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MATHEMATICAL MODELS FOR CONVENTIONAL AND MICROWAVE THERMAL DEACTIVATION OF *Enterococcus faecalis, Staphylococcus aureus* AND *Escherichia coli*

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Abstract – Temperature dependencies of survival fecal coliforms such as *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* in water were investigated between 25 - 65° C. Measured dependencies had "bell" shaped form with maximum bacterial viability at 35 - 45 °C. The rates of growth and decay of bacterial viability depend on specific forms of bacteria. At temperatures of 60 - 65 °C the number of viable bacteria decreased in one hundred times in comparison with the maximum value. Similar "bell" shape forms were found for dependencies between bacterial viability and time of microwave (dielectric) heating of water. The dependencies had maximum value at 1 - 2 min of microwave heating. Then, the number of viable bacteria decreased, and at 4 - 5 min of microwave heating, became insignificantly small. The proposed mathematical models for conventional and microwave heating took into account "growth" and "death" factors of bacteria, and had forms of second degree polynomial functions. The results showed good relationships (with coefficient correlation 0.84 - 0.99) between the proposed mathematical models and experimental data for both conventional and microwave heating.

Key words: Microwave or Dielectric Heating, Conventional Heating, Bacteria, Mathematical Model, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*

INTRODUCTION

Recognized as environmental indicators, fecal coliforms such as *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* are used by health professionals to denote unsanitary conditions [4,5]. Recently, World Health Organization (WHO) reported that the deactivation of these fecal coliforms is a vital step in reduction of water related diseases, which are major causes of morbidity and mortality worldwide [2]. Therefore, ways are being sought for address this problem. In the present report we examine the potential of thermal disinfection. Thermal disinfection (deactivation) is among the earliest and most common techniques used for water purification. Currently, there are two methods used for thermal disinfection: conventional radiant heating and microwave or dielectric heating.

Kamau et al. [9] studied thermal destruction of *Listeria* monocytogenes and *Staphylococus aureus* in milk at temperature of 55.2 °C and found that the lactoperoxidase system enhanced this destruction. Moats [16] investigated viability of *Streptococcus faecalis* at water temperature of 60°C, which depended on time of heating. He found a significant decrease in bacterial viability after 18 minutes. King et al.[11] reported data (in accompany with data of other scientists) which were devoted to calculations of bacterial death rates for different forms of bacteria during conventional heating of water at constant temperatures. Mattick et al.[15] studied

Abbreviations: **CFU**, *Colony* Forming Unit*;* **CoC**, *Coefficient* of correlation; E**.** ***coli*** , *Escherichia coli;* ***E.faecalis***, *Enterococcus faecalis;* **min**, minute; **mL**, milliliters; ***S. aureus***, *Staphylococcus aureus*; **TSA**, Tryptic Soy Agar; **TSB**, Tryptic Soy Broth; **WHO**, World Health Organization.

dependencies between the number of viable *Salmonella* bacteria and time of conventional water heating for several temperatures intervals ranging from of 55 - 80 °C. They found that power functions could be used for dependencies between the logarithmic number of viable bacteria and time of heating. Abraham et al.[1] studied thermal destruction of *Bacillus stearothermophilus* in the temperature interval of 105 - 130 °C. In contrast with other scientists, they found that dependencies between natural logarithm of number of viable bacteria and time of heating at constant temperature had initial growth and then decay ("shoulder form"). They decided that high temperature level and short duration of the heat treatment prevent any interpretation of an increase in population as growth. They explain this initial growth of viable bacteria with the presence of uncountable dormant spores, which could be activated during heat treatment and then destroyed successively.

Goldblith and Wang [8] compared the deactivation of *Escherichia coli* and *Bacillus subtilis* spores by using conventional heating and microwave irradiation. They found that the deactivation of these bacteria was a result of the dielectric heating and not due to any secondary effect of the microwave irradiation. Lechowich et al.[14], Vela et al.[19], and Fujikawa et al.[7], also suggested that the deactivation of *Streptococcus faecalis* (later classified as *Enterococcus faecalis*) and *Saccharomyces cerevisiae* when using microwave irradiation resulted from the dielectric heating . Shin and Pyun [17] compared the results of deactivation of *Lactobacillus plantarum* by conventional heating at 50 °C, continuous microwave heating, and pulsed higher power microwave heating during 30 min. They found greater reduction of viable bacteria for pulsed microwave heating compared to conventional and continuous microwave heating. Based upon their results, they suggested a secondary non-thermal mechanism that assisted in the deactivation of bacteria during higher power of microwaves. In addition, Culkin and Fung [6] and Kozempel et al.[12], also, suggested that a secondary non-thermal mechanism could assist in the deactivation of certain species of bacteria such as *Escherichia coli and Salmonella typhimurium.*

Currently, there are two major mathematical models of deactivation of bacteria, mechanistic and vitalist [13]. The mechanistic assumes that deactivation of bacteria occurs in an exponential form and, therefore, predicts straight lines. The vitalist approach assumes differences in population sensitivity thereby allowing for varied deactivation patterns. Although the mechanistic view of bacterial deactivation is still found in the literature, the fact that research data does not closely resemble this model has weakened support for this approach [10].

Most mathematical models were prepared for explanation of dependencies between bacterial survival and time of conventional heating. These models cover bacterial strains such as *Clostridium botulinum, Salmonella enterica, Pseudomonas viscosa, Bacillus stearothermophilus, Salmonella typhimurium, Brochothrix thermosphacta* and *Listeria monocytogenes, Streptococcus faecalis* (later name Enterococcus faecalis) along with several other bacteria [1, 3, 9, 10, 13, 16, 18]. Several models [1,18] have attempted to explain the growth of the bacteria in these dependencies with time at the beginning of treatment by using exponential laws. Environmental and genetic variations in a given bacteria require the frequent revision of these mathematical models. Factors such as increased sensitivity or resistance can dramatically alter the growth and death of a given bacterial population, making the model obsolete and no longer able to accurately predict the life cycle [16].

As can be seen from the work described above, papers that were devoted to the investigation of water temperature influence on deactivation of bacteria were conducted into higher temperature intervals (more than 50 °C). Besides, in most of them only dependencies between bacterial survival and time of heating were measured at constant water temperature. Meanwhile, it is very important to investigate the behavior of the bacterial life cycle at low temperatures. This, in accompany with investigation at higher temperatures, gives the picture of influence of the temperature on growth of bacteria and stability of them to temperature changing.

Also, it is important to compare investigations of conventional and microwave heating that were conducted on the same forms of bacteria. This led us to build mathematical models for conventional and microwave heating which became the purpose of our paper.

MATERIALS AND METHODS

Conventional Heating of bacteria.

Stock cultures of *E. faecalis, S. aureus* and *E. coli* were created by growing in TSB (Tryptic Soy Broth) overnight in a Labline Imperial III Incubator (model # 305) at temperature 37°C. A 10µL aliquot of each stock culture was then placed into 250 mL Erlenmeyer vials containing 100 mL of distilled deionized water purified by Barnstead e-Pure System (model # D4641) preheated to 25°C, 37°C, 45°C, 55°C and 65°C. The bacteria were then incubated for 5 minutes. 50µL of the test solutions were plated in exponential spiral fashion on to 10 TSA (Tryptic Soy Agar) plates for each sample group using a Spiral Biotech Autoplate 4000 (model # AP 4000). The plates were then incubated overnight in a Labline Imperial III Incubator (model # 305). Finally, the colony forming units were counted using a Spiral Biotech Q-count (model # 510) auto-plate reader set for a 50µL exponential spiral pate setting. The CFUs (colony forming units) were then calculated and graphed using Grapad Prism®.

Microwave/Dielectric Heating of bacteria.

Stock cultures of *E. faecalis, S. aureus* and *E. coli* (Ward Natural Scientific, Rochester, NY) were created by growing them in TSB (Tryptic Soy Broth) overnight in a Labline Imperial III Incubator (model # 305) at temperature of 37°C. An aliquot of 10µL of stock culture was then placed into 250 mL sample vials containing 100 mL of distilled deionized water purified by a Barnsted e-Pure System (model # D4641). The sample vials were then microwaved in a Panasonic Inverter Microwave (model # NN-S543BF) at power 130 W for 1 -5 minutes. Results were compared with control samples which were not microwaved. The CFUs into an aliquot of 50µL of the test solutions were calculated by the same method as for conventional heating.

RESULTS

Effect of Conventional Heating on the Deactivation of E. faecalis, S. aureus and E. coli.

Experimental results for conventional heating of *E. faecalis, S. aureus and E. coli* are summarized into Table 1 and Figs.1-3. The data shown in Figs.1-3 can be divided into two distinct regions or processes. The first of them is increasing of number of viable bacteria with initial increasing of water temperature. The second region can be observed after passing a maximum value for bacterial viability and is responsible for decrease in of bacterial survival with increasing of water temperature. Rates of growth for the first process, rate of decay for the second, location of the optimum temperature for bacterial viability depend on bacterial form.

For *E. faecalis* Fig.1 showed an increase in the number of viable bacteria was found in the temperature range between 25°C and 37°C. In this region of the graph, a 12°C increase in temperature resulted in a 25.4% increase in bacterial viability. The maximum number of viable *E. faecalis* was found between 37°C and 45°C. This data suggests that 25°C is not the optimal temperature for certain critical process to occur. For water temperature above 45°C number of viable bacteria was decreased with increasing of water temperature from 104.5% for temperature of 45°C to 0.01% at 65°C. These two distinct regions, the growth and decay, are again observed in Fig.2 for *S. aureus*. Increasing water temperature from 25°C to 37°C lead to a 55.7% increase in bacterial viability. An maximum value of viable *S. aureus* was found at 37°C. The following increasing of water temperature from 37°C to 65°C gave result of a 99.9% decrease in *S. aureus* viability. Temperature dependencies for *E. coli* also showed existence of these two processes (Fig.3). The growth of number of viable bacteria was found in the range of 25 - 37°C where a 10.9% increase in *E. coli* viability can be observed. A 99.9% reduction in *E. coli* viability is found between 37 - 65°C.

Table 1. Survival of *E. faecalis, E. coli* and *S. aureus* at various temperatures using conventional radiant heating.

Bacteria	Temperature (°C)	Bacterial Survival (CFUs/mL)	% Bacterial Survival
E. faecalis	25	1.398E+08	74.6
	37	1.873E+08	100.0
	45	1.958E+08	104.5
	55	1.209E+08	64.5
	65	2.267E+04	0.01
S. aureus	25	1.338E+08	44.3
	37	3.017E+08	100.0
	45	1.446E+08	47.9
	55	5.599E+07	18.6
	65	8.180E+04	0.03
E. coli	25	8.933E+08	89.1
	37	1.003E+09	100.0
	45	9.337E+08	93.1
	55	5.876E+07	5.9
	65	6.130E+04	0.01

The results showed that *E. faecalis* is more thermal stable than either *S. aureus* or *E. coli*. A temperature of 55°C was required to reduce *E. faecalis* viability by 35.5% with 99.9% reduction occurring at 65°C. *S. aureus* appeared to be somewhat less thermal stable than E. faecalis requiring a temperature of 45°C to bring about a 52.1% reduction in bacterial viability with 99.9% reduction occurring at 65°C. *E. coli* appeared to be the most thermal sensitive of all bacteria tested. The data suggests a temperature of 55°C was required to cause a 94.2 % reduction in *S.*

aureus bacterial viability with 99.9% occurring at 65°C.

Mathematical Treatment of Bacterial Survival of Conventional Heating

Consider the kinetics of bacterial survival which depends on water temperature that contained these bacteria. Two processes must be considered for this model. The first of them is the "growth factor". This term refers to the growth of bacterial number with an optimal water temperature range that contributes to bacterial growth. When the temperature is out side this range we must consider the second factor named "death factor". This factor is related to the reduction of bacterial number with increasing of water temperature due to bacteria death. With the starting stage the "growth factor" is dominant but eventually the "death factor" has more influence on bacterial number.

In the first approximation, we can assume that the rate of change of bacterial survival number into both of these processes can be written as linear function of water temperature.

$$\beta = a_1 T + b_1 \qquad (1)$$

$$\delta = a_2 T + b_2 \qquad (2)$$

where β and δ - growth and decay of bacterial number with changing water temperature on 1 C related with "growth factor" and "death factor", respectively, T -water temperature, a_1, b_1, a_2, b_2 constant coefficients depend on form of bacteria.

By using this assumption rate the change dN/dT of survival number with water temperature T will be

$$dN/dT = \beta - \delta \qquad (3)$$

After substitution formulas (1) and (2) into expressions (3) we will receive

$$dN/dT = A_1 T + B_1 \qquad (4)$$

where $A_1 = a_1 - a_2$, $B_1 = b_1 - b_2$ - coefficients that depend on bacterial forms.

To find the number of survival bacteria at given water temperature T we first performed simple algebraic rearrangement and then took the integral of the rearranged expression (4)

$$N(T) = \int (A_1 T + B_1) dT = AT^2 + BT + C \qquad (5)$$

where, the terms A - C are coefficients that depend on bacterial forms. Therefore, we can expect that a relationship between number N of viable bacteria and water temperature T will be given by second order polynomial function (5).

In accordance with this theory, we performed approximation of experimental results with polynomial function (5) (solid curves on Figs. 1-3). The values of the coefficients A, B, C that gave the best correlation with experimental results were calculated by using the least square method and placed into Table 3.

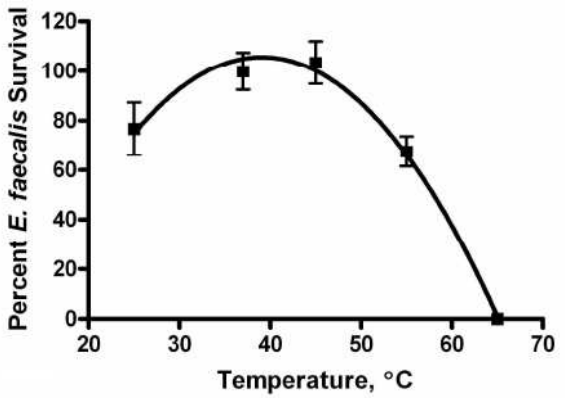

Figure 1. Effect of Conventional heating on *E. faecalis* Survival.

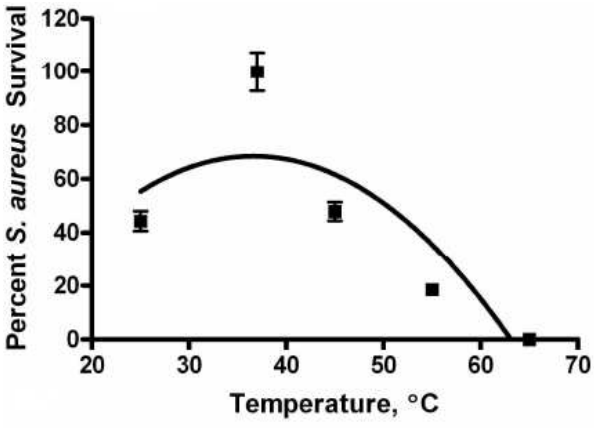

Figure 2. Effect of Conventional heating on *S. aureus* Survival.

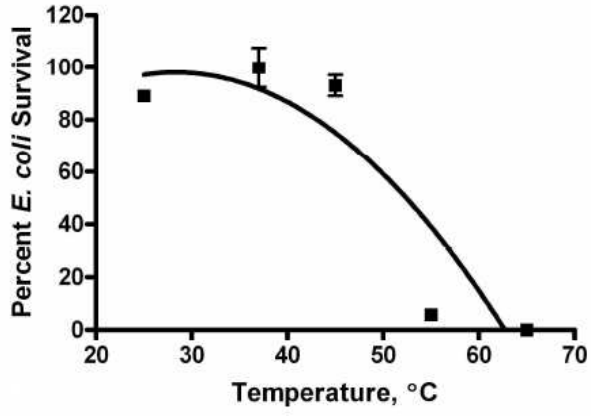

Figure 3. Effect of Conventional heating on *E. coli* Survival.

Effect of Microwave/Dielectric Heating on the Deactivation of E. faecalis, S. aureus and E. coli.

Experimental results were summarized into Table 2 and Figs.4-6.

Table 2. Survival of *E. faecalis*, *E. coli* and *S. aureus* at various times using microwave heating

Bacteria	Microwave time (min)	Bacterial Survival (CFUs/ml)	% Bacterial Survival
E. faecalis	Control sample	1.496E+08	100.0
	1	1.799E+08	120.3
	2	1.676E+08	112.0
	3	1.774E+08	118.6
	4	4.787E+07	32.0
	5	4.190E+05	0.28
S. aureus	Control sample	1.708E+08	100.0
	1	2.369E+08	138.7
	2	1.611E+08	94.34
	3	1.250E+08	73.20
	4	1.022E+06	0.60
	5	4.090E+04	0.02
E. coli	Control Sample	5.198E+08	100.0
	1	6.065E+08	116.7
	2	5.652E+08	108.7
	3	1.292E+08	24.9
	4	2.040E+04	0.004

The results showed that *E. coli* was the most thermal sensitive of all tested bacteria. It required only three minutes of microwave time to bring about a 75.1 % reduction in the bacterial viability with 99.9% reduction occurring after four minutes. *E. faecalis* appeared to be the most thermal stable of all the bacteria tested. The data suggested that four minutes of microwave time were required to cause a 68.0 % reduction in bacterial viability and five minutes resulting in a 99.3% reduction. *S. aureus* required three minutes to show a 26.8% reduction in viability with 99.4% occurring after four minutes.

Similar to the result found with conventional heating, dielectric heating showed the "growth factor" and "death factor" processes. For *E. faecalis* the "growth factor" was found between

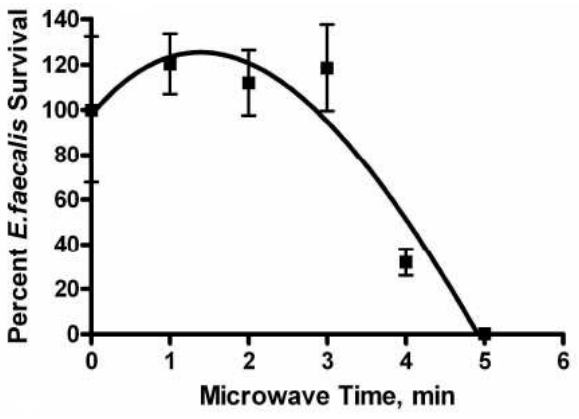

Figure 4. Effect of Dielectric Heating on *E. faecalis* Survival.

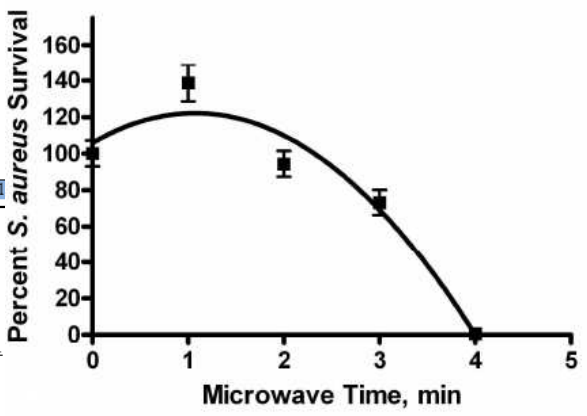

Figure 5. Effect of Dielectric Heating on *S. aureus* Survival.

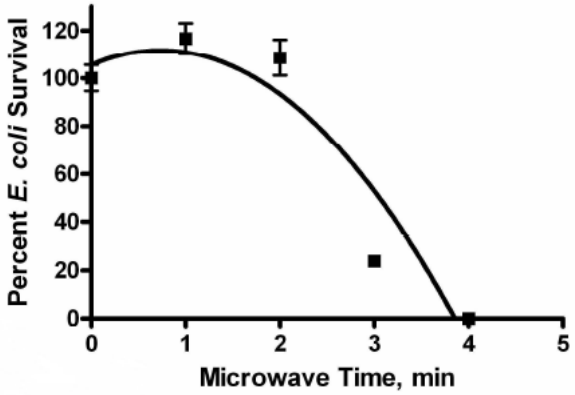

Figure 6. Effect of Dielectric Heating on *E. coli* Survival.

the control sample (t = 0 min) and first minute of microwave heating showing a 20.3% increase in bacterial viability. After this initial increase, *E. faecalis* viability was stable until three minutes with no significant change in viability. The "death factor" occurs between three and five minutes where there was a 118.3% bacterial viability reduction. *S. aureus* showed a "growth factor" process over the first minute with a

38.7% increase in bacterial viability. The "death factor" for *S. aureus* was observed between one to five minutes where the bacterial viability was reduced from 138.7% to 0.02%. For *E. coli* the "growth factor" was found within the first minute where bacterial viability was increased by 16.5%. A short stabilization phase was found at the second minute with the "death factor" process occurring between three and four minutes showing a 108% reduction in bacterial viability.

Table 3. Coefficients of Mathematical Model (Equation 5) for Conventional Heating

Bacteria	A	B	C	CoC
E. faecalis	-0.158	12.35	-136.7	0.99
S. aureus	-0.098	7.16	-62.7	0.84
E. coli	-0.085	4.94	25.4	0.91

Mathematical Treatment of Bacterial Survival of Microwave Heating

Let's consider relationship between number N of bacterial survival and microwave heating time *t*. All experiments were conducted at constant power of microwave (P = 130 W). In this case, energy *E* that transferred to sample of water placed into microwave during time *t* will be

$$E = Pt \qquad (6)$$

Part of this energy transformed to heat ΔQ

$$\Delta Q = kE = kPt \qquad (7)$$

where k – constant coefficient if water mass unchanged.
But heat ΔQ is related to the changing of water temperature ΔT

$$\Delta Q = c_w m \Delta T = c_w m(T-T_i) \qquad (8)$$

where T and T_i – current and initial water temperature, respectively,
c_w – specific heat of water, m – mass of water.
By using formulas (7) and (8) we can write

$$c_w mT - c_w mT_i = kPt \qquad (9)$$

From formula

$$T = T_i + (kP/c_w m)t = k_1 + k_2 t \qquad (10)$$

where k_1 and k_2 – constants for a given mass of water, its initial temperature, and microwave power during heating.

Now, we can replace temperature T into expression (5) with formula (10), and receive for number of viable bacteria

$$N(t) = A(k_1 + k_2 t)^2 + B(k_1 + k_2 t) + C =$$
$$= Ak_2^2 t^2 + (2Ak_1 k_2 + Bk_2)t + Ak^1{}_2 + Bk_1 + C = \qquad (11)$$
$$= A_2 t^2 + B_2 t + C_2$$

where $A_2 = Ak_2^2$, $B_2 = 2Ak_1k_2 + Bk_2$, $C_2 = Ak_1^2 + Bk_1 + C$ - constant coefficients depend on forms of bacteria. Therefore, we can expect that the relationship between number *N* of viable bacteria and microwave time will be given by second order polynomial function (11). In accordance with this theory, we performed approximation of experimental results with polynomial function (11) (solid curves on Figs. 4-6). The values of the coefficients A_2, B_2, C_2 that gave the best correlation with experimental results were calculated by using the least squares method and placed into Table 4.

Table 4. Coefficients of Mathematical Model (Equation 11) for Microwave Heating

Bacteria	A_2	B_2	C_2	CoC
E. faecalis	-10.01	28.80	100.7	0.95
S. aureus	-14.24	30.51	105.7	0.98
E. coli	-11.28	15.86	105.8	0.95

DISCUSSION

Temperature dependencies of survival bacteria that were recorded in our experiments show the existence of two factors: growth of number of viable bacteria with initial increasing of water temperature, passing through maximum value at 35- 45 °C, concluding in significant decreases in numbers of viable bacteria at temperatures between 60 - 65 °C. These two processes lead to "bell" shape form for temperature dependencies of bacterial survival. The rate of growth and decay depended on specific forms of bacteria. This observation is in agreement with the results of Abraham et al.[1]. However, our experiments in contrast with [1] began with increasing water temperature from low values. For this case, we can decide that growth of viable bacteria with initial increasing of water temperature relates with improving conditions for bacteria reproduction.Similar "bell" shape forms were found for bacterial viability and the time of microwave heating. After 1-2 min of microwave heating the number of bacteria decreased and between 4-5 minutes of microwave heating became insignificantly small.

Similarity of forms of temperature dependencies for conventional heating and time dependencies for microwave heating bear witness of fact that the principal factor that affect bacterial behavior during microwave heating is transformation of electrical energy into heat energy, and its following influence on life cycle of bacteria. This is in agreement with the results of works [7, 8, 14, 19]. Absence of other non-thermal factors that have influence on bacteria during microwave heating as reported by Shin and Pyun [17], can be explained with comparable lower power level of microwave heating and time of microwave irradiation in our experiments in comparison with the work [17].

The proposed mathematical models relating bacterial survival to temperature dependencies of for conventional heating and time dependencies for microwave heating is consistent to experimental data (coefficient of correlation 0.84 - 0.99). This correlation confirms the assumptions made for the proposed mathematical models, particularly, about linear relationships between rates of "growth" or "death" and water temperature. Existence of mathematical models for conventional and microwave heating of water allows for a determination of bacterial survival levels at predetermined temperatures for conventional heating or times for microwave heating. The proposed mathematical models can be applied to items such as refrigerated foods where increased temperature is beneficial to bacterial growth until a given point where the "growth" factor becomes less than the "death" factor caused by the elevated temperature. These models can be used to determined optimal growth temperature or heating time for refrigerated foods.

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