three soup regions. With respect to any given exposure time, organisms in the top region of the TMB-sequence soup still had declined to the lowest survival values, organisms in the middle region had intermediate levels of survival, and organisms in the bottom of the soup showed the greatest survival. In terms of any given temperature, it was once again evident that organisms in the top had the least survival, organisms in the middle had the intermediate survival levels, organisms in the bottom had the greatest survival. Also there was a difference of up to 23° between the temperatures in the top and bottom of the TMB-sequenced soup for which the same percentage survivals in these regions was achieved. This compares to a difference of 22° in the soup which was sampled in the reverse (i.e., BMT) sequence.

Although the sequence of sample removal had no effect on the pattern of survival of *E. coli* in tomato soup, increasing the volume of soup to 600 ml did show a difference in some of the results. The temperature profile for the larger soup volume was similar to that for the smaller volume in that the middle was the warmest region, the bottom had the intermediate temperatures, and the top was the coolest region throughout the microwave exposure. Also, in terms of exposure times, the pattern of survival of *E. coli* in the triple volume was similar to the pattern in the single serving volume. For almost all of the heating period, organisms at the top had the lowest survival, those in the middle had intermediate values, and organisms sampled from the bottom had the greatest survival. However, with respect to temperatures in the three regions, there was a difference in the survival pattern. Although for any given temperature, organisms in the top declined to the lowest levels, the relative levels of survival for the middle and bottom regions are reversed in comparison to the findings for the smaller soup volume. In the triple serving, it was the bottom which showed intermediate levels and the middle showed the greatest survival. The difference in the survival between middle and bottom regions in all experiments was relatively small compared to the difference between these regions and the top region.

Survival of E. coli in microwave cooked tomato soup contained in a modified graduated cylinder

The temperature profile (data not shown) of tomato soup inoculated with *E. coli* during the microwave exposures reveals that position D (located between the middle and bottom; see Fig. 2) and position C (the middle) had the warmest temperatures throughout the heating period. Position B (between the top and middle) was consistently the coolest region. Positions E (the bottom) and A (the top) were the intermediate temperature regions. This profile is similar to that for the single and triple servings of tomato soup in which the middle was the warmest region, the bottom was intermediate, and the top was coolest. In terms of exposure time, the pattern of survival in the modified cylinder was also consistent with previous findings. Figure 8 shows, for any given time, the closer the sam-

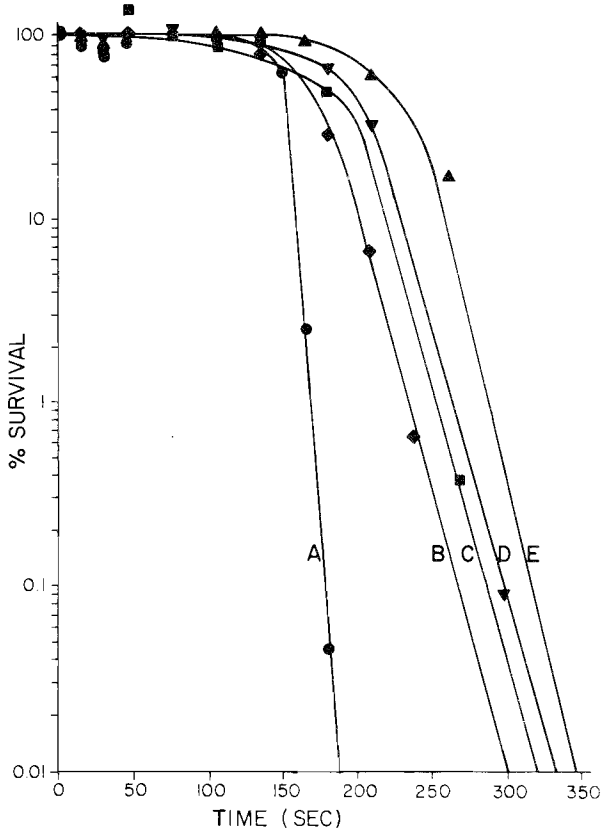

Figure 8. *Decrease in survival of* Escherichia coli *in tomato soup contained in the modified graduated cylinder with respect to microwave exposure time. Circles indicate Position A (Top), Diamonds indicate Postion B (Between Top and Middle), Squares indicate Position C (Middle), Inverted Triangles indicate Postion D (Between Middle and Bottom), and Triangles indicated Postion E (Bottom).*

pling level was to the top of the liquid the greater the decrease in survival of the organisms.

In terms of the temperatures reached during the exposure, the survival pattern in the five regions in this experiment agree well with the previously found patterns for the single serving of tomato soup. Figure 9 indicates

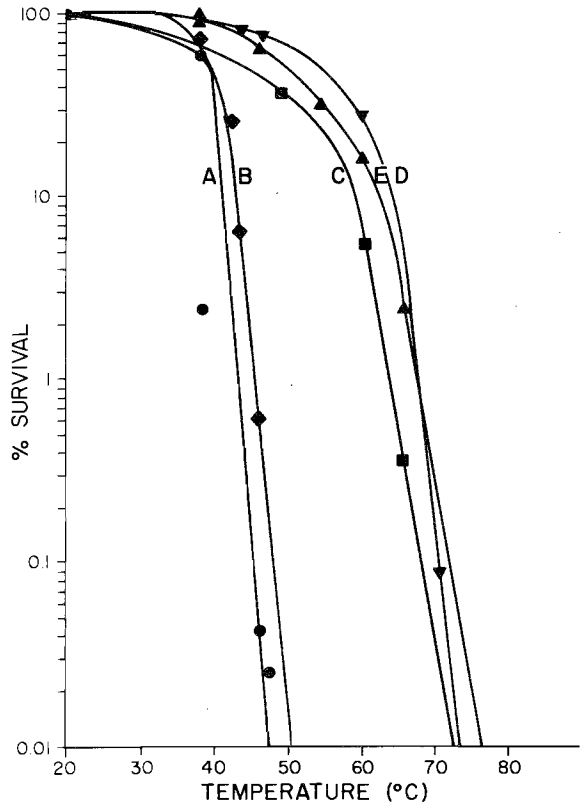

Figure 9. *Decrease in survival in* Escherichia coli *in tomato soup contained in the modified graduated cylinder with respect to temperatures reached during microwave exposure. Circles indicate Postion A (Top), Diamonds indicate Postion B (Between Top and Middle), Squares indicate Position C (Middle), Inverted Triangles indicate Position D (Between Middles and Bottom), and Triangles indicated Postion E (Bottom).*

that, for any given temperature, organisms in the two top regions (A and B) had declined to the lowest levels, the middle region (C) showed the intermediate level of survival, and organisms in the bottom regions (D and E) had the least decrease in survival. Also, there was again a difference of up to 23° in the temperature between the top and bottom regions for which a given value of survival was obtained. Organisms in the top two regions of the cylinder had declined to nondetectable levels by the time the temperature had reached ca. 55 C. In contrast, survivors were detectable in the middle and bottom regions until the temperatures had reached ca. 75-80 C.

DISCUSSION

Many of the literature reports concerning the biological effects of microwaves had attempted to ascertain whether the lethality of such radiation for microorganisms is solely due to the generated heat. In this study, experiments were done to investigate the relationship between temperatures generated in liquid foods and destruction of inoculated bacteria in these foods. To account for uneven energy distribution, temperatures achieved in several regions of the foods during the microwave exposure were measured and correlated with the percentage of surviving bacteria in

those regions. Copson (*4*) had determined that the temperature profile of agar cylinders heated with 915 MHz microwaves revealed that the interior or "core" section of the cylinders were heated to higher temperatures than the peripheral or surface regions. Heating of tomato, vegetable, and beef broth soups with 915 MHz microwaves in this work also revealed a similar profile in that the middle region of the soup was the warmest region throughout the exposrure period. In tomato and in vegetable soups, the finding that the top portion of the soup was the coolest region may have been a consequence of heat loss from the uncovered, non-insulated surface of these liquids to the cool, unheated oven air. However, in beef broth, the bottom region was the coolest portion of the soup. This different pattern of heating in beef broth may have been due to differences in the conduction and convection patterns that were induced in this soup as compared to the other two soups. Although conduction and convection are not responsible for the primary heating effect in microwave cooked foods, they do serve to redistribute the heat within the food once the primary heating has occurred (*4*).

If the lethal action of microwave energy on microorganisms were solely due to the heat generated by the waves, it would be expected that organisms sampled from the warmest region of the soups would have declined to the lowest survival values at any given time. Conversely, those sampled from the coolest region should show the greatest survival. However, results of microwave cooking of soups in this study indicate that a factor other than heat generated in the surroundings of the bacteria may be responsible for the inactivation of the inoculated organisms. Regardless of soup type or volume, sampling procedure, test organism, or relative temperatures of the sampled positions, the consistent finding was that, for any given exposure time, the closer the sampled region was to the top of the container, the greater the decrease in microbial survival. In terms of the temperatures reached in any of the given soup positions, it was also evident that organisms in the top regions of the liquids declined to given levels of survival at temperatures lower than those required to reduce organisms in the middle and bottom regions to the same given levels.

In contrast to the findings in this study, Goldblith and Wang (*9*) concluded that the lethal effects of 2450 MHz radiation for *E. coli* and *B. subtilis* spores were solely due to the heat generated by the microwaves. Lechowich et al. (*12*) also concluded that the inactivation of *S. faecalis* and *S. cerevisiae* by 2450 MHz microwaves could be explained solely in terms of the heat generated during the exposures.

Nevertheless, precedent does exist for the demonstration that temperature increase alone is insufficient in explaining the detrimental effects of microwaves for microorganisms and several mechanisms have been suggested to elucidate the nature of such apparently "non-thermal" effects. For instance, Olsen (*14*) postulated that microorganisms present a "preferential target" for the action of microwaves. That is, since microorganisms usually contain high intracellular concentrations of ionizable compounds, they heat extremely well and may reach higher temperatures than the surrounding matrix when placed in a microwave field of suitable strength. Carroll and Lopez (*3*) also stated that, depending on the relative chemical composition of microbial cells and their surrounding medium, the cells may be selectively heated by microwaves.

However, it has also been postulated that some of the apparent "non-thermal" effects of microwaves may actually be due to molecular level responses of the biological systems to the input of thermal energy. For instance, Vogelhut (*16*) has demonstrated how the input of microwave energy may effect structural changes in the bound-water layer surrounding biological macromolecules. Such changes can be expected to alter the stability and function of the macromolecules and, consequently, the biological processes in the cell itself. Carroll and Lopez (*3*) have proposed that microwave frequency radiation may be selectively absorbed by certain essential biochemical molecules. If such resonance frequencies exist, configurational changes may occur so that the molecule would be irreversibly denatured. Illinger (*11*) has considered some of the molecular mechanisms for microwave absorption by biological systems and has undertaken theoretical predictions, based on energy requirements and frequency dependencies, of which mechanisms could be significant.

In addition to inducing molecular level changes, it has also been suggested that microwaves may affect biological systems on a mechanical level. Copson (*4*) stated that it is to be expected that the net charge of bacterial cells will influence their behavior in an electromagnetic field. Carroll and Lopez (*3*) speculated that the presence of this charge may cause the cells to oscillate rapidly in a high frequency field. Mechanical disruption of cells would occur if the oscillations were rapid enough and of sufficient displacement to exceed the elastic limitations of the cell wall. Teixeira-Pinto et al. (*15*) demonstrated eventual rupturing of *Amoeba limax* with 27 MHz radiation as the field strength in their experiment was progressively increased.

It seems evident from the results in this study that two lethal effects of microwaves for bacteria were demonstrated. First of all, heat was generated which undoubtedly contributed much to inactivation of the inoculated organisms. In addition, the greater decrease in survival of organisms in the top regions of the liquids as compared to the decreases in survival in the middle and bottom regions indicates that the microwaves' irradiation also effected an inactivation of bacteria that could not be explained solely in terms of the relative temperatures generated in the soup regions. This greater decrease in survival in the top regions may be a reflection of the fact that the microwave field intensity was greater there than in the other soup regions. The waves entered from the top of the oven, and as they were absorbed by

successive regions in the liquid, the intensity would decrease. (It might be expected therefore that the top region would be the warmest region due to the greater intensity, but there is also much more heat dissipation from this region.) Whether the nature of the lethality of the microwave radiation for bacteria as noted in this study was molecular, mechanical, or a selective heating effect, it is likely that the effect would be greater for greater intensities. Results of this study do not indicate the exact nature of the lethal effects of microwaves for bacteria. However, they do cast doubt on the theory that heat alone as generated by microwaves in the surroundings of the bacteria is fully adequate to account for the destruction of the bacteria. In light of the fact that the exact nature of the biologic effects of microwave radiation for any living system are still not completely understood, it seems that continuing research in this area is definitely warranted.

ACKNOWLEDGMENTS

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