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Continuous-Flow Microwave Heating Inactivation Kinetics of α-Amylase from *Bacillus subtilis* and a Comparison with Conventional Heating Conditions

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Featured Application: This manuscript describes the microwave inactivation kinetics of α-amylase enzyme for application as a time-temperature integrator in conventional or microwave heating. The demonstrated pH-dependent inactivation kinetics of α-amylase are valuable because they show that the thermal resistance of α-amylase varies with pH, and, therefore, a suitable pH can be used to employ α-amylase as a model for testing the efficiency of different thermal processes.

Abstract: The inactivation kinetics of an α-amylase enzymatic time-temperature integrator (TTI) from *Bacillus subtilis* (BAA) under continuous-flow microwave (MW) and conventional heating conditions were evaluated and compared in this study. The TTI dispersed in a buffer solution (pH 5.0 to 6.9) at 20 °C initially, and it was continuously circulated through two helical coils connected in a series for heating. The two coils were positioned in two domestic microwave ovens (2450 MHz and 1000 W nominal capacity each) and connected by a short tube. The sample flow rates were adjusted to result in a specific exit temperature in the range of 65 to 80 °C. A short fully insulated helical coil at the exit of the second oven was used as a holding tube. Test samples were drawn either at the exit of the second MW oven or immediately after the holding tube. The decimal reduction times obtained under conventional batch heating conditions decreased from 66 to 24 s as the temperature changed from 70 to 75 °C at pH 5.0 while they decreased from 8 to 5 s under MW in the same temperature range, but at pH 6.0, they increased both under conventional and microwave heating conditions (138 to 120 s and 89 to 61 s, respectively). The D-values under conventional thermal holding were four–eight times higher than under a continuous-flow MW heating condition. By varying the pH, the D-values could be modified to suit the validation of appropriate processing conditions.

Keywords: microwave heating; kinetics; inactivation; enzyme; α-amylase; D- and z-values

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1. Introduction

Conventional pasteurization of liquid foods (milk, juices, and other beverages) is carried out in continuous flow high-temperature short-time (HTST) or ultra-high-temperature (UHT) heating systems using heat exchangers (tubular/plate) and then through a set of holding tubes, followed by subsequent cooling in heat exchangers, and is then packaged, usually under aseptic conditions. A common problem encountered in these continuous HTST pasteurization/UHT processes is the contact surface fouling caused by the exposure of fluids to a high surface temperature. This is caused by the heat denaturation of proteins or the gelatinization of starches present in the system. The surface scale (foul) not only results in the deterioration of heat transfer but also tends to form off flavors. Recent innovations, such as scraped or swept surface heat exchangers, help to minimize fouling problems by continuously sweeping the hot surfaces with scraper blades. Scraper blades prevent the continuous accumulation of the proteins and carbohydrates along the heat transfer

surface. Microwave (MW) heating could provide an alternative for the continuous pasteurization of liquid food products. Microwaves heat by a different mechanism compared to conventional thermal heating: the heat is generated internally due to molecular vibrations of dipole and ionic components in response to the changing electric fields created by the magnetrons. The dipole materials undergo flip-flop rotations, while the ionic components undergo a back-and-forth motion. With domestic microwaves operating at 2450 MHz, these oscillations could occur millions of times in a second, creating kinetic motion and internal friction heating. The microwaves pass through the relatively cooler heat exchanger surface and the product is heated internally, and, as a result, the fouling problem is largely eliminated. The greater penetration depth and the faster heating rates associated with microwave heating have been recognized as potential factors to improve the retention of thermo-labile constituents in liquid foods, such as milk and fruit juices [1,2]. A potential advantage of continuous-flow sterilization or pasteurization of fluid food over conventional sterilization methods for canned foods is also that product quality can be improved due to higher nutrient retention.

Several studies have reported the successful microwave pasteurization of milk [3,4]. Microwave inactivation of enzymes and microorganisms in fruit juices have also been studied [5]. Tajchakavit and Ramaswamy [6–9] applied microwave heating for inactivation of pectin methyl esterase (PME) in orange juice and the destruction of spoilage microorganisms (*S. cerevisiae* and *L. plantarum*), and found that the inactivation/destruction followed typical first-order reaction kinetics that showed a linear destruction rate on a logarithmic plot of residual activity/survivors vs. residence times. In these studies, microwave heating was shown to inactivate the enzyme and destroy the microorganisms by an order of magnitude faster than conventional thermal heating. However, the two selected strains were very sensitive to microwave heating and rapid destruction was achieved at a relatively low temperature and short heating times, and, as a result, these are not suitable for high-temperature studies.

A time-temperature indicator (TTI) is defined as a small, inexpensive device that is time- and temperature-dependent and easily measurable, and irreversible change can be correlated to the changes of a target attribute of a food undergoing the same variable temperature exposure. Therefore, a TTI with high thermo-stability is desired if one were to use enzymatic inactivation as a high temperature-time integrator (TTI).

Several studies have been carried out to evaluate the effect of microwave heating on biological and chemical systems using different approaches, experimental designs, and techniques. Various strains of microorganisms and enzymes have been employed in these studies. Some studies [10–12] have associated the microwave effects to be mainly due to the thermal effects of the dipolar molecular friction heating. Other studies [6–9,13–15] have observed the non-thermal or enhanced thermal effects of microwaves. Chen et al. [16] have explored the possibility of non-thermal effects of microwave heating on the inactivation of wheat germ lipase, and they found microwave inactivation to be more effective than the conventional kind, while Xu et al. [17] found no difference between thermal and microwave heating. The results of some studies [18,19] have demonstrated that the microwave-assisted thermal process was more effective than the conventional one under the same conditions for inactivating methylesterase, polyphenol oxidase, and peroxidase in cloudy apple juice, indicating the possibility of some additive non-thermal effects. A recent review [20] also provided additional details on the non-thermal effects of MW inactivation kinetics. Another recent study [21] found the combination of MW and ultrasound to be the most effective enzyme activity and quality parameters. It is well recognized that the activity and the thermal stability of many enzymes as well as microorganisms are also pH dependent. In one study, it was demonstrated that the α-amylase activity in apple juice was dependent on pH, heat, and calcium ions [22].

From the preceding sections, it is clear that there is a need for a TTI that can be used under different heating conditions, such as pasteurization (low thermal resistance) or

sterilization (high thermal resistance). α-amylase has been found to be a good enzymatic TTI for kinetic studies.

The objectives of this study were (1) to evaluate the kinetic parameters (decimal reduction time, D-values, and temperature sensitivity parameter Z-values) for the inactivation of α-amylase-based TTI in different pH solutions during continuous-flow non-isothermal microwave heating conditions, and (2) to compare the results with those obtained from the conventional batch thermal treatment as well as the continuous thermal holding time kinetics.

2. Materials and Methods

2.1. Enzyme and Different Buffers

Commercial α-amylase (Validase BAA 1200L, Valley Research, Inc., South Bend, IN, USA) was used to prepare the enzyme solution. Sodium and potassium phosphates (Sigma) were used to prepare the buffers of different pH from 5.0 to 7.0, and were used as needed.

2.2. Microwave Heating System

The microwave heating system used for subjecting the enzyme solution to continuous-flow treatments is detailed in Tajchakavit and Ramswamy [6] and LeBail et al. [23]. A schematic diagram of the system is shown in Figure 1. Briefly, it consisted of two 1.4 cubic feet volume, 1000 W, 2450 MHz domestic microwave ovens (SHARP Carousel, Model. R411AWC; Goldstar Waveplus II, Sharp Corporation, Osaka, Japan) with two helical glass coil heat exchangers (one in each) centrally located inside each oven cavity. The enzyme solution was circulated through the helical coils made from Pyrex glass tubing (inside diameter, 0.5 cm, with a volume of coil 78.6 mL (first oven) and 80 mL (second oven)). These two ovens were connected by a short plastic tubing. The flow of the test solution in the system was adjusted using a calibrated variable-speed metering pump (Cole-Parmer Instrument Company, Masterflex®, Cole-Parmer, QC, Canada). The direction of the fluid flow in the tube was upward in order to have a better control of flow rate. Inlet, outlet, and middle temperatures were continuously gathered by using thin wire copper-constantan thermocouples centrally inserted in the tubes and attached to the data-logger (HP-3497üA DVM +HP- 34.901A multiplexer). In order to obtain a better mixing condition and to reduce the temperature gradient across the radius of the tube, two static mixers were installed at the outlet of the first and second microwave oven.

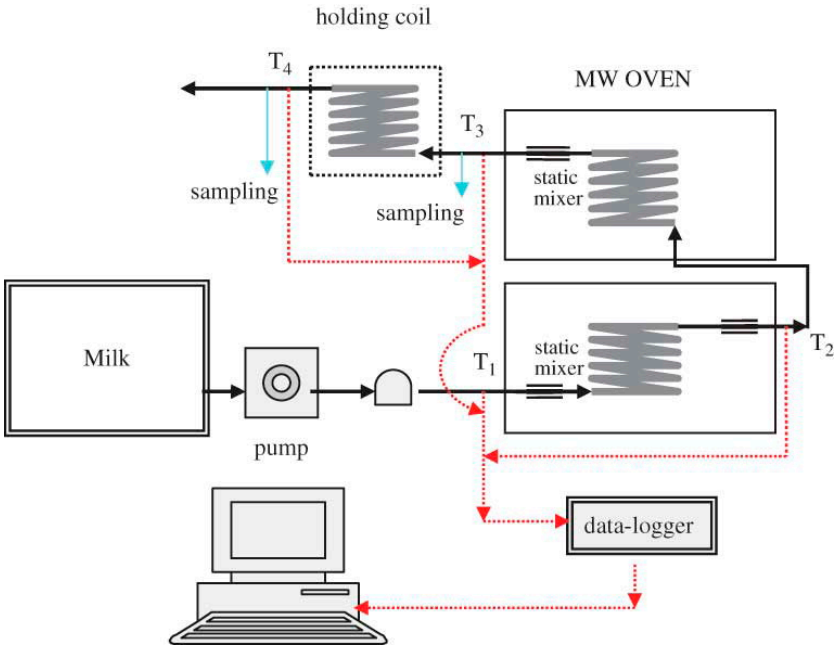


Figure 1. Schematics of the microwave heating set-up.

The average residence time of the test liquid in the microwave heat exchanger was obtained by dividing the total volume of the test sample inside the oven (which was subjected to microwave heating) by the steady state volumetric flow rate of the liquid through the system. After the second oven, the sample exiting from the microwave oven was run through an isothermal holding tube made of Pyrex glass tubing (volume: 39.6 mL; inside diameter: 0.9 cm). The tubing was insulated to prevent heat loss. The length of the tubing and the flow rate were pre-adjusted based on preliminary runs to obtain the desired exit temperatures. Heat-treated test samples were withdrawn both at the exit of the second microwave oven and at the exit of the holding tube during steady-state heating periods, with exit temperatures in a range from 65 to 80 °C. Each exit temperature was achieved by pre-adjusting and changing the flow rate. At the flow rate used, the fluid flow profile was expected to be essentially laminar. However, the use of helical coils creates secondary flow, which can result in thorough mixing of the fluid as it passes through the system. In order to assess the flow characteristics of the system, several parameters associated with the heating system were evaluated. The heating parameters and the formulas used for its calculation are detailed in Table 1.

Table 1. Summary of operating temperatures, flow rates, heating rates, and absorbed power (initial temperature: 20 °C).

Exit Temperature, °C	Flow Rate, mL/s	Residence Time, s	Heating Rate, °C/s	Power Absorbed, W	Efficiency, %	Re/De Number
95	4.17	64.8	1.09	1228	61	600/178
88	5.00	54.0	1.18	1327	66	720/214
81	5.50	49.1	1.14	1287	64	792/236
75	6.67	40.5	1.23	1395	69	960/286
70	7.50	36.0	1.25	1413	71	1080/322
66	8.33	32.4	1.27	1441	72	1200/357

Re = Reynold's number = $VD\rho/\mu$; V = fluid velocity; D tube diameter; ρ fluid density; and μ fluid viscosity. De = Dean's number = Re $(Ri/Re)^{1/2}$; Ri is the tube radius; and Re is the coil radius. Power absorbed = V ρ C_p ΔT, where V is the volumetric flow rate, ρ is density, and ΔT is temperature difference.

2.3. Kinetic Data Analysis

The destruction of microorganisms is generally modeled based on the first-order rate reaction kinetics, as detailed in Tajchakavit and Ramaswamy [7,8]. Briefly, the D-values at the exit temperatures were first calculated from the regression of the log residual numbers of survivors vs. uncorrected heating time (nominal heating time) (Equation (1)), and then the z-value was obtained as the negative reciprocal slope of log D vs. temperature (Equation (2)). Mathematically, the D-value can be represented using Equation (1) (survivor curve):

$$\log_{10}\frac{N}{N_0} = -\frac{t}{D};\ \text{Slope} = -1/D \quad (1)$$

where N is the residual activity of the enzyme at time 't' and N_0 is the initial activity. The D-value is also called the 'decimal reduction time' because at any particular temperature, D represents the heating time required for a reduction in the microbial population by one decimal reduction or 90%.

The second parameter used to define the thermal inactivation kinetics is the temperature sensitivity indicator, which is denoted by z. The z-value is the difference in the thermal treatment temperature that causes an increase or A decrease in the D-value by one decimal. The temperature sensitivity indicator was obtained as the negative reciprocal of the thermal resistance curve, which is a plot of $\log_{10}D$ versus the heat treatment temperatures (Equation (2)).

$$z = \frac{T_2 - T_1}{\log_{10} D_1 - \log_{10} D_2} \quad (2)$$

where D_1 and D_2 are D-values at the temperatures T_1 and T_2, respectively.

Using the calculated *z*-value, the heating times are corrected as effective times (Equation (3)), and new *D*-values and, subsequently, the new *z*-value are computed. This step is repeated as many times as necessary until the convergence of the *z*-value:

$$\text{Effective heating time} = \int 10^{((T-121.1)/z)}\, dt \quad (3)$$

3. Results

3.1. Microwave Heating Characteristics

The typical evolution of temperature as a function of time at different mean fluid flow rates during continuous-flow microwave heating is shown in Figure 2, and it indicates typical lag periods prior to achieving a steady state. As explained in Kudra et al. [4], the non-linearity in the time-temperature profile during the early phase of heating is contributed by the coil and the environment within the oven cavity. In the microwave heating set-up used, it took about a minute after turning the microwave on to reach the target temperature in the range 65 to 95 °C, while equilibrated steady-state exit temperatures were achieved after about 2 min.

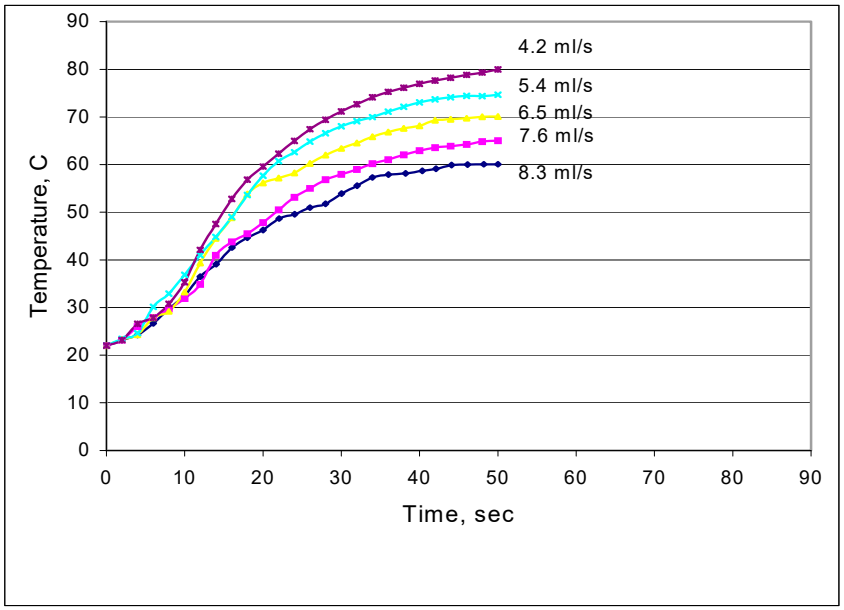


Figure 2. Typical microwave heating profiles in the MW system as a function of flow rate.

The mean exit temperature at the end of the first and the second oven as a function of flow rate and residence time are shown in Figure 3a,b. As expected, the exit temperatures after the second oven were higher than in the first due to the continuous flow of liquid from the first to the second oven. Further, higher flow rates resulted in lower exit temperatures. Figure 3b demonstrates the continuous evolution of temperatures through the first and the second ovens as a function of the overall residence time, ranging from 15–65 s, and reaching 50 °C to 95 °C after heating in the first and the second microwave ovens. The associated data on Reynolds and Dean numbers, mean heating rate, power absorbed, and power absorption efficiency are summarized in Table 1.

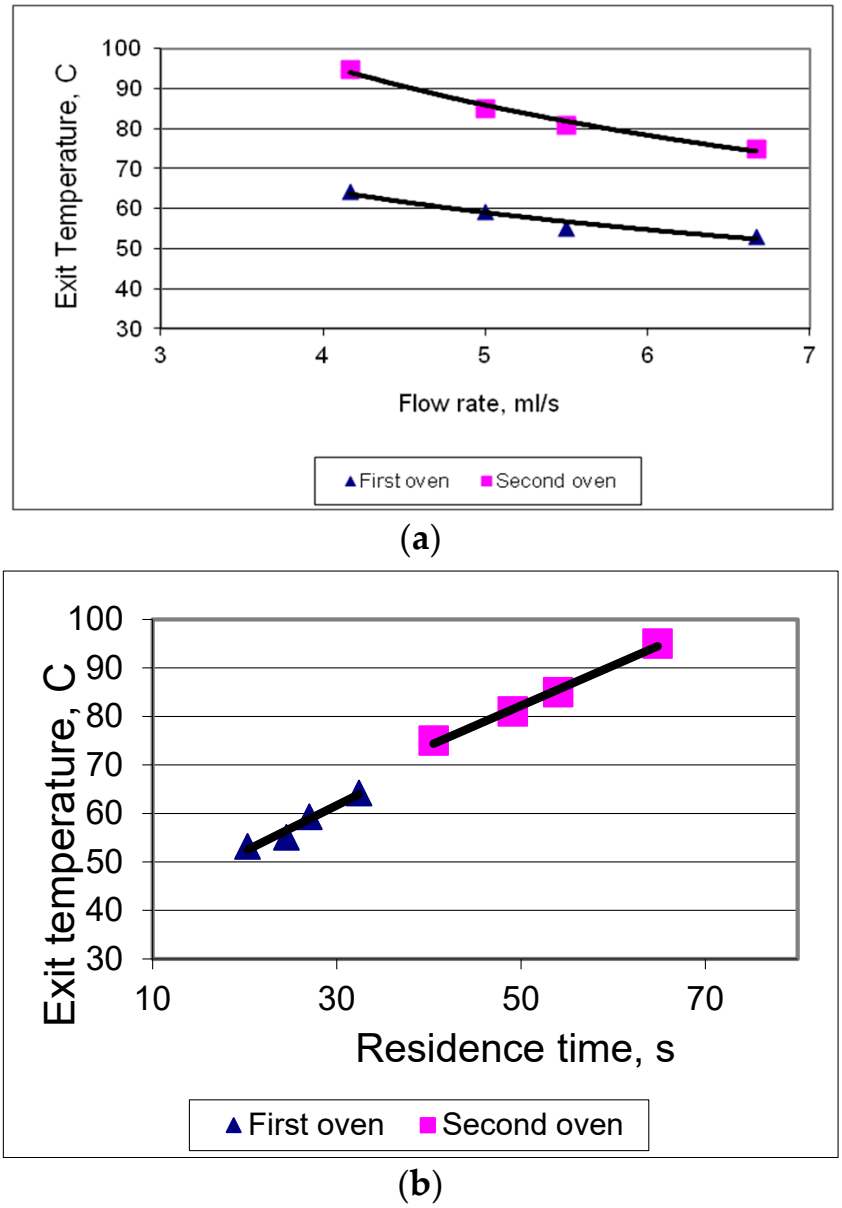
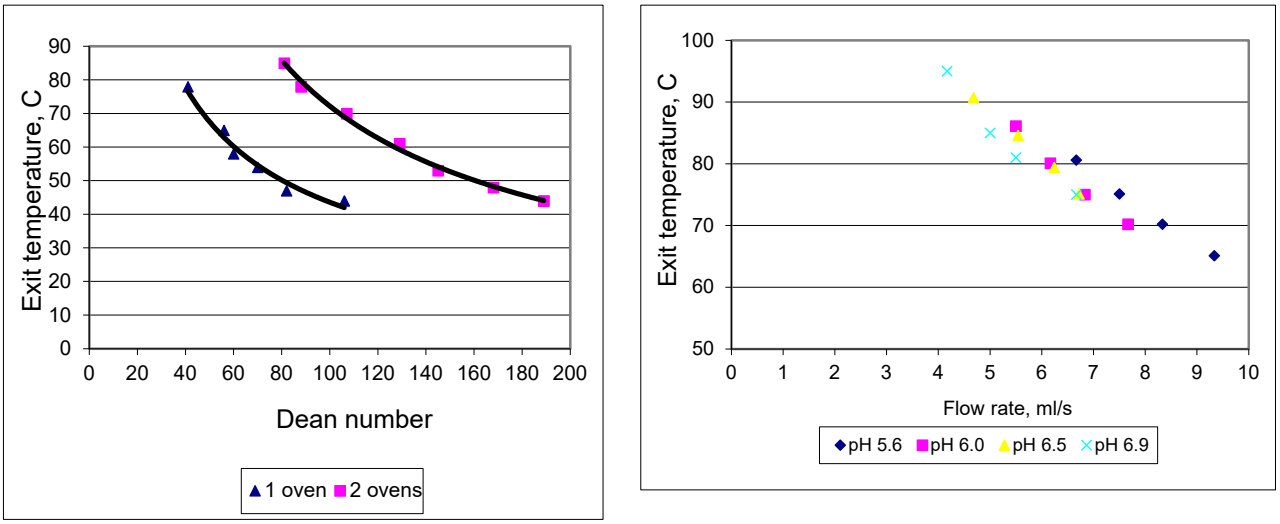


Figure 3. Sample temperatures at the microwave exit as a function of (**a**) residence time and (**b**) flow rate.

The heating rates varied from 1.09 to 1.27 °C/s depending on the flow rate and the exit temperatures. The microwave power absorption efficiency varied from 61% to 72%, with lower levels associated with higher exit temperatures and lower flow rates. This can be easily explained by the higher loss of heat to the microwave oven environment at the higher temperatures. The associated Reynolds numbers were considerably lower than the 2100, indicating the flow to be essentially laminar. However, the use of a helical coil creates secondary flow, which results in thorough mixing of the fluid as it passes through the system. It has been argued that, at high Dean numbers, the secondary mixing of fluids within a helical coil provides a "perfectly mixed or plug flow profile" for the flowing liquid [24]. The Dean numbers associated with the flow varied from 178–357 in the present studies. The exit temperatures achieved as a function of the Dean number and the flow rate numbers are shown in Figure 4a. The exit temperature as a function of flow rate at two pH levels are shown in Figure 4b.



(**a**) (**b**)

Figure 4. Sample temperatures at the microwave exit as a function of (**a**) Dean number and (**b**) flow rate.

The microwave heating patterns also depended on the pH of the test solutions. The mean exit temperatures (a) and the power absorbed (b) as a function of the Reynold number and the flow rate at different pHs are shown in Figures 5 and 6, respectively. The power absorbed and, hence, the exit temperatures were higher at a lower pH. Clearly, pH was an influencing factor. The differences in the heating patterns at different pHs were ascribed to the differences in the concentrations of various chemicals used for the preparation of the buffers and their influence on MW heating.

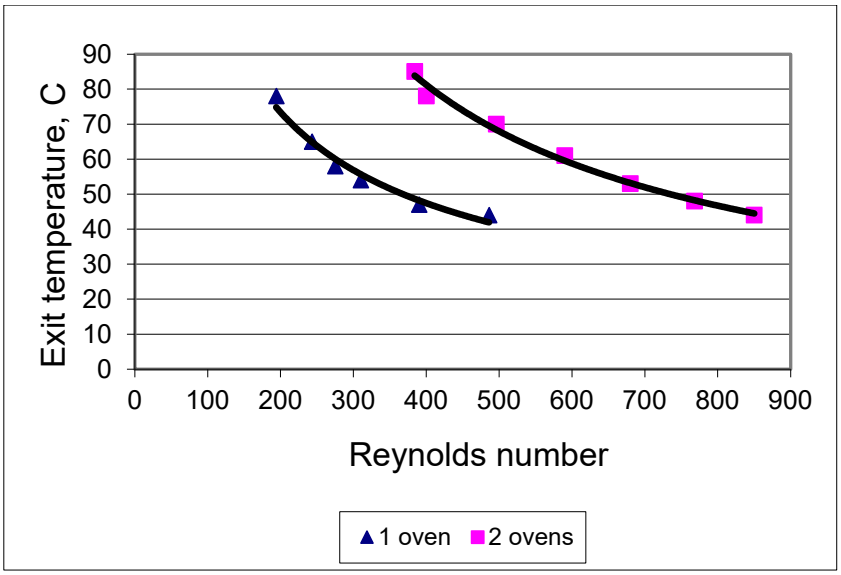


Figure 5. Sample temperatures at the microwave exit as a function of Reynolds number.

Figure 7 shows the residual α-amylase activity at the different sample exit temperatures as they exit from the MW oven. What is not apparent from the conventional residual activity plotted against temperature (Figure 7) is the fact that the flow rates employed for achieving the different temperatures (at each pH) are different. For the same exit temperature, the residence times under different heating conditions were also different. Obviously, the residence time at each pH steadily increases as the exit temperature is elevated, thus giving a compounded destruction effect due to higher temperature and the longer residence

time combination. The steeper drop in residual activity shown in Figure 7 is the result of such a combination effect. Quantitative comparisons under these heating conditions can be made using computed D-values [D = heating time/(log reduction in residual activity)]. The heating time, however, should be the effective time computed as before by integrating the kinetics into the heat penetration data. Come-up time-temperature profiles under the heating conditions are needed for this purpose. Since the come-down was almost instantaneous (with small volumes of heated test solution collected directly in ice-chilled conical flasks), the lethality accumulated during the come-down period can be neglected.

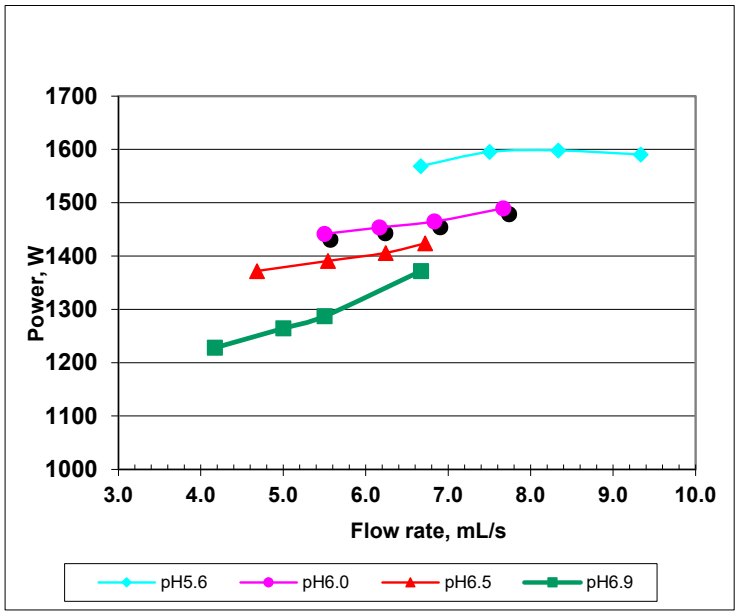


Figure 6. Microwave power absorbed as a function of flow rate at different pH levels.

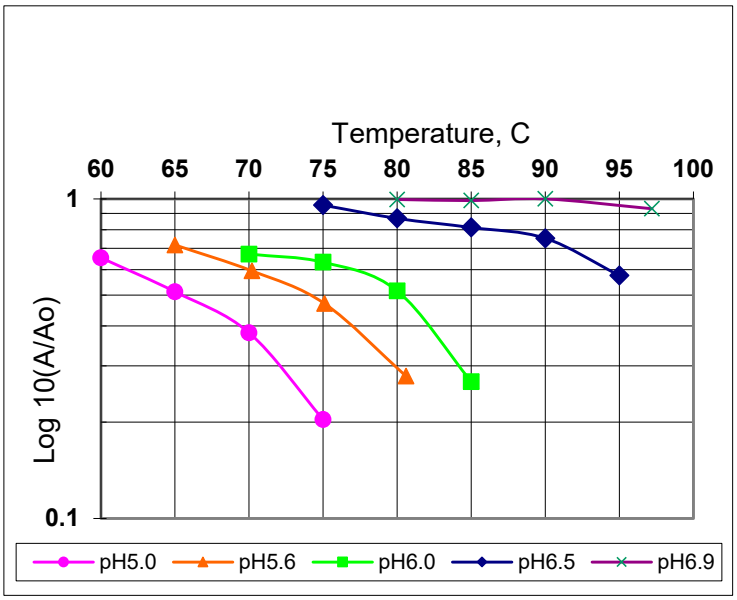


Figure 7. Inactivation rate of TTI from BBA at different pH under continuous-flow microwave condition.

The experimentally determined time-temperature profile for the microwave and conventional heating conditions are shown in Figure 8. Relative to the somewhat exponential conventional heating, the heating profile under the microwave has been reported to be fairly linear, which is also apparent from the temperature continuously measured at the mid-point of the system (between the two microwave ovens) in this study. The linear

profile between the initial and final temperatures over the come-up period was subdivided to 100 elements for accurate computation of accumulated lethality during the come-up period. The effective D- and z-values were computed as explained earlier [7,8].

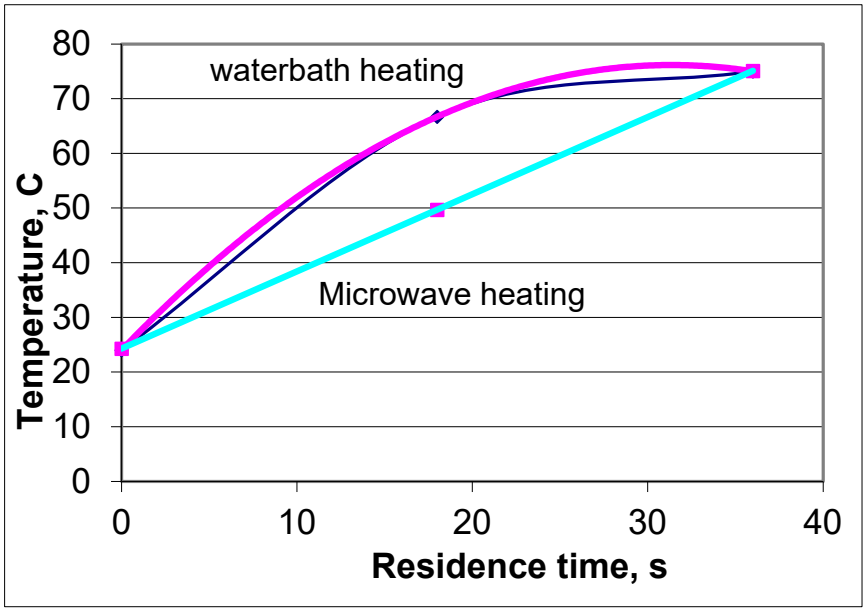


Figure 8. Time-temperature profile of the continuous-flow microwave vs. the water-bath heating condition at exit temperature 70 °C. The black and pink lines demonstrate curve fitting.

3.2. Decimal Reduction Time Comparisons

The D-values obtained and corrected from the experimental survivor data at different temperatures and pHs are shown in Table 2. D-values from the conventional batch system and microwave heating are summarized, which provides some very interesting comparisons between the enzyme inactivation in the two heating systems.

Table 2. Thermal kinetics parameters (D-, z-values) comparison of TTI from BBA under conventional batch heating and MW continuous-flow heating conditions.

Temperature °C	**D-Values (s)**					
	at pH 6.0		**at pH 5.6**		**at pH 5.0**	
	Microwave continuous flow	Thermal batch	Microwave continuous flow	Thermal batch	Microwave continuous flow	Thermal batch
65	N/A	N/A	27	198	16	102
70	89	138	15	114	8	66
75	61	120	12	66	5	24
80	43	66	6	50	N/A	N/A
85	22	N/A	N/A	N/A	N/A	N/A
z-value °C	25	33	23	30	20	21

The D-values under the microwave heating condition ranged from 16 s to 5 s at pH 5.0 between 65 and 75 °C, from 27 to 6 s at pH 5.6 between 60 and 80 °C, and from 89 to 22 s at pH 6.0 between 70 and 85 °C, respectively. This clearly indicated that the D-values decreased with an increase in temperature but increased with an increase in the pH values. D-values during the conventional thermal heating varied from 102 to 24 s

at pH 5.0 between 65 and 75 °C, from 198 to 50 s at pH 5.6 between 65 and 80 °C, and from 138 to 66 s at pH 6.0 between 70 and 80 °C. Comparing the common temperature ranges of 65–75 °C at pH 5.0–5.6 or 70–80 °C at pH 5.6–6.0, the D-values associated with microwave heating were much lower than those obtained for conventional batch heating. These are clearly illustrated in Figures 9 and 10 as D-value curves. Most importantly, the D-values obtained under conventional batch heating were four to eight times higher than those obtained under microwave heating at both pH 5.6 and 5.0. This indicates that the microwave continuous-flow heating condition is much more efficient at inactivating enzymes than the conventional thermal treatment. The temperature sensitivity indicator, z-value, ranged from 20 to 25 °C for microwave heating and 21 to 33 °C for thermal heating, again increasing with pH, but also demonstrating small differences.

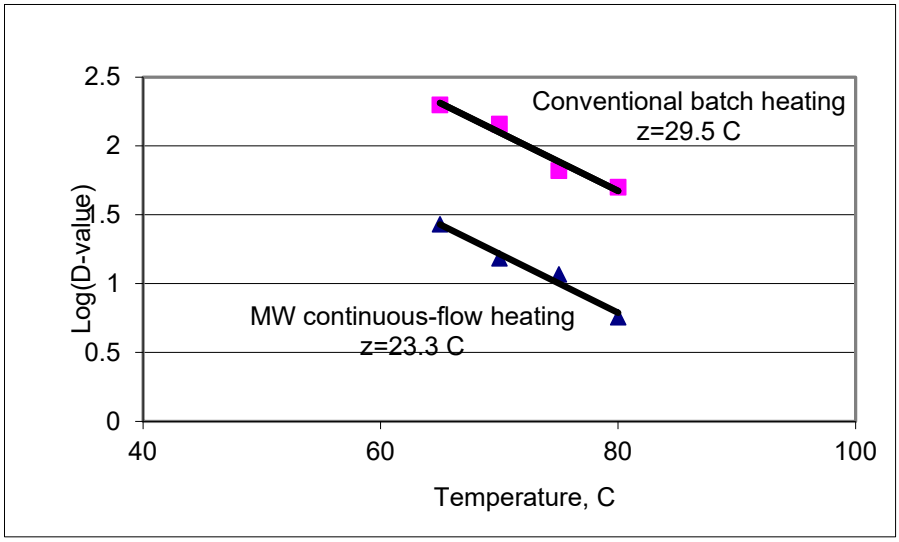


Figure 9. Temperature sensitivity comparison between continuous-flow microwave heating and conventional batch heating at pH 5.6.

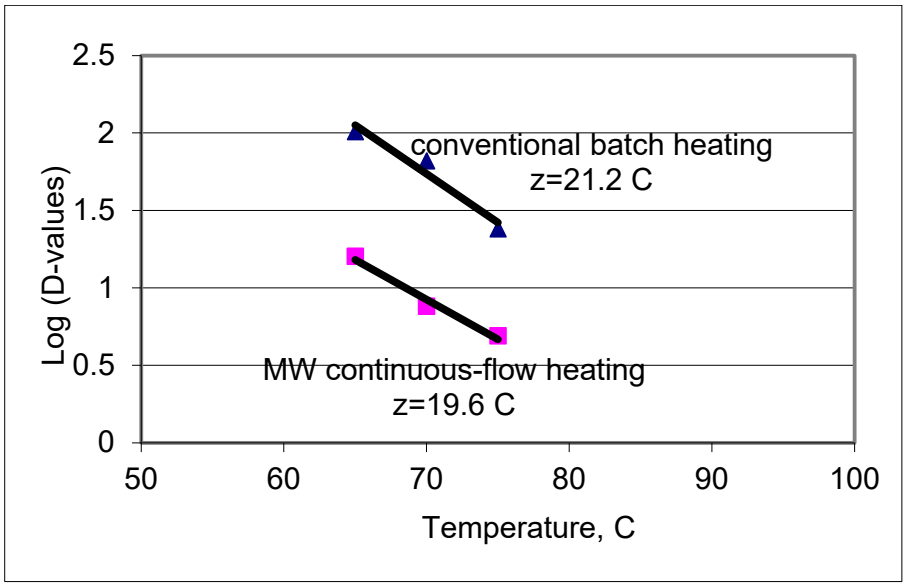


Figure 10. Temperature sensitivity comparison between continuous-flow microwave heating and conventional batch heating at pH 5.0.

Earlier studies also reported the D-values associated with microwave heating to be considerably lower than the corresponding values found under both conventional batch

and even continuous-flow thermal holding conditions [25]. Riva et al. [26] attributed the differences found in the destruction kinetics between the conventional and microwave heating to different heating kinetics and to non-uniform local temperature distributions during microwave heating rather than the existence of non-thermal effects. Aktas and Ozilgen [27] evaluated the injury of *E. coli* during pasteurization with microwaves in a tubular flow reactor, and they indicated that the destruction effect is the influence of the flow behavior and the other experimental conditions. They also suggested that microbial death might be caused through damage to a different sub-cellular part under each experimental condition. Proteins, as complex macro-molecules, generally have numerous polar and/or charged moities (i.e., COO–, and H+), which can be affected by the electrical component of the microwave field [2]. Although the microwave energy may be insufficient to disrupt covalent bonds, the noncovalent bonds, such as hydrophobic, electrostatic, and hydrogen bonds, may well be disrupted. Thus, the direct microwave effect could be more pronounced, immediate, and specific than the random kinetic energy mechanism associated with conventional heating.

3.3. Enzyme Inactivation Profiles under Continuous-flow Thermal Hold Section

As indicated in Figure 1, the enzyme test solutions after microwave heating were passed through a small holding tube. Only a short residence time was accommodated in this section, since the test solution entering the holding tube is at its highest temperature. There was only a small drop in temperature (maximum 2 °C) in the holding tube since it was well insulated. The drop in temperature was likewise accommodated, as in the case of the come-up time for the microwave heating, and from the logarithmic reduction in the residual activity estimated within the hold tube section, the associated D-values were computed. Table 3 compares the D-values between the two continuous-flow systems—microwave heating and thermal holding at pH 5.6. The data in Table 3 compare the three modes of heating for their effect on α-amylase activity—thermal under continuous flow in the hold tube, conventional batch heating, and continuous flow microwave heating conditions at pH 5.6. The D-values obtained under continuous flow thermal holding were 39, 30, and 18 s at 65, 70, and 75 °C, respectively, at pH 5.6, which were lower than similar data under the thermal batch, which were 114, 66, and 50 s at 65, 70, and 75 °C, respectively, at pH 5.6 heating conditions. The difference between the last two was discussed earlier with respect to temperature as well as pH. This difference between continuous thermal hold vs. batch was mainly attributed to the two heating modes. While it is possible to carry out predominantly isothermal experiments under batch heating conditions employing small sample volumes, the continuous flow systems are necessarily non-isothermal, especially in the absence of a holding time. The uncertain residence time and temperature distribution in the continuous-flow systems and the conservative averages employed generally yield lower D-values.

Table 3. Kinetics parameter (D- and z-values) comparison of α-amylase under batch, continuous-flow thermal holding, and MW heating conditions.

Temperature (°C)	D-Value (s) at pH 5.6	D-Value (s) at pH 5.6	D-Value (s) at pH 5.6
	Thermal—continuous flow heating (holding)	Thermal—batch heating	Microwave—continuous flow heating
65	39	114	27
70	30	66	15
75	18	50	118
80	N/A	N/A	6
z-value (°C)	32	30	23

An important finding is the dependence of D-values on pH. At lower pH, the D- values are relatively very short, and the enzyme is very heat labile. The D-values increased as the

pH is elevated (2–10 times) for both the conventional and microwave heating. As a TTI, it can recognized that α-amylase activity and thermal resistance can be suitably tailored by adjusting the pH to suit the validation time-temperature range or the thermal severity.

4. Discussion

Gathering thermal destruction kinetic data is routine, and it has been practiced by numerous researchers. It simply requires collecting data on destruction kinetics at different temperatures when heated to different lengths of time. Often, one of the kinetic models is used, and the most common is the first-order log-linear model. The slope index of the logarithm of concentration reduction vs time is designated as a decimal reduction time or D-value (negative reciprocal slope). The D-value represents one logarithmic reduction in the original concentration or 90% reduction/destruction. Since the D-value is temperature dependent, a temperature dependency of the D-value parameter, defined as a z-value, is also computed as the negative reciprocal slope of the log D-value vs the temperature curve. There are some recent models that rely on non-log-linear curves and use Weibul type models [28].

In the above kinetic considerations, the heating times are generally all assumed to be given to the sample at the target temperatures. Obviously, this is not possible under practical conditions, and, therefore, the early part of the heating time until the sample reaches the target temperature is generally discarded. In high-temperature destruction kinetics, this frequently may not be possible. In this case, suitable corrections are made to the come-up time to only take the effective portion of the come-up time. Such concepts are routinely used in thermal process calculations [29] in order to expand the time span for the kinetics and to accommodate some temperature fluctuations during the data gathering.

Unlike thermal kinetics, in which a significant portion of the heating time exists at a constant target temperature, microwave destruction kinetics always occur at unsteady-state heating conditions. This implies that as long as the MW energy is absorbed, the temperature of the sample continues to increase. If attempting to hold a constant temperature by using an on/off mechanism to the magnetron or using a low power MW to maintain the temperature drop, very little MW energy is used during the holding period, and conditions would mostly represent "thermal hold" conditions. Hence, MW inactivation or destruction kinetics will need to be entirely carried out with MW "on" period, and, therefore, entirely carried out under non-isothermal conditions. Hence, the effective time concept described earlier (Equation (3)) needs to be adopted, as it is in this study, and, here, significant differences were observed between the thermal and MW kinetics.

Furthermore, the destruction kinetics of α-amylase are very much pH sensitive. At low pH, the inactivation rates are considerably lower, and α-amylase as a TTI under this condition can be used for pasteurization. At higher pH levels, the enzyme is much more resistant to heat inactivation, and such higher pH levels can be adopted for using the same enzyme as a TTI for high-temperature applications, such as sterilization.

5. Conclusions

The application of microwave energy for the destruction of an α-amylase in the continuous-flow condition was studied to assess its suitability as a TTI. Inactivation kinetics under conventional thermal heating, the continuous flow microwave heating system, and the continuous-flow thermal holding period demonstrated that α-amylase was found to be a suitable TTI for a wide range of temperature applications. Notably, the inactivation kinetics were pH dependent, with the TTI at a higher pH demonstrating greater heat stability and suitability for higher temperature applications. This study further demonstrated that MW heating was more effective than conventional heating for the TTI inactivation at all temperatures and pH conditions, and it also indicated that there is the possibility of the existence of non-thermal or enhanced thermal microwave effects on enzyme inactivation.

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