THERMAL DESTRUCTION KINETICS OF HEAT RESISTANT MICROBIAL SPORES AT DIFFERENT pH VALUES

By

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Abstract

Thermal processing is an application of heat for the purpose of shelf life extension and promotion of safety of food. *Clostridium botulinum* is one of the most important pathogens used as a target for thermal processing, especially for low-acid canned food. However, *C. botulinum* can produce neurotoxin. Thus, *C. sporogenes* and *Geobacillus stearothermophilus* are commonly used as surrogates of *C. botulinum* in research studies. This research was aimed to determine the thermal destruction kinetics of *C. sporogenes* ATCC 7955 and *G. stearothermophilus* ATCC10149 at different pH values. Destruction kinetics were evaluated at 110, 115 and 120℃, with spores suspended in distilled water, phosphate and the McIlvaine buffers. Initially, the first-order kinetic model was used to model the results. Results showed that spores had highest D and lowest Z values in distilled water. Spores had greater heat sensitivity in the McIlvaine buffer than in phosphate buffer. It might have been caused by the synergic effect between citric acid and phosphate. D values of both spores were somewhat maximum at pH 7.0 and decreased along both sides. However, no significant change ($P > 0.05$) was observed for Z value with pH. Temperature had greater effect on influencing heat sensitivity of microorganisms than pH.

Since some deviations from linearity were observed with the semi-logarithmic survival curves while using the first order model, the thermal destruction behavior of *C. sporogenes* and *G. stearothermophilus* was also evaluated by using the Weibull model. Comparison of fitness on predicting experimental data between the Weibull and the first-order kinetics models was determined by using the residual plots and scale-location plots. It was observed that first order model was better for modeling of the experimental data than the Weibull model in this study.

Résumé

Le traitement thermique est une application de chaleur dans le but de prolonger la durée de conservation et la promotion de la sécurité des aliments. *Clostridium Botulinum* est l'un des agents pathogènes les plus importants utilisés comme cible pour le traitement thermique, en particulier pour les bas-acide conserves. Cependant, *C. botulinum* peut produire neurotoxine. Ainsi, *C. sporogenes* et *Bacillus stearothermophilus* sont couramment utilisés comme substituts de *C. botulinum* dans les études de recherche. Cette recherche a été conçu pour déterminer la cinétique de destruction thermique de *C. sporogenes* ATCC 7955 et *G. stearothermophilus* ATCC 10149 à différentes valeurs de pH. Cinétiques de destruction ont été évalués à 110, 115 et 120 ℃, avec des spores en suspension dans l'eau distillée, le phosphate et les tampons McIlvaine. Dans un premier temps, le modèle cinétique de premier ordre a été utilisé pour modéliser les résultats. Les résultats ont montré que les spores avaient des valeurs de Z le plus élevé et le plus bas D dans de l'eau distillée. Les spores ont une plus grande sensibilité à la chaleur dans le tampon McIlvaine que dans un tampon phosphate. Cela pourrait être possible en raison de l'effet synergique entre l'acide citrique et de phosphate. La valeur D des deux spores était un peu au maximum à un pH de 7.0 et diminue le long des deux côtés. Toutefois, aucun changement significatif (P> 0,05) a été observé pour la valeur Z avec le pH. La température a eu un plus grand effet sur la sensibilité à la chaleur des micro-organismes que le pH.

Certains écarts par rapport à la linéarité ont été observés avec les courbes de survie semi-logarithmique en utilisant le premier modèle de commande, le comportement de destruction thermique de *C. sporogenes* et de *G. stearothermophilus* a également été évalué en utilisant le modèle de Weibull. Comparaison de remise en forme sur la prévision des données expérimentales entre le modèle Weibull et la cinétique de premier ordre a été déterminée en utilisant les parcelles résiduelles et échelle localisation des parcelles. Il a été observé que le modèle du premier order est meilleure pour la modélisation des données expérimentales que le modèle de Weibull dans cette recherche.

Contributions of authors

Parts of the research presented in this thesis are being prepared for publication and presentation in scientific conference. The authors involved in the thesis and their contributions to the thesis are as follow:

Mengting Xu is the M.Sc. candidate who designed and conducted all experiments and data analysis under guidance from the supervisor. She prepared the draft of all manuscripts for scientific publications.

Dr. H. S. Ramaswamy is the thesis supervisor, under whose guidance the experiment was conducted successfully. He directed the candidate in designing and conducting the research work, also correcting, editing and proofreading the manuscripts for publications.

List of publications and presentations

Part of this thesis has been prepared as manuscripts for publications in referred scientific journals:

Mengting Xu and Hosahalli S. Ramaswamy, Thermal destruction kinetics of *Clostridium sporogenes* ATCC 7955 at different pH values (in preparation).

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CHAPTER 1

INTRODUCTION

Thermal processing is a common processing method for providing quality and safety of food products (Hassan and Ramaswamy, 2011). There are several methods of thermal processing, some conventional and others novel. Conventional methods are mostly based on heat conduction/convection processes using traditional heating media like steam and water, while novel methods are based on other heat sources such as microwave, radio frequency and ohmic heating. Heat contributes most in this processing method, not only because it can modify texture of products to satisfy consumers, but also it can destroy undesirable microorganisms, including pathogens, spoilage and enzymes (Richardson, 2001). In respect of inactivating microorganisms, thermal processing can be classified into two groups: pasteurization and sterilization.

In order to tell effectiveness of microbial inactivation, standpoints of sterilization are introduced. Food can be classified into low-acid food (pH above 4.5), acid food (pH range from 4.0 to 4.5), and high-acid food (pH below 4.0). Standpoints of sterilization are different from different groups. Among them, facultative anaerobic and obligate anaerobic spores in low-acid food are the most heat resistant microorganism (Stumbo, 1973). *Clostridium botulinum* is a significant food spoilage spore forming bacterium in low-acid food, as it could produce neurotoxin which has high lethality. *C. botulinum* is a rod-shaped, gram-positive anaerobic bacterium. Botulinum neurotoxin produced by this bacterium can cause human botulism. It became a health concern since the first outbreak of botulism by digesting "blood sausage" in the late 1700s (Ting and Freiman, 2004; Lund and Peck, 2013). In industries, 12 decimal reduction of number of *C. botulinum* in low-acid food is needed to achieve commercial sterilized products (Shao and Ramaswamy, 2011). Hence, knowledge of thermal destruction characteristic of this microorganism is crucial. However, as *C. botulinum* is a pathogenic bacterium, it is not safe to use this bacterium directly in inoculated studies. Therefore, nontoxic surrogates of proteolytic *C. botulinum* are needed for research studies.

Spore forms of *C. botulinum* with higher heat resistance than the bacterium are frequently used as indicators of commercial sterilization, including *Clostridium sporogenes* (Brown et al., 2012). *C. sporogenes* is often used as surrogate of proteolytic *C. botulinum* because of non-toxigenicity and similar heat resistance of *C. botulinum*. It is a gram positive and putrefactive anaerobic spore. The decimal reduction time (D value) at 121.1℃ is around

1min and the Z value can be ranged from 10 to 12℃ (Luechapattanaporn et al., 2004). Likewise, the Z value of proteolytic *C. botulinum* is 10.2℃ and D value is 0.21min at 121.1℃. Abundant literatures show feasibility of *C. sporogenes* plays as substitutes in research studies (Carter and Peck, 2015; Diao et al., 2014; Mah et al., 2008; Paredes-Sabja et al., 2007; Taylor et al., 2013). Despite of killing *Clostridium* cells and spores, thermophilic bacteria, such as *Geobacillus stearothermophilus*, could still exist in commercially sterilized products. *G. stearothermophilus* is a gram positive and facultative aerobic spore. The optimum growth temperature ranges from 49 to 55℃ with 4 minutes as D-value at 121.1℃. The Z value is around 14 to 22℃ (Stumbo, 1973). Since *G. stearothermophilus* has greater heat resistance and similar Z-value as *C. botulinum* has, it is also utilized as a biological indicator of sterilization as well (Feeherry et al., 1987; Watanabe et al., 2003). Hence, thermal destruction kinetics of *C. sporogenes* and *G. stearothermophilus* should be both studied to ensure effectiveness of thermal inactivation.

Many factors could influence the thermal destruction for microbial spores. First of all, inherent heat resistance of microorganism is the most important factor. According to the optimum growth temperature, microorganism could be divided into three groups: psychrophiles, mesophiles and thermophiles (Ingraham and Stokes, 1959; Kristjansson and Stetter, 1992). In other words, relatively higher treated temperature need to be applied to kill mesophiles and thermophiles. Environmental factors could affect thermal destruction as well, including water activity, pH value, and so on. In practice, improvement of thermal processing effectiveness by changing these environmental factors is frequently found in food industries (Jay, 2000). Among these factors, temperature and treatment time are two basic elements of inactivation. In general, higher temperature and longer treatment time have greater effect on killing microorganism. Water activity could also influence heat sensitivity of microorganism. Basically, several literatures concluded that dried spores and vegetative cells are more heat resistant than the moist status of same species (Coroller et al., 2001; Laroche et al., 2005; Murrell and Scott, 1996). Akterian et al. (1999) concluded that pH value is important factor impacts strength of thermal inactivation next to temperature itself and water activity. For instance, the optimum growth pH value is different from different microbial species. However, heat sensitivity will be enhanced if acid or base added into food when pH value is differ from the optimum growth pH value (Smelt and Brul, 2014). Thus, less severity of thermal processing will be applied on food with lower or higher pH value at same temperature. Same effect of pH on changing heat sensitivity was found for *C. botulinum*

(Townsend et al., 1954). Several literatures reported that changing pH indeed could influence heat resistance of *C. sporogenes* and *G. stearothermophilus* (Cameron et al, 1980; Löwik and Anema, 1972; Mafart et al., 2001; Paredes‐Sabja et al., 2007; Periago et al., 1998; Rodrigo et al., 1999). Given that pH 4.5 is generally considered as the limitation of *C. botulinum* to growth and produce toxin, most of the studies only focus on a narrow range of pH value, from 5 to 7. Whereas, some literatures reported that *C. botulinum* can occasionally grow and excrete botulinum toxin at pH value lower than 4.5 (Lund et al., 1987). For example, *C. botulinum* was found in pasteurized soya protein at pH 4.0 could still grow and produce toxin (Smelt et al., 1982). Likewise, Smoot and Pierson (1979) reported that spores of *C. botulinum* type A were still active at pH 4.3. Therefore, the thermal destruction of heat resistant spores should be determined in a wider pH range rather than only around neutral pH values. Thermal destruction trend of *C. sporogenes* and *G. stearothermophilus* in more acidic pH values are needed to be determined.

According to survival curve, the thermal inactivation kinetics could be understood. In general, the first order kinetics used most. In this thermal inactivation model, all cells or spores are supposed to have same sensitivity to heat. It is highly suitable for linear logarithmic survival curve. The decimal reduction time (D value) and the thermal destruction indicator (Z value) are two elementary parameters for analyzing thermal destruction trend (Peleg and Cole, 1998). However, only using this model is not enough as deviation may appear when the survival curve is non-linear. In fact, one microbial community contains different subpopulations with their own inactivation kinetics. Thus, introduction of other non-linear inactivation modellings are necessary, including Weibull model, Modified Gompertz model, and log-logistic model. Weibull model offers an alternative parametric approach for describing microbial surviving trend (Carroll, 2003). Modified Gompertz model was used for describing asymmetrical sigmoidal shape of microbial growth curves (Chen and Hoover, 2003). Log-logistic model was first introduced by Cole et al. (1993) which fits for non-linear curve with peaks and long tails. All these non-linear models had been well proved to analyze thermal inactivation behavior of different microorganism, including *C. sporogenes* and *G. stearothermophilus* (van Boekel, 2002; Mafart et al., 2001; Mafart et al., 2002; Linton et al., 1995; de W Blackburn et al., 1997).

The following general objectives were formulated for this study:

1. To determine thermal inactivation kinetics of *C. sporogenes* and *G. stearothermophilus* spores as influenced by different pH in buffers.
2. To ascertain the effect of pH value on the thermal destruction behavior of *C. sporogenes* and *G. stearothermophilus*.
3. To compare the thermal destruction behavior of *C. sporogenes* and *G. stearothermophilus* by using first-order and Weibull models.

CHAPTER 2

LITERATURE REVIEW

2.1Thermal processing of food

Thermal processing, defined as “the application of heat for the purpose of shelf life extension and promotion of safety of food”, is a popular concept both in scientific researches and design of food industries (Hassan and Ramaswamy, 2011). It is a very common processing method for improving food safety and stability.

Heat contributes most in thermal processing for food preparation, as it could modify texture and sensory properties of the products (Richardson, 2001). Since heat could induce reaction between food composition, flavor and color of food could be changed. For example, the Maillard reaction between amino compounds and reducing sugar under high temperature plays the most important role in enhancing tastes and browning of cooked food (Mottram, 2007). In contrast, heat may also cause negative effects on food quality, especially when using conventional thermal processing methods. As it takes long time for the center of the food to reach target temperature, off-flavor, color change, loss in nutrients and freshness are among the most common problems after thermal processing (Ling et al., 2014).

On the other hand, heat could kill microorganisms, including bacteria, endospores, fungi, and inactivate undesirable enzymes. Thus, safe and shelf stable commercially sterile foods could be offered after processing, which is also the main objective of thermal processing. According to definition and requirement given by the United States Food and Drug Administration (FDA), commercially sterile means products are free from pathogens and undesirable microorganisms under proper storage or distribution condition (Awuah et al., 2007). Therefore, thermal processes are widely used in food industries to kill pathogenic and/or more resistant spoilage microorganism. For instance, thermal sterilization is always applied on low-acid canned food to remove *Clostridium botulinum* spores, while pasteurization is common for milk products which eliminates vegetative pathogens (Gálvez et al., 2014; Ghani et al., 2001). Due to various properties and compositions of products, the severity of thermal processing is dependent on many different factors (Awuah et al., 2007). Hence, choosing the optimum severity and procedure of thermal processing is important in food industry.

2.1.1 Pasteurization

Pasteurization is a mild thermal processing by using relatively lower temperatures up to 100^{o}C, to inactivate viable pathogens to below a certain adaptable number. Most of heat-resistant enzyme and microorganisms would be destroyed. Usually, treated temperature would be lower than 100℃. After processing, the shelf-life of products could be last from several days to several months (Fellows, 2000; Jay, 2000). It is widely used for liquid products, such as inactivating *Mycobacterium paratuberculosis* in milk (Grant et al., 1998), minimizing possible health hazard in fruit juice (Aguilar-Rosas et al., 2007; Yeom et al., 2000) and destroying *Salmonella* in liquid egg product (Wong et al., 1996). However, in order to slow down the growth of spoilage microorganisms, the product after pasteurization often needs to be refrigerated. In addition, pasteurization could have effect on influencing sensory properties of products. As heat could cause denaturation of protein, thermal pasteurized liquid egg white has higher foam drainage and relatively unstable viscosity compared to irradiated egg white (Wong et al., 1996). Lee and Coates (2003) reported that there was significant loss of total carotenoid pigment contents in orange juice after thermal pasteurization at 90℃ for 30s. In addition, lighter and more saturated juice was obtained after treated. Gama and de Sylos (2007) found out only content of violaxanthin and lutein decreased significantly. In order to reduce loss of nutrition and sensory quality of food products, high-temperature short-time (HTST) condition was introduced. It is also named as “flash pasteurization” (Fellows, 2000).

2.1.2 Heat Sterilization

Sterilization means the destruction of all life. Sterilizing agent could be either physical or chemical (Pflug et al., 2001). Heat sterilization is one kind of physical sterilization methods to destroy all undesirable microorganisms by applying sufficiently high temperature for a long time, including killing heat resistant microorganisms and inactivating enzymes. In general, commercial sterilized products have shelf-life more than six months. The length of processing time depends on many factors, such as heat resistance of target microorganism, heat condition during processing, pH value of food, size of container and physical state of food (Fellows, 2000). Deindoerfer (1957) found out a method for calculating heat sterilization time by using microbial thermal destruction kinetics. On this base, analytical methods for calculating treatment time of heat sterilization were developed. However, when using these methods, the basic assumption should be followed, which is microbial destruction

rates can be correlated by an Arrhenius-type relationship over the temperature range of the sterilization (Deindoerfer and Humphrey, 1959). Heat sterilization has the same problem as pasteurization has, that is loss of nutrients and sensory properties during high temperature treatment. An obvious degradation of free amino acid was found in autoclaved infant formula. In addition, sterilized infant formula has 19.5% lower amount of total protein than conventional preparing infant formula has (Yeung et al., 2006). Hence, studies focus on developing methods to reduce damage on content of nutrients and maintain sensory characteristics are needed.

2.2 Thermal processing methods

2.2.1 Conventional thermal processing methods

Basically, thermal processing methods could be simply divided into two groups, in-pack and in-line processing (Tucker and Featherstone, 2010). For in-pack processing, retort technologies are the widest used technology among manufactures. The basic theory of steam retort system is shown in Figure 2.1. First in-pack products, such as canned products, should be loaded in crates. Design and material of crates should guarantee full-through heat penetration to all products. After loading, lid is closed and steam supply system is opened. Venting is necessary to remove entrapped air in the retort. Normally, the steam enters and exits are at the opposite sides of equipment, so that steam could be filled the vessel without any air. Vent valve will be closed after sufficient venting time. Then, temperature and pressure inside the vessel will increase. Once the target temperature has been reached, it would be hold for a period of time. After that, the retort will be cool down by water supply until reaching target cooling temperature. At the end, residual pressure will be released. The final temperature for products would be around 4℃, which is enough for drying container and inhibiting reproduce of thermophilic bacteria (Richardson, 2001). Actually, steam, air and water can be applied as the heat medium in retort systems. Hence, according to different heat mediums and retort patterns, there are several kinds of retort systems, including condensing steam retorts, crateless retorts, water immersion retorts, water spray and cascade, combined steam-air retorts, hydrostatic retorts, spiral retorts, and so on. Although retort technology is easy to apply, it takes long time to make product thermal center to reach the target temperature. Meanwhile, requirements of containers are strict as shape and size should be maintained after heating and cooling (Tucker and Featherstone, 2010).

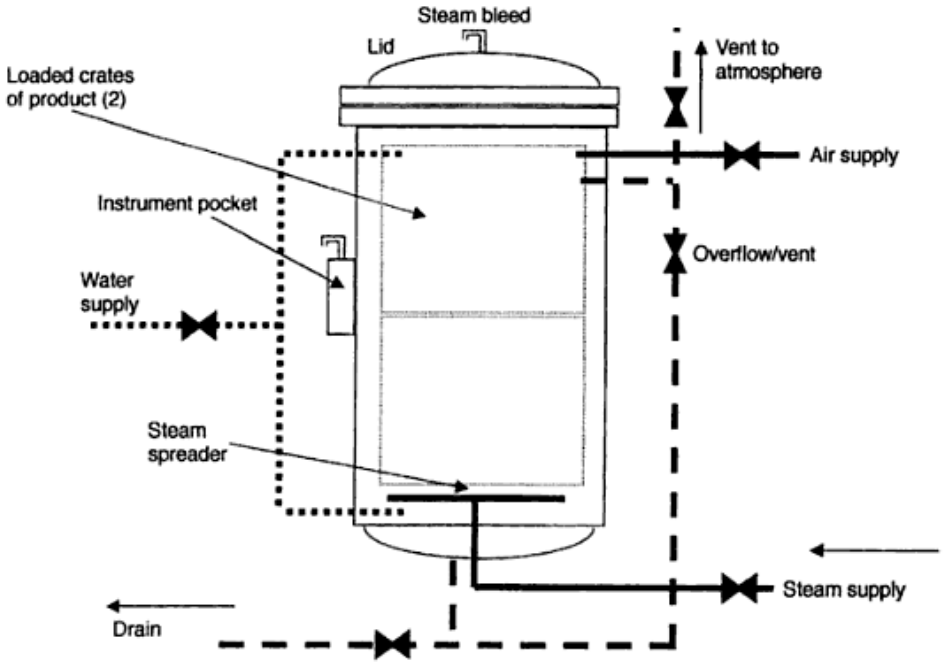


Figure 2.1 Schematic of steam retort system

However, when processing liquid or semi-liquid products, long time and low temperature batch retort methods may influence food quality, such as caramelized flavor and color changed. Instead of batch processing, in-line processing method, or continuous heating processing, are more suitable for the liquid and semi-liquid products. The basic theory of this method is pumped products through a continuous system, which could heated and then cooled down the products. After processing, the end-product will be packaged by a filling machine. The most famous in-line processing method is Ultra High Temperature (UHT) for milk (Richardson, 2001). Preparation module, heat exchanger and filling machine are the main three parts for in-line processing. Among that, heat exchanger influences most on food quality. Given to flow behavior and inherent characteristics of products, different heat exchangers will be applied for different food materials (Tucker and Featherstone, 2010). The in-line processing technologies could be classified into indirect and direct methods. The indirect method needs a heat transfer surface between products and heat medium, while the direct one does not (Richardson, 2001).

2.2.2 Novel thermal processing methods

In order to satisfy customers, more new thermal processing methods that can retain flavor,

texture and color of end-products are developed. Most of these novel methods are based on electro-technologies, such as radio frequency (RF) heating, ohmic heating and microwave heating. RF heating involves high frequency of electromagnetic energy to lead frictional interaction between molecules in food products. Food is loaded between two capacitor plates, which are charged by high frequency alternating electric field. Thus, only food may be heated. The surround atmosphere still maintains room temperature. This method could save energy and offer rapid and sufficient heat pattern as well. Nevertheless, the RF electromagnetic energy may be harmful to human health if it emits from poor designed equipment. Microwave heating is similar to the RF heating, but with higher frequency of energy, range from 950 to 2450 Hz. Water content of food is a crucial factor for both RF heating and microwave heating. As RF has lower frequency of energy, longer wavelength energy wave would be provided. Thus, RF heating may not lead to overheating on surface of food (Piyasena et al., 2003). However, microwave heating could be applied for food with package, which may reduce post-contamination after sterilization. Disadvantage of microwave heating is relatively high cost of equipment. In addition, microwave heating is highly dependent on food properties. Ununiformed temperatures of food are commonly found after microwave heating (Fakhouri and Ramaswamy, 1993). In terms of ohmic heating, the electric current is directly applied on food. Therefore, food could be rapidly heated. Nutrition and sensory properties could be maintained maximally. The only limitation is that there is no rapid cooling method able to use (Tucker and Featherstone, 2010).

Apart from that, infrared, another electromagnetic energy, is also a good source for heating food. The energy of infrared could be absorbed by food molecules. Vibrational state of the molecules could be changed, which can produce heat and increase temperature of food. This technology has been successfully applied on baking and roasting procedures. However, reports showed that infrared is not enough for eliminating microorganism. The infrared heating is more suitable for partial pasteurization, such as surface pasteurization (Sakai and Hanzawa, 1994). A new technology was introduced to tempering frozen food by combining microwave and infrared heating (Seyhun et al., 2009).

High pressure could inactivate vegetative cells by destroying cell membrane, denaturing protein, and decreasing intracellular pH value. Whereas only use high-pressure cannot remove heat resistant spores or enzymes. Hence, high pressure sterilization is more efficient for providing shelf-stable products (Matser et al., 2004). However, synergic effect between pressure and temperature on inactivating microorganisms and biological reactions was proved. Combination of high-pressure and moderated temperature shows good effect on destroying

heat resistance spores, including *C. sporogenes* and *G. stearothermophilus* (Patazca et al., 2006; Ramaswamy et al., 2010; Shao et al., 2010).

2.3 Standpoint of sterilization for food products

Generally, food could be divided into three groups according to pH value. There are low-acid food (pH above 4.5), acid food (pH range from 4.0 to 4.5), and high-acid food (pH below 4.0). The pH value of 4.5 is very significant as *C. botulinum* could produce neurotoxin in the condition of pH as low as 4.6. As different microorganism may growth under different pH value, the standpoints of sterilization are different from different group. For both low-acid and acid food, spore-bearing bacteria are the standpoints. In contrast, non-spore-bearing bacteria are for high-acid food (Stumbo, 1973).

2.3.1 Spore-bearing bacteria

Bacterial spore is a resistant structure of bacteria used for survival under unfavorable conditions. Exospore and endospore are two different categories of bacterial spores. Exospore is formed by differentiation of the entire bacterial cell into a spore, which is less able to survive with environmental changes than endospore. Endospore is a dormant, tough, and non-reproductive structure produced by bacteria by a response to profound environmental changes, such as nutrient starvation. The formation of endospore is named as sporulation. It is highly resistant to ultra violet radiation, desiccation, high temperature, extreme freezing, high pressure, and chemical disinfectants. Once the environment changes back to favorable condition, the endospore could geminate to vegetative cells (Abel-Santos, 2014). Spore forming bacteria would be distributed into three groups according to requirement of oxygen: obligate aerobes, facultative anaerobes and obligate anaerobes.

Obligatory aerobic spores need oxygen to support their growth. Most of them are not heat resistant. Apart from that, current food manufacturers use modified atmosphere package (MAP) technologies to reduce occupation of oxygen in package. Thus, obligate aerobes are least important for the standpoint of sterilization in spore-bearing bacteria (Stumbo, 1973).

Facultative anaerobic spores could generate energy aerobically and anaerobically. However, they may prefer oxygen more as aerobic respiration could generate more energy. This kind of microorganism is quite important for processing canned food because they may cause flat-sour spoilage (Cameron and Esty, 1926). In order words, they could induce spoilage by producing acid nearly without producing gas. The genus *Bacillus* is a typical type

of facultative anaerobes which could produce acidic substrate in canned food (Jansen and Aschehoug, 1951). For low-acid food, thermophilic *Geobacillus stearothermophilus* and related species are the most significant microorganism. The decimal reduction time, D-value, at 121℃ is approximately 4 minutes. For acid food, *Bacillus coagulans* is the most important one, particularly for tomatoes and relative products (Stumbo, 1973).

Obligate anaerobic spores are poisoned by oxygen. They could only live in anaerobic environment. Most spores are heat-resistant, either mesophilic or thermophilic. In canned food, anaerobic microorganism could cause swell with improper sterilization (Cameron and Esty, 1926). However, Nakayama and Sumiko (1980) found out that obligate anaerobes could also cause flat-sour spoilage in canned drinks. In low-acid food, anaerobic *C. botulinum* is regarded to be the most significant bacteria. Spores of *C. botulinum*, such as *C. sporogenes* and relative species are more resistant than *C. botulinum*. Hence, stricter requirements for food processing to prevent spoilage caused by *C. sporogenes* are necessary. For acid food, spores such as *C. pasteurianum* and relative species are important, but less heat resistant than *C. sporogenes* (Stumbo, 1973).

2.3.2Non-spore-bearing bacteria, yeast and molds

This group of microorganisms is important for high-acid food. The representative non-spore-bearing bacteria are *Lacobacillus* and *Leuconostoc spp.*. Basically, these microorganisms attach food products via container leakage. Meanwhile, all microorganisms in this group would be easily killed by mild thermal processing, such as pasteurization. They are less heat resistant compared to spore-bearing bacteria (Stumbo, 1973).

2.4 Factors influence heat resistance in microorganisms

2.4.1 Inherent resistance of microorganism

Many factors could influence the effect of thermal destruction on microorganisms. First of all, inherent characteristics of microorganisms play important roles. Classification of microorganism could be also followed the optimum temperature for growth. They could be divided into three groups: psychrophiles, mesophiles and thermophiles. The optimum growth temperature ranges for these three groups are -20 to 10℃, 20 to 45℃, and more than 50℃, respectively (Ingraham and Stokes, 1959; Kristjansson and Stetter, 1992). Basically, microorganisms have greater heat resistance with higher optimum growth temperature.

Meanwhile, growth stage and number of the microorganism could influence the heat resistance as well. Reports showed that bacterial cells in the stationary phase seem more heat stable than the ones in the logarithmic phase. More cells in the mixture also have higher heat resistance. It is supposed that metabolic compounds extruded by cells could protect them from heat treatment (Jay, 2000). In addition, vegetative cells are less heat stable than their endospores. In each species, temperature of treatment for endospores is nearly 40℃ higher than that for vegetative cells to reach the same comparative D-value (Pflug et al., 2001).

2.4.2 Treatment temperature and time

Apart from inherent factors, environmental factors also contribute to the heat resistance of microorganism, including treatment severity, composition of the medium, pH, and water activity.

It had been widely acknowledged that temperature and time of heat treatment are the two basic elements for killing microorganism in food. The most common inactivation model is the Bigelow linear model, also called the first order reduction. In this inactivation model, the number of microorganism reduced proportionally in every unit of time at certain treating temperature (Sala et al., 1995). All in all, higher temperature and longer treatment time have greater effect on killing microorganism. In addition, size and material of product containers could affect the heat conduction. Hence, large containers need more time or higher temperature than the small ones to reach the same microbial inactivation effect (Jay, 2000).

2.4.3 pH value

According to most research studies, bacteria or spores in neutral heating medium have the maximum heat resistance. Different species may have their own maximum resistance at different pH value. For example, greatest resistance of *C. tetani* appears at pH 7.0, while *B. anthracis* appears at pH 8.0 (Jay, 2000; Amaha and Sakaguchi, 1954; Walker, 1964). However, heat sensitivity will be enhanced if acid or base added into the medium. Thus, less severity of thermal processing will be applied on food with lower or higher pH value at same temperature. Bahçeci and Acar (2007) reported that pH value could influence heat resistance of *Alicyclobacillu acidoterrestis* in fruit juice, though the effect is not as obvious as temperature has. Hutton et al. (1991) reported *Clostridium sporogenes* has twice greater heat resistance at pH 7 than at pH 5. The effect of pH on enhancing heat sensitivity of microorganism had been widely used in food processing in heat treatments, especially in canned food. Acidified canned with neutral pH value by acidulant could milder the thermal

processing, which could reduce influence of heat on sensory properties. Several acids could be applied as acidulant, including citric acid, phosphoric acid, tartaric acid, malic acid, lactic acid, fumaric acid, adipic acid, acetic acid, and so on (Gardner, 1973; Powers and Niven Jr, 1976; Farber and Pagotto, 1992).

2.4.4 Water activity

Water is an essential element for microorganisms to grow. Water activity (a_w) is the ratio of vapor pressure of water to vapor pressure of pure water at the certain temperature in one system (Rahman and Sablani, 2009). The higher a_w of food or medium indicates more free water could be used by microorganism. Basically, several literatures concluded that dried spores and vegetative cells are more heat resistant than the moist status of same species. Lower a_w of heat medium leads to less heat inactivation effect on microorganism (Jay, 2000; Laroche et al., 2005; Coroller et al., 2001; Murrell and Scott, 1966). The reason is that rate of protein denaturation is faster when heated with more water. Presence of carbohydrates could lead to lower a_w and consequently strengthen heat resistance of microorganism. Salt composition could also change a_w of heat medium. However, different salts may have different effects. Some salts could decrease a_w which could protect the microorganism, including Ca^{2+} and Mg^{2+}. In contrast, some salts may increase a_w and consequently increase microbial sensitivity, such as phosphate (Jay, 2000). Besides, it also depends on type of microorganisms. For example, sodium chloride could protect most heat-sensitive bacteria, but destroy most heat-stable bacteria (Coroller et al., 2001).

2.4.5 Fat

Fat is another medium composition which could alter water content. Higher percent of fat in the medium simply reduces a_w and consequently enhance heat resistance of microorganism. Ahmed et al. (1995) proved that *Escherichia coli* O157:H7 had higher D-values in higher fat level meat products than in low level. Meanwhile, long-chain fatty acids have greater fat protection than short-chain fatty acids (Jay, 2000). Nevertheless, fat could also prevent growth of microorganism by entrapping the cells (Hansen and Riemann, 1963).

2.4.6 Proteins

Proteins in heat medium enhance heat resistance of microorganism as well. High level protein foods need higher temperature or longer thermal processing time than low level

products (Jay, 2000). Unfortunately, the mechanism of protection from proteins is unclear. It is supposed that proteins could protect microorganisms by attaching the surface of cell, but evidences were insufficient (Hansen and Riemann, 1963).

2.5 Thermal destruction of heat resistance spores

2.5.1 Thermal destruction of *Clostridium sporogenes* at different pH

Generally, bacterial spores, or endospores, are the hardest life form in the world. They can stand for various physical insults, such as wet, dry heating, UV and gamma radiation (Nicholson et al., 2000). This life form is especially important for *Clostridium* and *Bacillus* as dormant cellular structure (Yang and Ponce, 2009). In food industries, *C. botulinum* spores are used as an indicator of commercial sterilization. For example, 12 decimal reduction of *C. botulinum* spore number should be achieved for low-acid food thermal processing. Given to neurotoxins secreted by *C. botulinum*, endospores, such as *C. sporogenes*, are widely used as surrogate (Shao and Ramaswamy, 2011). *C. sporogenes* are gram-positive, mesophilic, and putrefactive anaerobic spores. The D value at 121.1℃ is around 1 minute. Besides, *C. sporogens* are easy to cultivate, which is good for researches (Stumbo, 1973).

Several researches were done for finding out pH influence on heat resistance of *C. sporogenes* (Table 2.1). Brown et al. (2012) reported a review of heat resistance data of *C. sporogenes* based on literatures over the past 100 years. Most of the results showed that lower pH could reduce D-value. The reduction effect is more pronounced at lower temperature, but the exact relationship is still unknown. Santos and Zarzo (1995) used citric acid and glucono-δ-lactone (GDL) as acidulants in asparagus puree. The pH values were adjusted to 4.5, 5.0 and 5.5 respectively. Compared to natural asparagus puree (pH 5.8), *C. sporogenes* in acidified matrix had relatively lower D-value indeed. Obvious reduction was found when treatment temperature range from 121 to 127℃. While Rodrigo et al (1993) suggested that acidification do not reduce D-value at the temperature range from 121 to 143℃. In contrast, Löwik and Anema (1972) did a study on heat resistance of *C. sporogenes* with Ravioli minced meat. The result came out as only absolute D value decreased with lower pH medium but not relative D value. In other words, the treatment temperature cannot disturb the reduction effect of pH value on D values. In addition, different acidulants may have different efforts on reducing D-value at the same pH. Previous research showed that citric acid had greater effect on enhancing heat sensitivity and decreasing recovery ability of *C. sporogenes* than GDL (Silla et al., 1992; Santos and Zarzo, 1996).

Table 2.1 Thermal destruction of *C. sporogenes* ATCC 7955 at different pH

Matrix		Temperature(℃)	D-value (min)	Reference
Substrate	**pH**			
carrot-alginate particles acidified with GDL	5	80	36.5	Naim et al, 2008
	4	80	13.23	
acidifed minced meat	6.0	110	about 11	Löwik and Anema, 1972
	5.0		about 9	
	4.0		about 7	
mushroom extract acidified with citric acid	6.2	125	0.92	Fernandez et al., 1996
	4.6		0.89	
0.067M Sorensen phosphate buffer	7.0	110	15.9	Cameron et al., 1980
		121	2.6	
	6.5	110	17.8	
		121	1.8	
	6.2	110	12.3	
		121	1.8	
	6.0	110	15.0	
		121	2.0	
	5.50	110	13.5	
		121	1.6	
	5.00	110	10.6	
		121	1.1	
Acidified asparagus with citric acid	5.47	110	10.28	Silla et al., 1992
		115	7.96	
		118	3.25	
		120	1.26	
	5.06	110	10.45	
		115	3.10	
		118	2.40	
		120	1.56	
	4.77	110	7.57	
		115	4.03	
		118	2.09	
		120	1.26	
	4.50	110	7.93	
		115	3.06	
		118	3.14	
		120	1.28	

Table 2.1 Thermal destruction of *C. sporogenes* ATCC 7955 at different pH (continued)

Matrix		Temperature(℃)	D-value (min)	Reference
Substrate	pH			
Acidified asparagus with GDL	5.42	110	18.31	Silla et al., 1992
		115	5.52	
		118	2.63	
		120	1.61	
	5.12	110	10.21	
		115	4.65	
		118	2.66	
		120	1.04	
	4.83	110	12.52	
		115	4.84	
		118	1.97	
		120	1.47	
	4.57	110	6.54	
		115	3.03	
		118	1.55	
		120	0.92	
Acidified mushroom extract with citric acid	6.70	110	8.47	Ocio et al., 1994
		115	1.74	
		118	1.22	
		121	0.67	
	6.22	110	16.17	
		115	2.10	
		118	1.50	
		121	0.79	
	5.34	110	9.14	
		115	2.04	
		118	1.30	
		121	0.67	
	4.65	110	6.00	
		115	2.01	
		118	1.30	
		121	0.72	

Table 2.1 Thermal destruction of *C. sporogenes* ATCC 7955 at different pH (continued)

Matrix		Temperature(℃)	D-value (min)	Reference
Substrate	pH			
Acidified mushroom extract with GDL	6.70	110	8.47	Ocio et al., 1994
		115	1.74	
		118	1.22	
		121	0.67	
	6.22	110	14.42	
		115	2.71	
		118	1.16	
		121	0.68	
	5.34	110	10.05	
		115	2.27	
		118	0.98	
		121	0.51	
	4.65	110	7.29	
		115	1.46	
		118	1.11	
		121	0.56	
Acidified asparagus with citric acid	5.50	121	1.11	Santos and Zarzo, 1995
		130	0.09	
		140	0.03	
	5.00	121	1.08	
		130	0.12	
		140	0.04	
	4.50	121	1.08	
		130	0.06	
		140	0.02	
Acidified asparagus with GDL	5.50	121	1.49	
		130	0.10	
		140	0.04	
	5.00	121	1.32	
		130	0.08	
		140	0.02	
	4.50	121	1.27	
		130	0.10	
		140	0.03	

2.5.2 Thermal destruction of *Geobacillus stearothermophilus* at different pH

Although several literatures reveal that sufficient thermal processing which can diminish *Clostridium* cells and spores, thermophilic bacteria could still exist in commercially sterilized products, including *G. stearothermophilus* (Feeherry et al., 1987). *G. stearothermophilus*, also named as *B. steatothermophilus*, is a kind of endospore form of *Bacillus*. It is also a particular interest in food industries since this spore form is extremely heat resistant. Meanwhile, it could induce flat sour spoilage for low-acid canned food. The optimum growth temperature could range from 49 to 55℃ with 4 minutes as D value at 121.1℃. What is more, germinated vegetative cells could live at temperature more than 70℃ (Finley and Fields, 1962; Nazina et al., 2001; Stumbo, 1973). Because of similar Z-value as *C. botulinum* has, *G. stearothermophilus* is utilized as a biological indicator of sterilization as well. Normally, 5 decimal reduction of *G. stearothermophilus* spore number should be achieved for commercial applications (Hassan and Ramaswamy, 2011; Watanabe et al., 2003).

Inactivation kinetics of *G. stearothermophilus* was studied in the past decades, including high pressure processing and thermal processing. Researches related to thermal destruction of *G. stearothermophilus* ATCC 12980 with different pH matrix are listed in Table 2.2. The same as other bacteria, heat resistance of *G. stearothermophilus* is also decreased with lower pH matrix. The reduction effect is pronounced at lower temperature. López et al. (1996) revealed that no reduction effect on D value at temperature high as to 135℃. Rodrigo et al. (1999) found out no influence at temperature more than 130℃. Different results may be due to different strains were used for studies. Apart from that, types and concentration of acidulants could affect the efficiency of reduction. Comparison of organic acids was finished by Lynch and Potter (1988). The greatest effects were appeared at pH 4.6 with lactic acid, citric acid, ad acetic acid. In addition, higher concentration of acidulant in matrix seems to have higher reduction effect. It was found that 0.5M phosphate buffer had greatest effect on increasing heat sensitivity of *G. stearothermophilus*, whereas buffers with concentration lower than M/80 had no effect. The reason was supposed as lower concentration phosphate buffer could induce germination and

outgrowth of spores. Hence, heat resistance of *G. stearothermophilus* would not be influenced, but could be even enhanced (Finley and Fields, 1962).

2.6 Thermal inactivation kinetic models

As only visible survival microorganism could be analyzed after thermal destruction, microbial inactivation kinetic models turn to be significant for understanding trend of cell death. It is really important in the field of food quantitative risk assessment (Peleg and Cole, 1998). The trend of inactivation is always presented by survival curves, which shows the relationship between logarithm of survival numbers and exposure time (Anderson et al., 1996). According to the shape of logarithmic survival curve, there are four types of curves: linear curves, curves with a shoulder, curves with a tailing and sigmoidal curves (Xiong et al., 1999 b). Basically, the first order kinetics used most, as it is highly suitable for linear survival curves. It is assumed that all cells or spores in the system have equal sensitivity to heat. However, it is a chance of a quantum of heat influence on cells death rate (Anderson et al., 1996; Peleg and Cole, 1998). Deviation of the first order kinetics had been frequently reported since this model does not fit for non-linear curves. Other kinds of model are established for non-linear curves, such as modified Gompertz model, Weibull model, log-logistic model and so on (Xiong et al., 1999 a).

Table 2.2 Thermal destruction of *G. stearothermophilus* ATCC 12980 at different pH

Matrix		Temperature(℃)	D-value (min)	Reference
Substrate	**pH**			
Acidified mushroom extract with GDL	6.2	130	0.14	Fernandez et al., 1996
	5.3		0.11	
McIlvaine buffer	7	115	11.36	López et al., 1996
		120	2.31	
		130	0.096	
	6	115	3.99	
		120	1.24	
		130	0.092	
	5	115	3.29	
		120	0.98	
		130	0.07	
	4	115	1.28	
		120	0.44	
		130	0.023	
Food without oil or vinegar	5.26	115	8.7	Rodrigo et al., 1999
		118	3.5	
		121	1.41	
		125	0.42	
Food without vinegar and with oil	5.26	115	-	
		118	-	
		121	1.41	
		125	-	
Food without oil and with vinegar	4.81	115	6.53	
		118	2.89	
		121	1.28	
		125	0.43	
Food without oil and vinegar	4.81	115	3.86	
		118	1.85	
		121	0.93	
		125	0.37	
Acidified mushroom extract with GDL and 1.5% NaCl	6.7	123	0.93	Periago et al., 1998
	6.35		0.63	
	5.9		0.35	

2.6.1 First order kinetics

This model assumes that cells or spores have identical resistance to heat. Thus, the inactivation trend is linear reduction at a certain temperature treatment. The differential equation could be expressed as followed:

$$-\frac{dN}{dt} = \mathrm{kN} \quad (1)$$

where N is the survival number of cells or spores after treatment, t is the exposure time, and k is the reaction-rate constant, which equals slope of the survival curve and highly depending on temperature (Peleg and Cole, 1998; Pflug et al., 2001).

The equation (1) could transfer to an equation with logarithms to the base "10":

$$\log\mathrm{N} = -\frac{t}{D_T} + \log N_0 \quad (2)$$

where N_0 is the number of initial count, D_T is the decimal reduction time, which equals to -1/k (Pflug et al., 2001).

The decimal reduction time, also called D-value, is time at certain temperature for reducing 10 fold of target treated microbial number. Mostly, the D-value presents in minutes. As it is the negative reciprocal of reaction-rate constant, it depends on the temperature as well. Relationship between temperature and D-value supposed to be log linear (Peleg et al., 2005). Different microbe may have different D-value at the same temperature. This concept has been commonly used as a criterion for determining efficiency of thermal processing, such as pasteurization and sterilization (Katzin et al., 1943).

In order to express the temperature sensitivity of D-value, logarithms D-values are plotted as a function of temperature. Expected result is a straight line. The slope of the graph relates to another value, named as Z-value, indicating a temperature interval that could induce 10-fold increase or decrease of D-value. It could be presented in ℃ or ℉ (Peleg et al., 2005; Pflug et al.,

2001).

$$Z_T = \frac{(T_2 - T_1)}{[\log(D_1) - \log(D_2)]} \quad (3)$$

where D_1 and D_2 are D-values at temperature T_1 and T_2 respectively.

Apart from D-value, F value is frequently used as an indicator for efficiency of thermal processing too. F_T value defined as the equivalent minutes at temperature T which is able to destroy cells or spores of target microorganism as same effect of a specific z-value. For example, F_0 value means equivalent minutes at 250.0°F with a z-value of 18.0°F, or 121.1℃ with a z-value of 10.0℃ (Pflug et al., 2001; Jay 2000).

$$F_0 = D_{250.0°\mathrm{F}}(\log a - \log b) \quad (4)$$

where a is the initial count of microorganism and b is the final count.

However, the first order model is based on the assumption that all cells have same behavior to heat. The fact is that one microbial community contains different subpopulations with their own inactivation kinetics. It is one of the reasons that could explain appearance of non-linear survival curves (van Boekel, 2002). Hence, introduction of other non-linear inactivation models are necessary.

2.6.2 Weibull model

The Weibull model is a combination of accelerated failure-time model and parametric distribution. It offers an alternative parametric approach for describing microbial surviving trend (Carroll, 2003). For convenience, this model is based on the assumption that possibility of individual cells or spores die by treatment disperses according to Weibull distribution, while the survival curves are in cumulative form for the distribution of lethal events (Chen, 2007).

$$\log \frac{N}{N_0} = -bt^p \quad (5)$$

where b and p are the two parameters of distribution; b is a scale parameter, which is a characteristic time and p is the shape parameter. When p >1 indicates that the survival curve is concave upward, while p < 1 indicates concave downward. It could indicates linear survival curve when p = 1, which is the same as first order kinetics. These two parameters could be used to calculate mean, variance and coefficient of skewness of survival curves (Peleg and Cole, 1998).

This model had been successfully used on thermal destruction of *B. cereus*. It is also suitable for *C. botulinum* and *G. stearothermophilus* spores. Not only for thermal processing, the Weibull model is also applied to electronic and mechanical system, such as reduction of microorganism after treated with pressure (van Boekel, 2002; Mafart et al., 2002).

2.6.3 Modified Gompertz model

Originally, the modified Gompertz model was used for describing asymmetrical sigmoidal shape of microbial growth curves. As similarity between shape of growth and inactivation survival curve, this equation also utilized for thermal inactivation kinetics (Chen and Hoover, 2003). It could be applied for predicting non-linear survival microorganism dealing with time-varying temperature conditions (Gil et al., 2006). The equation was modified by dividing N_0 to avoid influences by different initial number in multiple experiments (Xiong et al., 1999 b).

$$\log \frac{N}{N_0} = Ce^{-e^{BM}} - Ce^{-e^{-B(t-M)}} \quad (6)$$

where C is the difference in value of upper and lower asymptote, the minus before C means reduction of microorganism. M is the time that reaches absolute greatest death rate, which is B.

Several literatures reported successfully application of modified Gompterz model on showing destruction of microorganism, including thermal destruction of *Listeria monocytogenes* in liver sausage slurry and inhibition of *Yersinia enterocolitica* ATCC 35669 under pressure (Xiong et al., 1999 a). In addition, the Gompterz model could be used for predicting microorganism under different environmental conditions, such as thermal destruction of *L. monocytogenes* in heating medium with different pH and different concentration of NaCl (Linton et al., 1995).

2.6.4 Log-logistic model

This model was first introduced by Cole et al. (1993) which fits for non-linear curve with peaks and long tails. Premise of this model is that heat resistance of cells or spores are individual and the differences are permanent. The equation shows as:

$$log_{10}(cfu\ ml^{-1}) = \alpha + \frac{\omega-\alpha}{1+exp\frac{(4\sigma(\tau-log_{10}t))}{(\omega-\alpha)}} \quad (7)$$

where t is the exposure time, usually in minutes, α is the upper asymptote ($log_{10}(cfu\ ml^{-1})$), ω is the lower asymptote ($log_{10}(cfu\ ml^{-1})$), σ is the maximum slope of inactivation curve (=$log_{10}(cfu\ ml^{-1})$ /$log_{10}t$), and τ is $log_{10}t$ at the position of maximum slope (log min).

This model had been utilized on predicting thermal destruction of *L. monocytogenes*, *C. botulinum*, *E. coli* O157:H7, *Y. enterocolitica* and so on (Chen and Hoover, 2003). What is more, the log-logistic model showed ability to quantified influence of heat resistance from pH and water activity (de W Blackburn et al., 1997). It also used for indicating inactivation of *Salmonella enterica* serovar under pulsed electric field (Chen, 2007).

PREFACE TO CHAPTER 3

Effectiveness of thermal processing on inactivating microorganisms is crucial for ensuring food safety. *Clostridium botulinum* is one the most important pathogens in food industry. A 12-fold degradation of *C. botulinum* is commonly considered as the standard for commercial sterilized products. As *C. botulinum* will produce toxin, spore form of *C. botulinum*, such as *C. sporogenes,* is frequently used as surrogate in research studies. Several factors can influence heat resistance of microorganisms. pH value is the major one. It is practical for industry to decrease heat resistance of microorganisms by changing the factors and consequently milder thermal processing. Basically, microorganisms are less resistant to heat at lower pH levels. Most existing literatures on thermal inactivation behavior of *C. sporogenes* only focused on pH value around neutral value, from 5 to 8. However, thermal destruction trend of spores is needed in a wider pH range for better understanding. In addition, substrates can also change heat sensitivity of microorganisms. Therefore, comparison between heat resistances of spores in different buffer solutions at the same pH value is necessary. The aim of this chapter is evaluating thermal destruction kinetics of *C. sporogenes* as influenced by buffer type and pH values.

Part of this research will be presented in the Northeast Agricultural and Biological Engineering Conference (NABEC) 2016 and prepared for publication in a scientific journal. The research was carried out by the candidate under the supervision of Dr. H.S. Ramaswamy.

CHAPTER 3

EFFECT OF BUFFER TYPE AND pH VALUE OF HEAT RESISTANCE OF *CLOSTRIDIUM SPOROGENES* ATCC 7955

3.1 Abstract

Thermal processing is an application of heat for the purpose of shelf life extension and promotion of safety of food. For low acid foods, destruction of *Clostridium botulinum* spores is deemed necessary from public health safety point of view. Since *C. botulinum* is pathogenic, a surrogate is often used for inactivation studies. *Clostridium sporogenes* is commonly used as surrogate of *C. botulinum*. Several factors influence heat resistance of microorganisms, the medium pH being a major one. In this study, the effect of pH and salt used in the buffer on the destruction kinetics of *C. sporogenes* ATCC 7955 was evaluated at 110, 115 and 120℃ respectively. Results demonstrated that *C. sporogenes* had lower heat resistance in phosphate and McIlvaine buffers than in distilled water. In McIlvaine buffer, *C. sporogenes* had a maximum heat resistance at pH 7.0, which is also the optimum pH value for their growth. D values decreased when pH value decreased or increased from 7.0. The pH effect on spore kill was more apparent at lower temperatures. However, the Z value was not related pH ($P>0.05$).

3.2 Introduction

Thermal processing is one of the most frequently used processing technologies in food industry to produce safe, shelf stable foods. Heat contributes most in this processing method. It could provide sensory characteristics by modifying texture and taste of food, such as Maillard reaction (Mottram, 2007). Meanwhile, heat could destroy undesirable microorganisms, including pathogens, endospores, and enzymes (Richardson, 2001). With respect to food preservation, thermal processing could be simply divided into two categories according to the temperature used for processing: pasteurization and sterilization. Vegetative pathogenic bacteria and some spoilage microorganisms would be destroyed or reduced to certain governmental approved level in pasteurization. However, for sterilization, all microorganisms capable of growing in processed canned foods should be killed (Jay, 2000). Requirement of thermal processing is different for different classes of food products. Generally, foods could be divided into three groups according to pH value. They are low-acid food (pH above 4.5), acid food (pH range from 4.0 to 4.5), and high-acid food (pH below 4.0). The pH value of 4.5 is very significant as *Clostridium botulinum* could produce neurotoxin only in the condition of pH above 4.5. Industrially, *C. botulinum* spores are commonly used as targets for commercial sterilization. For example, 12 decimal reduction of *C. botulinum* spore population should be achieved for low-acid food thermal processing (Hassan and Ramaswamy, 2011). Among these spores, *C. sporogenes* is often chose for research studies due its non-toxigenicity and similar heat resistance of *C. botulinum*.

Modifying severity of thermal processing is important for maintaining sensory characteristics of products as well as achieving requirement of microbial inactivation. Temperature, water activity and pH value are three factors of major importance with respect to the heat resistance of microorganisms. Several previous researchers have shown that lower pH could significantly reduce decimal reduction time (D value) of microorganisms at the same temperature (Bahçeci and Acar, 2007; Hutton et al., 1991). pH value also has a direct effect on influencing heat resistance of *C. sporogenes*, but the exact effect is still unknown. Most of literature shows that lower pH value could enhance heat sensitivity of *C. sporogenes*, especially

at relatively lower temperature (Naim et al, 2008; Cameron et al., 1980). Rodrigo et al. (1993) suggested that acidification do not reduce D-value at high temperature range from 121 to 143℃. In contrast, Löwik and Anema (1972) did a study on heat resistance of *C. sporogenes* with Ravioli minced meat. Their results show that temperature cannot disturb the reduction effect of pH value on D-values. Apart from pH value, different acidulants may have different influence on decreasing D value at same pH value. Citric acid is supposed to be more efficient than glucono-δ-lactone (GDL) (Santos and Zarzo, 1995; Santos and Zarzo, 1996; Silla et al., 1992). Although lots of previous research studied on effect of pH influencing microbial heat resistance, most of them only focus on a narrow pH range from 5.0 to 7.0. It is because of narrow pH range of phosphate buffer. What is more, seldom studies applied for pH lower than 4.5, as *C. botulinum* is acknowledged to be not significant below pH 4.5. However, some studies do report that *C. botulinum* could produce toxic in acid environment with pH lower than 4.5 (Lund et al., 1987; Smelt et al., 1982). Therefore, thermal inactivation trend of *C. sporogenes* in a wider pH range is desirable. In addition, there is no comparison of efficiency of reducing microbial heat resistance between phosphate buffer and McIlvaine buffer (citric acid and diosodium hydrogen phosphate).

Therefore the objectives of this study were to: 1) determine the influence of buffer type (phosphate vs McIlvaine buffer) on the heat resistance of *C. sporogenes* ATCC 7955 (PA 3679) at the same pH, and 2) determine thermal destruction kinetics of *C. sporogenes* ATCC 7955 (PA 3679) in McIlvaine buffer at different pH levels.

3.3 Materials and Methods

3.3.1 Spore preparation

Spore preparation was done according to the method reported by Shao and Ramaswamy (2011). Freeze-dried culture powder of *C. sporogenes* spores (ATCC-7955) was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and stored at -40℃ until use. The freeze-dried spore culture was hydrated with 10 mL of reinforced clostridial medium (RCM)

broth (Oxoid LTD., Hampshire, UK). The hydrated culture was anaerobically incubated at 37℃ for 24 h. The anaerobic condition was created by applying anaerogen sachat (Oxoid LTD., Hampshire, UK) to the anaerobic jar (BD Diagnostics, Microbiology, CA). This step was repeated for two times after which a sample of 0.1 mL of the hydrated spore broth was transferred into 50 mL of fresh prepared RCM broth and anaerobically incubated at 37℃ for 24 h. This step was repeated for two times. Then 0.2 mL of the culture was transferred and spread on Campdem Sporulating Agar (CSA) plate. The plates were anaerobically incubated at 37℃ for 7 days. Formulation of CSA plate involved dissolving 2.5g bacterial peptone (BD, CO., Spark, MD), 2.5g tryptone (Amresco Inc., Solon, OH), 0.5g lab lemco meat extract (Oxoid LTD., Hampshire, UK), 1g yeast extract (BD, CO., Spark, MD), 0.025g calcium chloride (EMD Chemicals Inc., Germany), 0.031g $MnSO_4 \cdot H_2O$ (Fisher Scientific, Fair Lawn, NJ), 0.5g glucose (Thermo Scientific, Logan, UT) and 7.5g bacterial agar (ACP Chemicals Inc., Montreal, QC) in 500 mL of distilled water.

3.3.2 Spore harvesting

Spores were collected by flooding the ager sporulated plate surface with approximately 10mL of buffer solution and scrapped with sterilized spreader. The wash step was repeated to ensure all colonies were collected. The mixture was collected in a sterilized 50mL centrifuge tube (Fisher Scientific, Fair Lawn, NJ). After harvesting, the spores were washed three times by centrifuging at 4000× g for 15 minutes at 4℃ and suspended in a buffer solution at the target pH value to give an initial microbial concentration of approximately 10^6 CFU/mL. The spore suspension was heat shocked at 80℃ with water bath for 10 minutes to remove potential vegetative cells. The spore suspension was stored at 4℃ until use. In this study, distilled water, 0.2M phosphate buffer at pH 7.0 and McIlvaine buffer at selected pH from 3.0 to 8.0 were used for spore harvesting. Formula of McIlvaine buffer is shown in Table 3.1.

Table 3.1 Formula for preparing 100mL of McIlvaine buffer

pH	0.2 M Na_2HPO_4 (mL)	0.1 M Citric Acid (mL)	pH	0.2 M Na_2HPO_4 (mL)	0.1 M Citric Acid (mL)
2.2	2.00	98.00	5.2	53.60	46.40
2.4	6.20	93.80	5.4	55.75	44.25
2.6	10.90	89.10	5.6	58.00	42.00
2.8	15.85	84.15	5.8	60.45	39.55
3.0	20.55	79.45	6.0	63.15	36.85
3.2	24.70	75.30	6.2	66.10	33.90
3.4	28.50	71.50	6.4	69.25	30.75
3.6	32.20	67.80	6.6	72.75	27.25
3.8	35.50	64.50	6.8	77.25	22.75
4.0	38.55	61.45	7.0	82.35	17.65
4.2	41.40	58.60	7.2	86.95	13.05
4.4	44.10	55.90	7.4	90.85	9.15
4.6	46.75	53.25	7.6	93.65	6.35
4.8	49.30	50.70	7.8	95.75	4.25
5.0	51.50	48.50	8.0	97.25	2.75

3.3.3 Thermal treatment

The spore suspension was first shaken for 15 minutes in an ultrasonic shaker before use. A temperature controlled oil bath (Polystat MDL. 1267-62, Cole Parmer Instrument Company, Chicago, IL) was used for giving the thermal treatment. The heat medium was pure glycerine G33-20 (Fisher Scientific, Fair Lawn, NJ). Temperature of the oil bath could be adjusted from 0 to 150℃. Stainless steel heating tubes with o-ring sealed screw caps were specially fabricated for this purpose (Figure 3.1). The come-up time in stainless steel caped test tube was approximately 1 minute thermocouple probes inserted through the tubes using special lids. Based on data of thermal destruction kinetics for *C. sporogenes* from previous literature, three temperatures (110, 115 and 120℃) were used for thermal treatment. For each temperature, at least four holding

times were used. The holding times did not include the come-up time. The intervals of holding times were different for each treatment temperature. For spores suspended in distilled water, the intervals were 10 minutes at 110℃, 4 minutes at 115℃ and 1 minute at 120℃ respectively. For spores suspended in 0.2M phosphate buffer (pH 7.0), the intervals were 8 minutes at 110℃, 2.5 minutes at 115℃ and 1 minute at 120℃ respectively. For spores suspended in McIlvaine buffer, the intervals were 4 minutes at 110℃, 2 minutes at 115℃ and 1 minute at 120℃ respectively. These times were established after some preliminary test runs to determine the approximate D value at each temperature. Duplicate of each treatment was necessary for insuring accuracy of test results. For each treatment, 1.5 mL of spore suspension was transferred into a previously autoclaved stainless steel caped test tube and sealed. The tubes were heated by suspending them in the oil bath for specified times. After treatment, the tubes were immersion cooled in ice water bath for 10 min.

3.3.4 Incubation and enumeration

The treated suspensions were poured into pre-sterilized micro-centrifuge tubes. Serial dilution was made with 0.1% peptone water and enumeration was done by using pour plate counting technique. The spores were anaerobically incubated on tryptic soy agar (TSA) plates (BD, CO., Spark, MD) at 37℃ for 48 h. After incubation, number of colonies was counted. The significant results were only chosen for those in the range of 30 to 300 colonies on one plate. The colony morphology of *C. sporogenes* is shown in Figure 3.2.

Figure 3.1 The stainless steel caped test tube used for oil bath

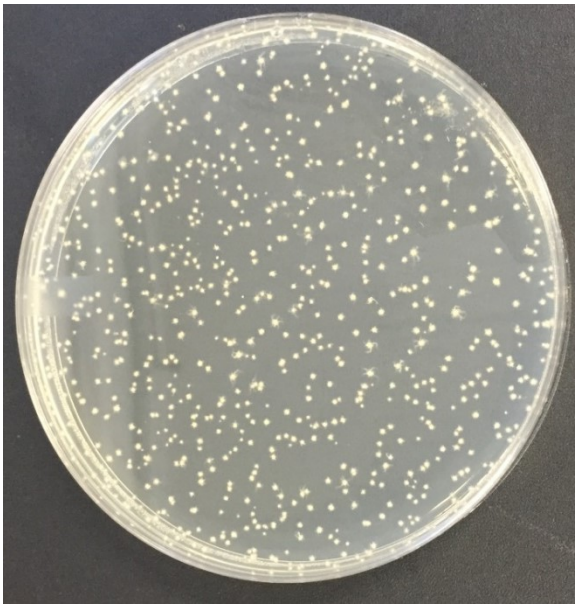


Figure 3.2 The colony morphology of *C. sporogenes* on tryptic soy agar plates

3.3.5 Data analysis

Test data were analyzed to determine parameters of thermal destruction kinetics based on the assumption that: (1) the inactivation of spores of *C. sporogenes* in buffer solution treated at any

temperature occurred at random in accordance with the first-order kinetics model, (2) all spores have identical resistance to heat.

According to previous assumptions, the decimal reduction time (D value) for each treatment groups could be calculated by using survival curves.

$$\log\ \mathrm{N} = -\frac{t}{D_T} + \log N_0 \tag{3.1}$$

where N is the survival number of cells or spores after treatment (CFU/mL), t is the exposure time (min), N_0 is the number of initial count (CFU/mL), and D_T is the decimal reduction time (min) at temperature T (℃), which is equal to the negative reciprocal slope of the semi-logarithmic survival curve and highly depending on temperature.

Therefore the D value can be obtained as the negative reciprocal of the linear regression slope of log (N/N0) versus time (min):

$$\mathrm{D} = -1/\mathrm{slope} \tag{3.2}$$

Apart from D value, the thermal resistance constant (Z value) was also calculated. The constant at a certain temperature was determined by plotting the decimal logarithm of decimal reduction time on the scale ($\log_{10}$ D value) versus the heating treatment temperature on the linear scale. The Z value is equal to the negative reciprocal of linear regression slope of thermal resistance curve (log D vs. T):

$$Z_T = \frac{(T_2 - T_1)}{[\log(D_1) - \log(D_2)]} \tag{3.3}$$

where D_1 and D_2 are D-values at temperature T_1 and T_2 respectively.

All experiments were repeated at least two times. The analysis of data was done using Microsoft Excel. Student's t test was used to determine the significance of differences and 95% confidence intervals of D values and Z values. One way ANOVA was used to estimate the effect of pH on influencing D values at certain temperature. In addition, difference parameter (Δ) and percentage relative difference parameter (Φ) were also utilized to analyze the differences of D values in different buffer solution. The equations of these parameters are shown followed:

$$\Delta = \mathrm{D_a} - \mathrm{D_b} \tag{3.4}$$

where D_a and D_b are D values of spores suspended in different buffer solutions at the same treatment temperature. The unit of difference parameter was min.

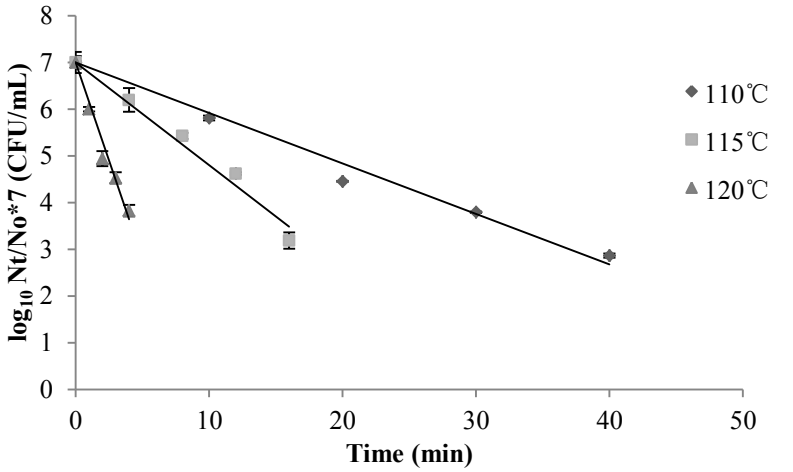
$$\Phi = (1-\frac{D_b}{D_a})\times 100 \quad (3.5)$$

where D_a and D_b are D values of spores suspended in different buffer solutions at the same treatment temperature and D_a is greater than D_b. The percentage relative difference parameter has no unit.

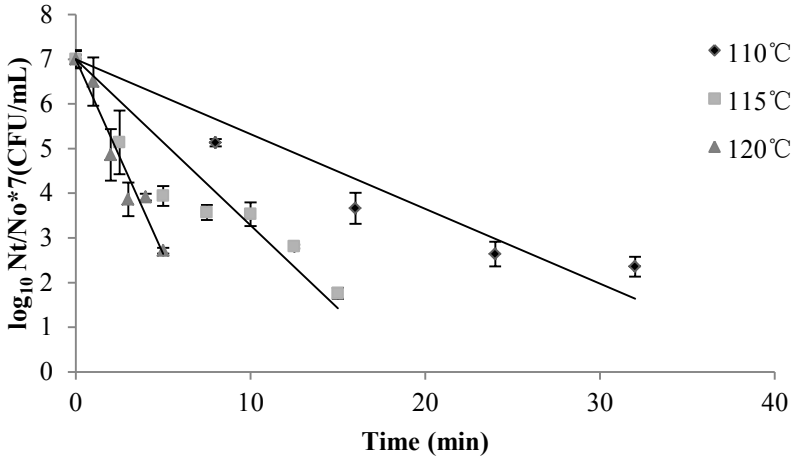
3.4 Results and Discussion

3.4.1 Effect of buffer type on influencing heat resistance of *C. sporogenes* ATCC 7955

Survival curves of spores were plotted as log10 (N_t/N_0) x 7 (so that the curves start from the nominal initial count of 10^7 CFU/ml) versus the holding time (min). The holding times did not include the come-up time (approximately 1min). Figure 3.3 (a) shows the survival curves of *C. sporogenes* ATCC 7955 in distilled water with the holding time range from 0 to 40 min while Figure 3.3 (b) shows the survival curves in 0.2M phosphate buffer at pH 7.0 with the holding time range from 0 to 32 min and Figure 3.3 (c) shows the survival curves in McIlvaine buffer at pH 7.0 with the holding time range from 0 to 20min. The linear regression line to the experimental data and, slope and the regression coefficients (R^2) of the regression line were obtained by using the Microsoft Excel program. The results are tabulated in Table 3.2 with the D values, R^2 and the 95% confidence interval for the D values of *C. sporogenes* in different heat media at treatment temperature 110, 115 and 120℃. In addition, the thermal resistance curves of *C. sporogenes* in different heat media are shown in Figure 3.4.



(a)



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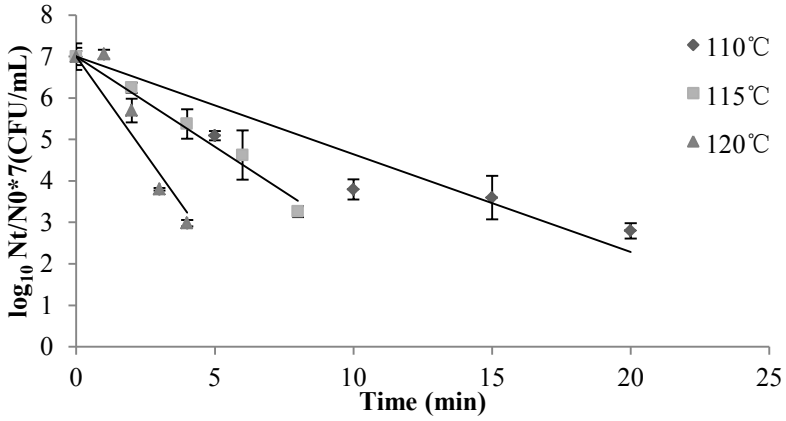


(c)

Figure 3.3 Survival curves of *C. sporogenes* in distilled water (a), phosphate buffer at pH 7.0 (b), and McIlvaine buffer at pH 7.0 (c) respectively, at different temperatures, fitted to the first-order model

Table 3.2 The D values ($\bar{x}\pm s$), R^2 and the 95% confidence interval for thermal destruction of *C. sporogenes* ATCC7955 in different buffer solutions

(a) D values of spores at 110℃

Marix	D_{110} （min）	R^2	95% confidence interval	
			lower limit (min)	upper limit (min)
Distilled water	10.03±0.028	0.986	9.98	10.07
Phosphate buffer pH 7.0	6.54±0.226	0.943	6.19	6.90
McIlvaine buffer pH 7.0	5.03±0.314	0.904	4.53	5.53

(b) D values of spores at 115℃

Marix	D_{115} （min）	R^2	95% confidence interval	
			lower limit (min)	upper limit (min)
Distilled water	4.58±0.165	0.983	4.31	4.84
Phosphate buffer pH 7.0	3.46±0.329	0.895	2.93	3.98
McIlvaine buffer pH 7.0	2.44±0.117	0.963	2.25	2.63

(c) D values of spores at 120℃

Marix	D_{120} （min）	R^2	95% confidence interval	
			lower limit (min)	upper limit (min)
Distilled water	1.31±0.035	0.974	1.25	1.36
Phosphate buffer pH 7.0	1.25±0.084	0.930	1.12	1.38
McIlvaine buffer pH 7.0	1.05±0.075	0.931	0.92	1.17

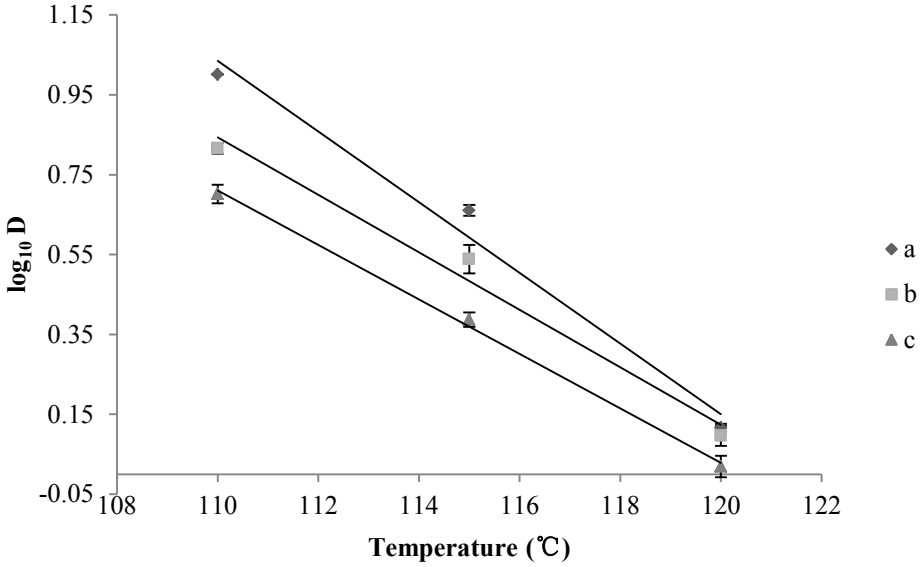


Figure 3.4 Thermal resistance curves of *C. sporogenes* ATCC7955 in distilled water (a), 0.2M phosphate buffer at pH 7.0 (b), and McIlvaine buffer at pH 7.0 (c)

Table 3.3 P values for the Student's t test to determine statistical significance of the difference of D values of *C. sporogenes* in different heat medium

Temperature(℃)	P value		
	a versus b	a versus c	b versus c
110	6.10×10^{-5}	6.59×10^{-8}	2.31×10^{-4}
115	2.63×10^{-3}	7.41×10^{-7}	5.35×10^{-3}
120	2.62×10^{-1}	8.00×10^{-4}	1.17×10^{-2}

*a: D values of *C. sporogenes* ATCC 7955 in distilled water

b: D values of *C. sporogenes* ATCC 7955 in Phosphate buffer at pH 7.0

c: D values of *C. sporogenes* ATCC 7955 in McIlvaine buffer at pH 7.0

Table 3.4 The difference parameter (Δ) and the percentage relative difference parameter (Φ) for the effect of heat medium influencing heat resistance of *C. sporogenes* ATCC 7955

(a)

Temperature (℃)	D_a (min)	D_b (min)	Δ (min)	Φ
110	10.03	6.54	3.49	34.80
115	4.58	3.46	1.12	24.51
120	1.31	1.25	0.06	4.51

(b)

Temperature (℃)	D_a (min)	D_c (min)	Δ (min)	Φ
110	10.03	5.03	5.00	49.83
115	4.58	2.44	2.14	46.67
120	1.31	1.05	0.26	20.04

(c)

Temperature (℃)	D_b (min)	D_c (min)	Δ (min)	Φ
110	6.54	5.03	1.51	23.06
115	3.46	2.44	1.02	29.35
120	1.23	1.05	0.18	16.27

*D_a: Mean of D values of *C.sporogenes* ATCC 7955 in distilled water

D_b: Mean of D values of *C.sporogenes* ATCC 7955 in Phosphate buffer at pH 7.0

D_c: Mean of D values of *C.sporogenes* ATCC 7955 in McIlvaine buffer at pH 7.0

The R^2 values of the survival curves of spores in distilled water ranged from 0.986 to 0.974, which means the first order model is a good fit for the spores. However, for spores in phosphate buffer at pH 7.0 and in McIlvaine buffer at pH 7.0, the R^2 values range from 0.943 to 0.895 and 0.963 to 0.904 respectively, slightly lower than in water. Thus, the first-order model has highest fitness in distilled water, followed with those in McIlvaine buffer and 0.2M phosphate buffer.

For the slope of the survival curves, trend of aggrandizement appear in all media when treatment temperature increased. In order words, *C. sporogenes* had lower D values at higher treatment temperatures, and the decrease is more than linear. The D values decreased from 10.03 to 1.31 min for spores dispersed in distilled water, from 6.54 to 1.25 min in 0.2M phosphate buffer at pH 7.0, and from 5.03 to 1.05 min in McIlvaine buffer at pH 7.0, respectively as the treatment temperature increased from 110 to 120℃.

According to previous research, the D value of *C. sporogenes* ATCC 7955 at 121.1℃ is around 1 min (Stumbo, 1973). In the current study, similar results were obtained. However, at lower treatment temperatures, the D values of *C. sporogenes* obtained in this study were relatively lower than those data reported before. For example, Cameron et al. (1980) reported that in 0.067M Sorensen's phosphate buffer at pH 7, the D values at 110 and 115℃ of *C. sporogenes* ATCC 7955 were 15.9 and 6.4min respectively. Ocio et al. (1994) got 12.78 and 5.2 min as D values at 110 and 115 ℃ in 1/15M phosphate buffer at pH 7 respectively. In this study, the D values at 110 and 115 ℃ of spore in 0.2M phosphate buffer at 7.0 were 6.54 and 3.46 min respectively. The differences between experimental data and previous data may be caused by the difference concentration of phosphate in the heat medium. Salts could change heat tolerance of spores by changing the water activity of heat medium. Phosphate could increase water activity and consequently increasing microbial sensitivity (Jay, 2000). In addition, concentration of salts could also influence the heat resistance of microorganisms. High concentration of salt in the heat medium may change difference of the osmotic pressure between inner and exterior of cells (Hansen and Riemann, 1963). Thus, the experimental data showed lower value than previous reported data as higher concentration of phosphate buffer was used in this study.

Table 3.3 gives the P values for the Student t test to determine statistical significance of the differences of D values of *C. sporogenes* in different heat medium. Table 3.4 gives the difference parameter (Δ) and the percentage relative difference parameter (Φ) for the effect of heat medium influencing heat resistance of *C. sporogenes*. The results show that the differences between D values in different heat medium were statistically significant ($P < 0.05$), except D values in phosphate buffer and McIlvaine buffer at 120℃ ($P > 0.05$). Generally, the difference parameters decreased as the temperature increased. The percentage relative difference parameters decreased when temperature increased as well, except for the comparison between phosphate buffer and McIlvaine buffer. It might be caused by the low fitness of the first-order kinetics for *C. sporogenes* in the phosphate buffer at 115℃, with R^2 value as 0.895. By carefully looking at these three tables, it can be observed that *C. sporogenes* is much more sensitive to heat in phosphate buffer and McIlvaine buffer than in distilled water. The reason is that possibly the phosphate and citric acid components in these buffer solutions could reduce microbial heat resistance (Jay, 2000). Nevertheless, the influence of buffers on reducing microbial heat resistance was minimal at higher temperatures. Furthermore, the McIlvaine buffer had greater effect on enhancing heat sensitivity of *C. sporogenes* than phosphate buffer. It is supposed that citric acid and phosphate might have synergistic effect on reducing heat resistance of *C. sporogenes*. As McIlvaine buffer contains both citric acid and phosphate, it would have greater effectiveness on reducing heat tolerance of microorganisms than phosphate buffer. However, further studies are needed to confirm this. Some synergistic effect between pH and NaCl has been reported with *C. sporogenes* with lower D values at higher percentage of NaCl in the heat medium at 112.8℃ and same pH (Hutton et al., 1991)

Apart from the decimal reduction time, the temperature sensitivity parameter, Z value was also calculated from the results. Table 3.5 shows the Z values, R^2 and the 95% confidence interval for the Z values of *C. sporogenes* in different heat medium. Table 3.6 gives the P value **f**or the Student's t test to determine statistical significance of the difference between Z values of *C. sporogenes* in different buffer solutions.

Table 3.5 The Z values ($\bar{x}\pm s$), R^2 and the 95% confidence interval for the thermal destruction of *C. sporogenes* ATCC 7955 in different buffer solutions

Marix	Z value （℃）	R^2	95% confidence interval	
			lower limit (℃)	upper limit (℃)
Distilled water	11.30±0.163	0.983	11.04	11.56
Phosphate buffer pH 7.0	13.93±0.832	0.987	12.60	15.25
McIlvaine buffer pH 7.0	14.72±1.222	0.999	12.78	16.67

Table 3.6 P values for the Student's t test to determine statistical significance of the difference between Z values of *C. sporogenes* in different buffer solutions as the heating medium

Groups	P value
a versus b	6.82×10^{-3}
a versus c	1.05×10^{-2}
b versus c	3.21×10^{-1}

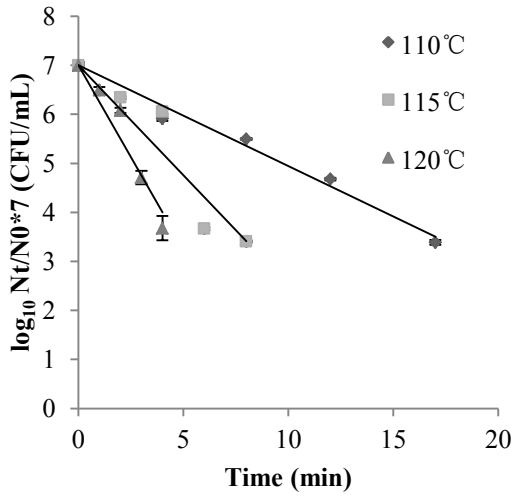
*a: Z values of *C. sporogenes* ATCC 7955 in distilled water
b: Z values of *C. sporogenes* ATCC 7955 in Phosphate buffer at pH 7.0
c: Z values of *C. sporogenes* ATCC 7955 in McIlvaine buffer at pH 7.0

According to previous research, Z value for *C. sporogenes* is around 10 to 12℃ in phosphate buffer (Luechapattanaporn et al., 2004). In this study, similar results were obtained. The Z value of *C. sporogenes* ATCC 7955 in distilled water was 11.30℃, which was significant lower than in other two group ($P<0.05$). Because of lower influence on reducing D value at higher temperature, slightly higher Z values were found in buffer solutions. Which means greater increment of temperature is needed to approach 10-log reduction of D values in buffer solutions

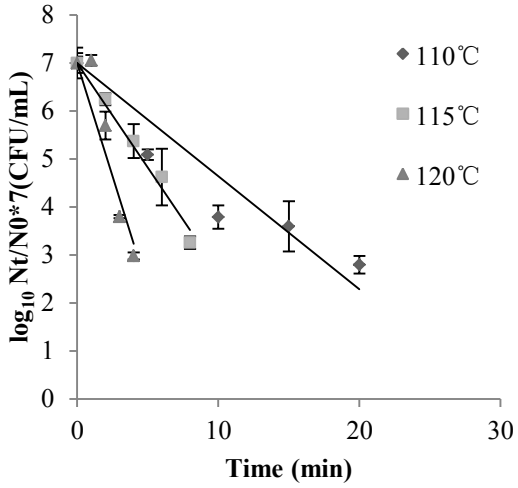
than in distilled water. On the other hand, the difference between Z value of *C. sporogenes* in phosphate buffer and McIlvaine buffer was not statistical significant (P >0.05). In order words, the effectiveness of phosphate buffer and McIlvaine buffer on influencing Z value of *C. sporogenes* is same.

3.4.2 Thermal destruction kinetics of *C. sporogenes* ATCC 7955 in McIlvaine buffer at different pH values

Figure 3.5 shows survivor curves of *C. sporogenes* in McIlvaine buffer at pH ranged from 3.0 to 8.0. Table 3.7 gives D values of *C. sporogenes* in McIlvaine buffer at different temperatures. One-way ANOVA was used for analyzing the significance of differences of D values at different pH value at same treatment temperature. At 110℃, spores had highest D value at pH 7.0. From pH 5.5 to 3.0, the D values gradually decreased with a decrease in pH value. Although the D values increased a little bit at pH 4.0, the difference was not significant. At 115℃, the highest D value was found at pH 7.0 as well. When pH value decreased, D values decreased with fluctuations. Ideally, similar degradation trend of D values should also result at 120℃. However, in this study, the results showed in contrast – the spores had less heat resistance at pH 7.0. These might be caused by low regression coefficients. However, according to previous researches, D value of *C. sporogenes* at 121.1℃ in low acid food could be widely range from 0.1 to 1.5 min (Stumbo, 1973; Luechapattanaporn et al., 2004). The differences between the highest and lowest D value was small - only 0.26 min, though it is significant in statistical analysis. Therefore, it could be considered that D values had no pH value influence at 120 ℃. The D value suddenly decreased at pH 6.0 at 110 and 115 ℃. This phenomenon has also been observed n previous studies. For example, Cameron et al. (1980) reported that D value was suddenly decreased at pH 6.2 in phosphate buffer, while Rodrigo et al. (1993) showed that *C. sporogenes* had lowest D value at pH 6.65 in acidified mushroom extract. In some cases, *C. sporogenes* might be more resistant to heat at pH around 5.5 (Rodrigo et al., 1993).



(a)



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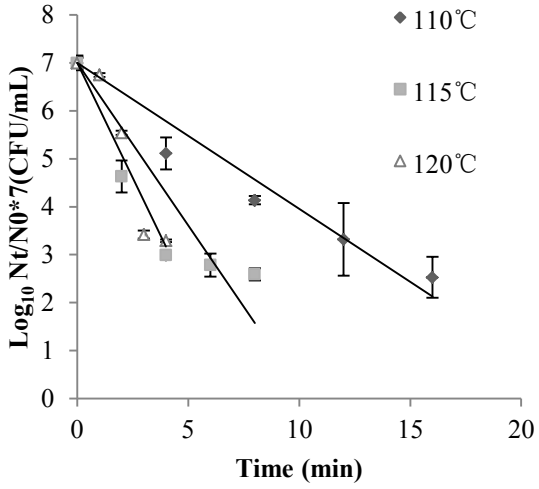

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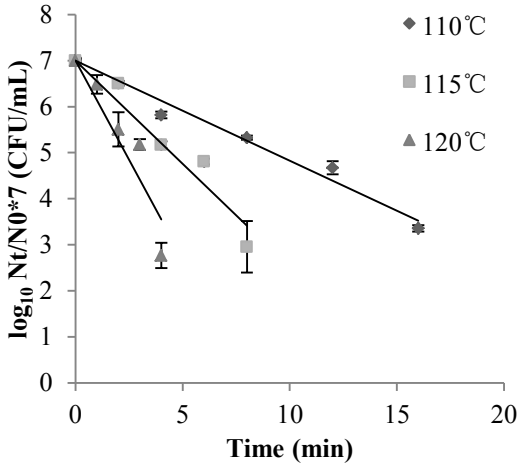
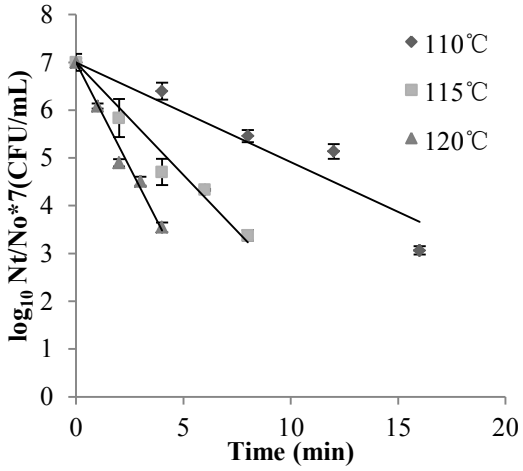


(d)

Figure 3.5 a-d . Survival curves of *C. sporogenes* in McIlvaine buffer at pH=8.0 (a), pH=7.0 (b), pH=6.0 (c), and pH=5.5 (d), respectively, at different temperatures, fitted to the first-order model



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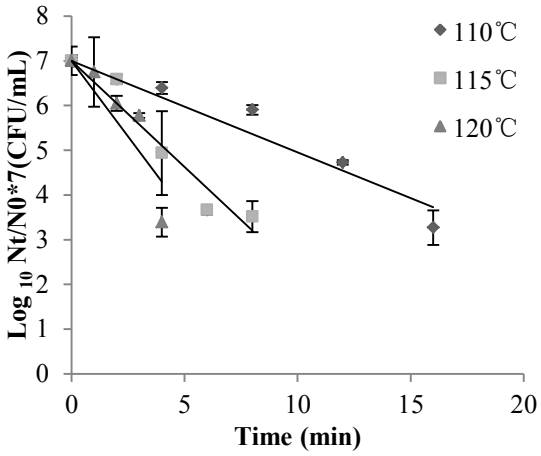

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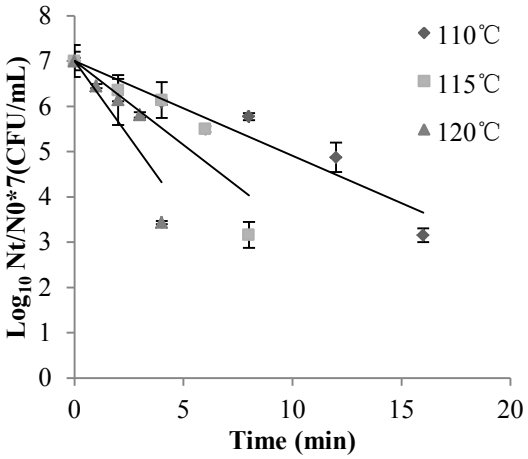

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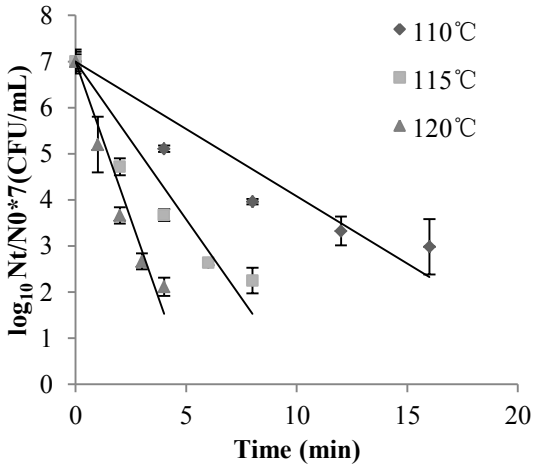


(h)

Figure 3.5 e-h. Survival curves of *C. sporogenes* in McIlvaine buffer at pH=5.0 (e), pH=4.5 (f), pH=4.0 (g), and pH=3.0 (h), respectively, at different temperatures, fitted to the first-order model

Table 3.7 D_T values (min) for thermal destruction of *C. sporogenes* ATCC7955 in McIlvaine buffer at different pH values as the heating medium

pH	D_T values (min)					
	110(℃)	R^2	115(℃)	R^2	120(℃)	R^2
8.0	4.36±0.040 ^{AB}	0.991	2.25±0.029 ^{DEF}	0.901	1.25±0.091 ^{IJ}	0.951
7.0	5.03±0.314 ^{C}	0.904	2.44±0.117 ^{F}	0.963	1.05±0.076 ^{G}	0.930
6.0	3.78±0.483 ^{A}	0.942	2.14±0.060 ^{DE}	0.826	1.11±0.021 ^{GH}	0.924
5.5	4.50±0.113 ^{BC}	0.977	2.32±0.325 ^{EF}	0.955	1.15±0.063 ^{GHI}	0.883
5.0	4.53±0.090 ^{BC}	0.925	2.05±0.093 ^{D}	0.973	1.21±0.033 ^{HIJ}	0.981
4.5	4.41±0.379 ^{B}	0.955	2.04±0.193 ^{D}	0.869	1.31±0.144 ^{J}	0.829
4.0	4.66±0.276 ^{BC}	0.895	2.35±0.126 ^{EF}	0.828	1.29±0.057 ^{J}	0.806
3.0	4.15±0.742 ^{AB}	0.895	2.22±0.146 ^{DEF}	0.933	1.11±0.083 ^{GH}	0.958

*Figures with the same letter are not significantly different (P>0.05)

Table 3.8 shows Z values of *C. sporogenes* at different pH values. One-way ANOVA was also used for analyzing the significance of pH value influencing Z value. Except at pH 7.0, there were not significant differences between Z values when pH was varied. Therefore, temperature has greater effectiveness on influencing thermal destruction of *C. sporogenes* ATCC 7955 than pH values. Similar results were reported by Löwik and Anema (1972). Compared to general Z value (10℃), the Z values in this study were higher (ranged from 14.73 to 19.37℃). However, relatively higher Z values were also reported in previous literatures. It was reported that Z value of *C. sporogenes* in low acid food could be ranged from 14 to 18℃ (Stumbo, 1973). Santos and Zarzo (1995) reported that Z value of *C. sporogenes* ATCC 7955 was 23.5℃ at treatment temperature ranged from 121 to 145℃. Naim et al. (2008) reported that Z values of *C. sporogenes* ATCC 11437 were 32.2 and 20.6℃ in phosphate buffer and carrot-alginate particles respectively. Much higher Z values for *Clostridium* species were reported by Casolari (1994). Since the Z value of *C. sporogenes* in distilled water and phosphate buffer were similar to those reported in previous research carried out in our laboratory, our research methodology was correct. In fact, several factors could influence the Z values, such as strain choice, heating substrate, range of treatment temperature and heating methods. It was reported that *C. sporogenes* had 2 times Z value by dry heating than moist heating (Russell, 2003).

Table 3.8 The Z values ($\bar{x}\pm s$), R^2 and the 95% confidence interval for the thermal destruction of *C. sporogenes* ATCC 7955 in McIlvaine buffer at different pH values

pH	Z value （℃）	R^2	95% confidence interval	
			lower limit (℃)	upper limit (℃)
8.0	18.50±1.079 ^{B}	0.999	16.78	20.22
7.0	14.73±1.222 ^{A}	0.994	12.78	16.67
6.0	19.05±2.039 ^{B}	0.998	15.81	22.29
5.5	16.82±0.600 ^{AB}	0.999	15.86	17.77
5.0	17.41±0.367 ^{AB}	0.999	16.83	17.8
4.5	19.37±3.114 ^{B}	0.962	14.41	24.32
4.0	18.01±1.422 ^{B}	0.998	15.74	20.27
3.0	18.06±3.477 ^{B}	0.992	12.53	23.59

*Figures with the same letter are not significantly different (P>0.05)

3.5 Conclusions

This study found out that *C. sporogenes* ATCC 7955 was less heat resistant in buffer solutions than in distilled water. In addition, McIlvaine buffer had slightly greater effect on enhancing heat sensitivity of *C. sporogenes* than phosphate buffer. However, when treatment temperature increased, effectiveness of substrate pH medium on influencing thermal destruction of *C. sporogenes* was reduced. Apart from that, pH value also changed the heat resistance of *C. sporogenes* when the environment is not optimum for growth of *C. sporogenes*. No matter the pH value changed from neutral to acidic or basic, D values of spores decreased. At 110 and 115℃, the D values of *C. sporogenes* ATCC 7955 were gradually decreased when pH value decreased in acid range. At pH 8.0, D values were lower than that at pH 7.0. However, pH value had no apparent effect on changing the D values at 120℃. In terms of Z values of *C. sporogenes*, no significant differences obtained when pH changed in the same buffer solution. In order words, temperature has greater effect on influencing D values of *C. sporogenes* compared to pH values.

PREFACE TO CHAPTER 4

In the previous chapter, thermal destruction behavior of *C. sporogenes* ATCC 7955 in different buffer solutions and pH values was determined. Results demonstrated that phosphate and citric acid could reduce heat resistance of *C. sporogenes*. In addition, D values of *C. sporogenes* were maximum at pH 7.0 and decreased on the both side of the neutral value. Greater effect of pH value on influencing heat resistance of *C. sporogenes* was found at lower temperatures. However, *C. sporogenes* is mesophile and has a lower thermal resistance of about 1 min at 121.1℃. At best, it can be used to validate a 5.0 min lethality process. However, there are several processes that are more severe and thermophile, which can survive under such processing conditions, is necessary as a surrogate for these studies. Therefore, thermal destruction behavior of *Geobacillus stearothermophilus* was evaluated in this chapter. As *G. stearothermophilus* has greater heat resistance than *C. sporogenes* but similar Z value as *C. botulinum*, it is also used as biological indicator for studying sterilization cycle (Fo up to 20 min). The aim of this study is evaluating thermal destruction kinetics of *G. stearothermophilus* as influenced by buffer type and pH values.

Part of this research will be presented in the Northeast Agricultural and Biological Engineering Conference (NABEC) 2016 and prepared for publication. The research was carried out by the candidate under the supervision of Dr. H.S. Ramaswamy.

CHAPTER 4

EFFECT OF BUFFER TYPE AND pH VALUE OF HEAT RESISTANCE OF *GEAOBACILLUS STEAROTHERMOPHILUS* ATCC 10149

4.1 Abstract

As detailed in previous chapter, thermal processing is an application of heat for the purpose of shelf life extension and promotion of safety of food. *Clostridium sporogenes* as the surrogate for *Clostridium botulinum* spores was detailed. *C. sporogenes* is appropriate when associated process lethality levels are low (<5.0 min). For situations involving higher lethalities, a thermophilic bactetrium, like *Geobacillus stearothermophilus,* with higher thermal resistance is used. Again, factors which can influence heat resistance of microorganisms include the medium pH. In this study, the effect of pH and type of buffer on the destruction kinetics of *G. stearothermophilus* ATCC 10149 was evaluated at 110, 115 and 120℃ respectively. Results showed that phosphate and citric acid present in these buffers could reduce heat resistance of *G. stearothermophilus* apparently at lower treatment temperatures. Consistent with C. *sporogenes*, *G. stearothermophilus* also had lower heat resistance in phosphate and McIlvaine buffers than in distilled water. In McIlvaine buffer, *G. stearothermophilus* had maximum heat resistance at neutral pH value. D values decreased at pH levels both higher and lower than 7.0. The pH effect on spore kill was more apparent at lower temperatures. However, the Z value was not related to pH ($P>0.05$).

4.2 Introduction

Thermal processing is frequently used for preserving and extending shelf life of products. The definition of thermal processing is the application of heat for the purpose of shelf life extension and promotion of safety of food (Hassan and Ramaswamy, 2011). In terms of preservation, most of undesirable microorganisms, including pathogens, endospores, and enzymes, could be inactivated by heat (Richardson, 2001). The heat severity of processing is divided into two categories: pasteurization and sterilization, first one is a mild treatment and the second one is a more severe treatment. As different food products have their own pH value, spoilage microorganisms could be different for different foods. *Clostridium botulinum* is one of the most concerned pathogens in food industry since it could produce neurotoxin in the condition of pH above 4.5. Due to its toxicity, nonpathogenic surrogates with similar or greater heat resistance are utilized in research studies, such as *C. sporogenes* (Shao and Ramaswamy, 2011). Basically, 12 decimal reduction of *C. botulinum* spore population should be achieved for low-acid food thermal processing. However, thermophiles could still survive in commercially sterilized products after sufficient thermal processing for destroying *Clostridium* cells and spores, such as *Geobacillus stearothermophilus* (Feeherry et al., 1987). *G. stearothermophilus*, also named as *B. steatothermophilus*, is a kind of endospore form of *Bacillus*. It is extremely resistant to heat with 4 to 5 min as decimal reduction time at 121.1℃, which is approximately 20 times more resistant than *C. botulinum* (Ghani et al., 2002; Stumbo, 1973). Because *G. stearothermophilus* has similar Z value as *C. botulinum* has, it is utilized as a biological indicator of sterilization as well, especially when higher sterility levels are required (Hassan and Ramaswamy, 2011; Watanabe et al., 2003). Normally, one decimal reduction of *G. stearothermophilus* spore population is equivalent to one process lethality (Fo) value of 5 min.

In order to maintain sensory characteristics of products as well as achieving requirement of microbial inactivation, modification of thermal processing is needed. Factors which could influence microbial heat resistance were commonly studied. Previous researchers have shown that pH value has effect on enhancing heat sensitivity of *G. stearothermophilus*. Fernandez et al.

(1996) reported that *G. stearothermophilus* has lower heat resistance in acidified mushroom extract with lower pH values. The effect of pH on heat sensitivity of spores has been studied by several researchers and pH sensitization of microbial heat resistance has been observed (Periago et al., 1998; López et al., 1996, Rodrigo et al., 1999; Iciek et al., 2006). Comparison between different organic acids on enhancing heat sensitivity of *G. stearothermophilus* has also been studied. Lynch and Potter (1988) reported that lactic acid, citric acid and acetic acid had greater effect than malic acid and hydrochloric acid at pH 4.6 on decreasing the D value of *G. stearothermophilus* in frankfurter emulsion slurry. Iciek et al. (2008) found that acidified red beet juice with acetic acid had lower D value of *G. stearothermophilus* at pH 4.0 than with citric acid. However, most of the studies have focused on neutral pH value rather than a wider pH range. Therefore, determining thermal destruction trend of *G. stearothermophilus* in a wider pH range is desirable.

Hence, the objectives of this study were to: 1) determine the influence of buffer type (phosphate vs McIlvaine buffer) on the heat resistance of *G. stearothermophilus* ATCC 10149 at the same pH, and 2) determine thermal destruction kinetics of *G. stearothermophilus* ATCC 10149 in McIlvaine buffer at different pH levels.

4.3 Materials and methods

4.3.1 Spore preparation

Spore preparation was prepared according to the method reported by Kim and Naylor (1966). Freeze-dried culture powder of *G. stearothermophilus* spores (ATCC 10149) was obtained from the American Type Culture Collection and stored at -40℃ until use. The freeze-dried *G. stearothermophilus* culture was hydrated with 10 mL of tryptone yeast extract glucose (TYG) broth. Formulation of TYG broth involved dissolving 5g tryptone, 2.5g yeast extract and 1g K_2HPO_4 (J.T. Baker Inc., Center Valley, PA) in 500 mL of distilled water and adjusted pH to 7.2. The hydrated culture was aerobically incubated at 55℃ for 24 h. This step

was repeated for two times. After that, a sample of 0.1 mL of the hydrated spore broth was transferred into 50 mL of fresh prepared TYG broth and aerobically incubated at 55℃ for 24 h. This procedure was repeated for two times. Then 0.2 mL of culture was transferred and spread on sporulation agar plate (SAP) and aerobically incubated at 55℃ for 5 days. Formulation of SAP medium involved dissolving 4g nutrient broth (Oxoid LTD., Hampshire, UK), 2g yeast extract, 0.05g $MnCl_2 \cdot 4H_2O$ (Acros Organics, Morris Plains, NJ), and 10g bacterial agar in 500 mL of distilled water.

4.3.2 Spore harvesting

Same as detailed for *C. sporogenes* ATCC 7955 in Chapter 3, except for incubation.

4.3.3 Thermal treatment

The spore suspension was first shaken for 15 min in an ultrasonic shaker before use. A temperature controlled oil bath was used for giving the thermal treatment. Stainless steel heating tubes with o-ring sealed screw caps were specially fabricated for this purpose. The come-up time in stainless steel caped test tube was approximately 1 min. It was tested by inserting thermocouple probes through the tubes with special lids (details shown in previous chapter). Based on literatures data on thermal destruction kinetics for *G. stearothermophilus*, three temperatures (110, 115 and 120℃) were used for the thermal treatment. For each temperature, at least four holding times were used, excluding the come-up time. The intervals of holding times were different for each treatment temerature. For spores suspended in distilled water, the intervals were 60 min at 110℃, 20 min at 115℃ and 5 min at 120℃ respectively. For spores suspended in 0.2M phosphate buffer (pH 7.0), the intervals were 50 min at 110℃, 15 min at 115℃ and 5 min at 120℃ respectively. For spores suspended in McIlvaine buffer, the intervals were 45 min at 110℃, 12 min at 115℃ and 4 min at 120℃ respectively. These times were established after some preliminary test runs to determine the approximate D value at each temperature. Duplicate of each treatment was necessary for insuring accuracy of test results. For each treatment, 1.5 mL of spore suspension was transferred into a previously autoclaved stainless

steel caped test tube and sealed. The tubes were heated by suspending them in the oil bath for specified times. After treatment, the tubes were immersion cooled in ice water bath for 10 min.

4.3.4 Incubation and enumeration

The treated suspensions were poured into pre-sterilized micro-centrifuge tubes. Serial dilution was made with 0.1% peptone water and enumeration was done by using pour plate counting technique. The spores were aerobically incubated on TSA plates at 55℃ for 24 h. After incubation, number of colonies was counted. The significant results were only chosen for those in the range of 30 to 300 colonies on one plate. The colony morphology of *G. stearothermophilus* is shown in Figure 4.1.

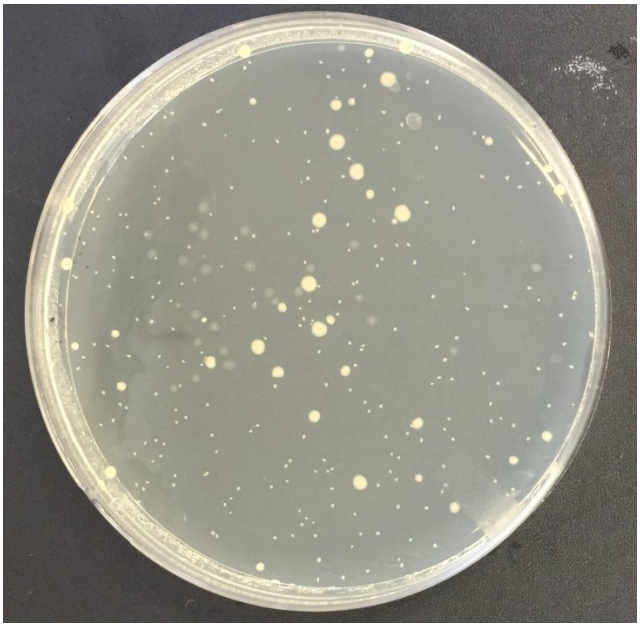


Figure 4.1 The colony morphology of *G. stearothermophilus* on tryptic soy agar plates

4.3.5 Data analysis

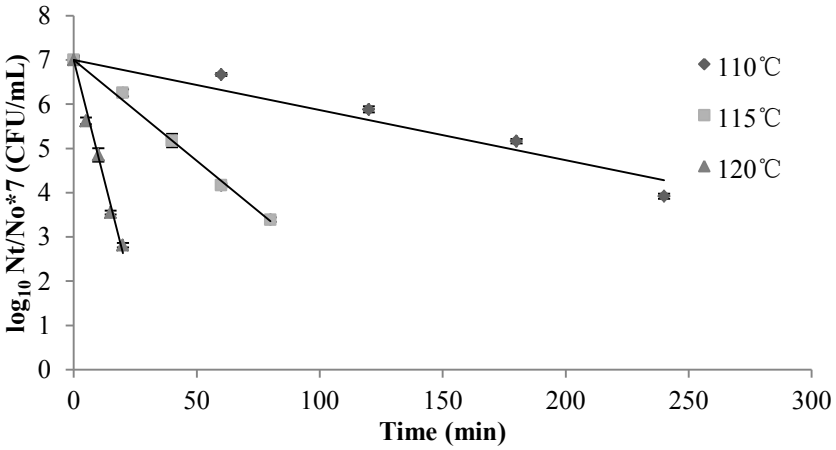
Same as detailed in Chapter 3.

4.4 Results and Discussion

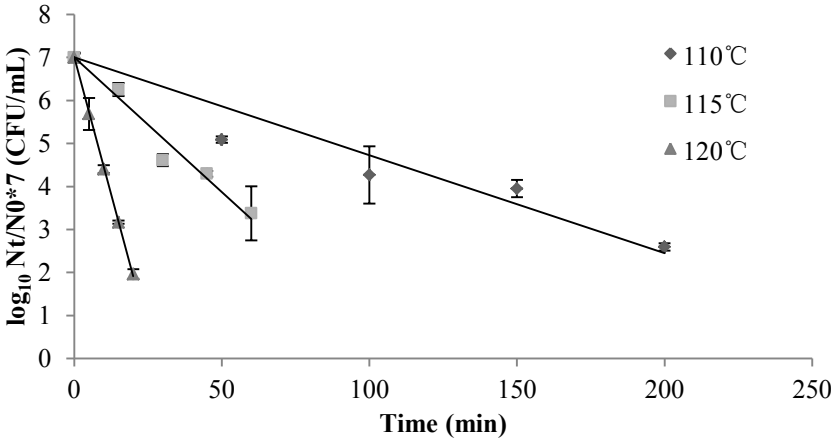
4.4.1 Effect of buffer type on influencing heat resistance of *G. stearothermophilus* ATCC 10149

Survival curves of spores were plotted as the log10 (N_t/N_0) $\times 7$ (so that the curves start from the nominal initial count of 10^7 CFU/ml) versus the holding time (min). The holding times did not include the come-up time (approximately 1min). Figure 4.2 (a) shows the survival curves of *G. stearothermophilus* ATCC 10149 in distilled water with holding time range from 0 to 240 min while Figure 4.2 (b) shows the survival curves in 0.2M phosphate buffer at pH 7.0 with the holding time range from 0 to 200 min and Figure 4.2 (c) shows the survival curves in McIlvaine buffer at pH 7.0 with the holding time range from 0 to 120 min. The linear regression line to the experimental data and, slope and the regression coefficients (R^2) of the regression line were obtained by using the Microsoft Excel program. The results are tabulated in Table 4.1 with the D values, R^2 and the 95% confidence interval for the D values of *G.stearothermophilus* ATCC10149 in different heat media at treatment temperature 110, 115 and 120℃. Figure 4.3 shows the thermal resistance curves of *G. stearothermophilus* ATCC 10149 in different heat media.

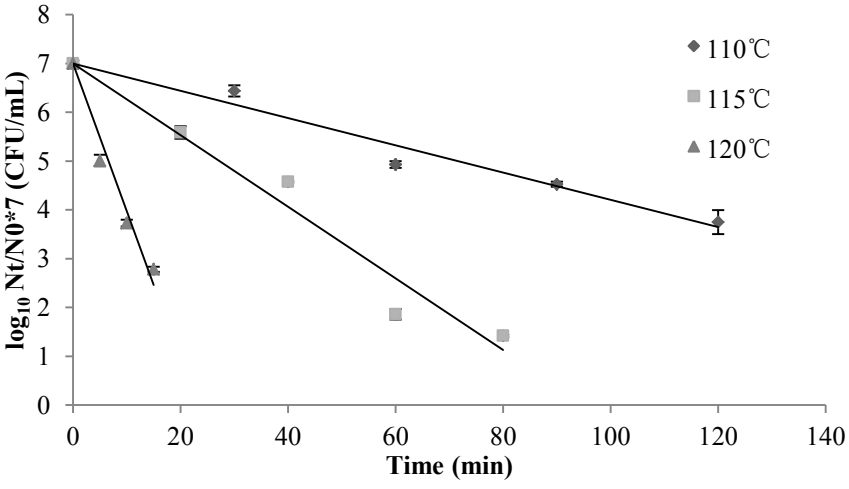
To elaborate, the R^2 values of the survival curves of *G. stearothermophilus* ATCC 10149 were quite high (ranged from 0.923 to 0.993), which means that the first-order kinetics has good fitness on analyzing thermal destruction kinetics of *G. stearothermophilus* ATCC 10149. The D values of *G. stearothermophilus* decreased when temperature increased in all cases. The D values decreased from 89.69 to 5.54 min for spores dispersed in distilled water, from 49.96 to 4.10 min in 0.2M phosphate buffer at pH 7.0, and from 43.73 to 3.96 min in McIlvaine buffer at pH 7.0 respectively as the treatment temperature increased from 110 to 120℃. Obviously, *G. stearothermophilus* ATCC 10149 had greater heat resistance when suspended in distilled water.



(a)



(b)



(c)

Figure 4.2 Survival curves of *G. stearothermophilus* in distilled water (a), phosphate buffer at pH 7.0 (b), and McIlvaine buffer at pH 7.0 (c) respectively, at different temperatures, fitted to the first-order model

Table 4.1 The D values ($\bar{x}\pm s$), R^2 and the 95% confidence interval for thermal destruction of *G. stearothermophilus* ATCC 10149 in different buffer solutions

(a) D values of spores at 110℃

Marix	D_{110} （min）	R2	95% confidence interval	
			lower limit (min)	upper limit (min)
Distilled water	89.69±0.464	0.974	88.95	90.43
Phosphate buffer pH 7.0	49.96±1.176	0.943	48.09	51.83
McIlvaine buffer pH 7.0	43.73±2.504	0.957	39.74	47.71

(b) D values of spores at 115℃

Marix	D_{115} （min）	R2	95% confidence interval	
			lower limit (min)	upper limit (min)
Distilled water	21.40±0.375	0.982	20.80	21.99
Phosphate buffer pH 7.0	16.33±2.050	0.964	13.07	19.59
McIlvaine buffer pH 7.0	15.63±0.111	0.923	15.45	15.80

(c) D values of spores at 120℃

Marix	D_{120} （min）	R2	95% confidence interval	
			lower limit (min)	upper limit (min)
Distilled water	5.54±0.035	0.980	5.49	5.60
Phosphate buffer pH 7.0	4.10±0.153	0.993	3.85	4.34
McIlvaine buffer pH 7.0	3.96±0.066	0.969	3.85	4.06

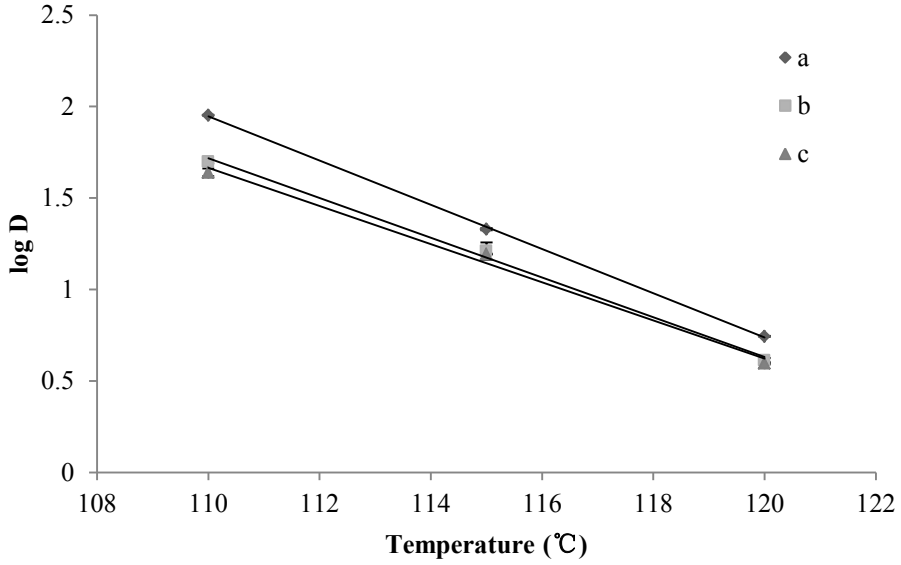


Figure 4.3 Thermal resistance curves of *G. stearothermophilus* ATCC 10149 in distilled water (a), 0.2M phosphate buffer at pH 7.0 (b), and McIlvaine buffer at pH 7.0 (c)

According to previous literature, D value of *G. stearothermophilus* ranges from 4.0 to 5.0 min at 121.1℃ and Z value ranges from 14 to 22℃ (Stumbo, 1973). However, there are differences between difference strains. For instance, López et al. (1996) made a comparison of heat resistance of *G. stearothermophilu*s ATCC 7953, 12980, 15951 and 15952 in McIlvaine buffer. Although there were no significant differences, different strains indeed had different D values at same treatment temperature and pH. *G. stearothermophilus* ATCC 12980 was frequently used for determining thermal destruction kinetics, with approximately 2 to 3 min as D value at 121.1℃ and 7 to 8℃ as Z value (Feeherry et al., 1987; Periago et al., 1998; Ting and Freiman, 2004). In the current study, more heat resistant *G. stearothermophilus* ATCC 10149 was used. For *G. stearothermophilus* ATCC 10149, Patazca et al. (2006) reported that the D value at 121.1℃ was 5.5 min under 0.2MPa pressure and the Z value was 10.8℃. Hassan and Ramaswamy (2011) reported that D values of *G. stearothermophilus* ATCC 10149 at 120℃

were 6.0min and 5.7min in carrot meat aligate purees respectively, while Z values were 11.5℃and 11.6℃ respectively. Similar results were obtained in the current study.

Table 4.2 gives the P values for the student's t test to determine statistical significance of the differences of D values of *G. stearothermophilus* ATCC 10149 in different heat medium. Table 4.3 gives the difference parameter (Δ) and the percentage relative difference parameter (Φ) for the effect of heat medium influencing heat resistance of *G. stearothermophilus* ATCC 10149. The results show that the effects of phosphate buffer and McIlvaine buffer on reducing tolerant of spores to heat were significant ($P < 0.05$). It is because of ability of phosphate to increase water activity and citric acid to reduce microbial heat sensitivity (Jay, 2000). In Table 4.3 (a) and (b), the difference parameters (Δ) decreased as temperature increased. In addition, the percentage relative difference parameters (Φ) were similar at 115 and 120℃. In order words, greater effect of heat medium on influencing thermal destruction was obtained at lower temperatures. On the other hand, McIlvaine buffer had greater effect on enhancing D values of *G. stearothermophilus* than phosphate buffer. However, the results of Student's t test show that the difference was significant only at 110℃ ($P < 0.05$). Thus, synergistic effect of citric acid and phosphate was not apparent on *G. stearothermophilus* ATCC 10149 at higher temperatures.

Table 4.2 P values for the Student's t test to determine statistical significance of the difference

Temperature(℃)	P value		
	1vs2	1vs 3	2vs3
110	1.09×10^{-9}	2.70×10^{-5}	9.32×10^{-3}
115	9.32×10^{-3}	1.01×10^{-7}	5.42×10^{-1}
120	1.92×10^{-4}	1.11×10^{-8}	1.65×10^{-1}

*1: D values of *G. stearothermophilus* ATCC 10149 in distilled water

2: D values of *G. stearothermophilus* ATCC 10149 in Phosphate buffer at pH 7.0

3: D values of *G. stearothermophilus* ATCC 10149 in McIlvaine buffer at pH 7.0

Table 4.3 The difference parameter (Δ) and the percentage relative difference parameter (Φ) for the effect of salt on influencing heat resistance of *G. stearothermophilus* ATCC 10149

(a)

Temperature (℃)	D_1 (min)	D_2 (min)	Δ (min)	Φ
110	89.68	49.96	39.72	44.30
115	21.40	16.33	5.07	23.70
120	5.54	4.10	1.44	26.09

(b)

Temperature (℃)	D_1 (min)	D_3 (min)	Δ (min)	Φ
110	89.68	43.73	45.95	51.24
115	21.40	15.63	5.77	276.96
120	5.54	3.96	1.58	28.63

(c)

Temperature (℃)	D_2 (min)	D_3 (min)	Δ (min)	Φ
110	49.96	43.73	6.23	12.47
115	16.33	15.63	0.70	4.29
120	4.10	3.96	0.14	3.44

*D_1: Mean of D values of *G. stearothermophilus* ATCC 10149 in distilled water

D_2: Mean of D values of *G. stearothermophilus* ATCC 10149 in Phosphate buffer at pH 7.0

D_3: Mean of D values of *G. stearothermophilus* ATCC 10149 in McIlvaine buffer at pH 7.0

Comparison of the temperature sensitivity parameter, Z values, of *G. stearothermophilus* ATCC 10149 in different medium were also obtained by using the Microsoft Excel program and the Student's t test. Table 4.4 shows the Z values, R^2 and the 95% confidence interval for the Z values of *G. stearothermophilus* ATCC 10149 in different heat medium. Table 3.6 gives the P value **f**or the Student's t test to determine statistical significance of the difference between Z values of *G. stearothermophilus* ATCC 10149 in different buffers. The results in Table 4.4 show

that spores had slightly higher Z values in buffer solution than in distilled water. The differences were 0.94℃ between spores in phosphate buffer and distilled water while 1.32℃ in McIlvaine buffer and distilled water. Furthermore, the Student's t test showed significance difference between these Z values. Nevertheless, there was no statistical significant difference between phosphate buffer and McIlvaine buffer on influencing Z value (P>0.05). Hence, the effectiveness of phosphate buffer and McIlvaine buffer on influencing Z value of *G. stearotherophilus* was same.

Table 4.4 The Z values ($\bar{x}\pm s$), R^2 and the 95% confidence interval for the thermal destruction of *G. stearothermophilus* ATCC 10149 in different buffer solutions

Matrix	Z value （℃）	R^2	95% confidence interval	
			lower limit (℃)	upper limit (℃)
Distilled water	8.27±0.003	0.999	8.27	8.28
Phosphate buffer pH 7.0	9.21±0.216	0.998	8.86	9.55
McIlvaine buffer pH 7.0	9.59±0.262	0.999	9.18	10.01

Table 4.5 P values for the Student's t test to determine statistical significance of the difference between z values of *G. stearothermophilus* ATCC 10149 in different buffer solutions as the heating medium

Groups	P value
1 versus 2	3.23×10^{-3}
1 versus 3	2.08×10^{-3}
2 versus 3	6.36×10^{-2}

*a: Z values of *G. stearothermophilus* ATCC 10149 in distilled water
b: Z values of *G. stearothermophilus* ATCC 10149 in Phosphate buffer at pH 7.0
c: Z values of *G. stearothermophilus* ATCC 10149 in McIlvaine buffer at pH 7.0

4.4.2 Thermal destruction kinetics of *G. stearothermophilus* ATCC 10149 in McIlvaine buffer at different pH values

Figure 4.4 shows survivor curves of *G. stearothermophilus* ATCC 10149 in McIlvaine buffer at pH ranged from 3.0 to 8.0. Table 4.6 gives D values of *G. stearothermophilus* ATCC 10149 in McIlvaine buffer at different temperature. One-way ANOVA was used for analyzing the significance of differences of D values at different pH value at same treatment temperature. At 110℃, the D values decreased slightly but without significant difference from pH 8.0 to 5.5 ($P>0.05$). When pH values were lower than 5.5, the D values decreased. However, at pH 3.0, the D values rebounded back to 40.83min, which was no difference compared to D values at pH from 8.0 to 5.5 ($P>0.05$). The lowest D value, 35.03 min, was obtained at pH 5.0. At 115℃, *G. stearothermophilus* had maximum resistance to heat at pH 7.0. The D values reduced when the pH value changed from pH 7.0 to acidic or basic. Greater degradation of D values was found when pH decreased from neutral to acidic. At 120℃, although significant degradation of D values was found at acidic pH values, the difference between the highest and the lowest D values was only 0.69 min. *G. stearothermophilus* ATCC 10149 had greatest heat resistance at pH 7.0 to

8.0 in all cases, which fits to previous research. It is pointed that the optimum pH value range for *G. stearothermophilus* to grow is usually about 7.0 or slightly above (Stumbo, 1973). Nazina et al., (2001) made a review of aerobic thermophilic bacilli, including *Geobacillus* and *Bacillus*. It showed that the optimum pH range for growth of *Geobacillus* was 6.5 to 7.0, and for Bacillus was 7.0 to 9.5 (Nazina et al., 2001). Thus, it is reasonable that the results obtained from the current study show that no apparent change for the D values of *G. stearothermophilus* around neutral pH value at 110 and 120℃ respectively. Meanwhile, similar D values were found for *G. stearothermophilus* suspended in buffer at pH value lower than 6.0 at all treatment temperatures. It might be caused by inherent characteristics of *G. stearothermophilus*. Since *G. stearothermophilus* is thermophile, temperature plays the most important role on destroying this spore. Thus, similar heat tolerance of *G. stearothermophilus* ATCC 10149 was obtained in low acidic and high acidic pH range at the same treatment temperature.

Table 4.7 shows Z values of *G. stearothermophilus* ATCC 10149 at different pH values. The results obtained in this study are similar to previous research (Hassan and Ramaswamy, 2011; Patazca et al., 2006). One-way ANOVA was also used for analyzing the significance of pH value on influencing Z value. Except at pH 5.0, there were not significant differences between Z values when pH was varied. However, the difference between the highest and lowest value was only 1.04℃. Hence, pH has no effect on influencing Z values of *G. stearothermophilus* ATCC 10149. Same results were described by López et al. (1996). Therefore, temperature has greater effectiveness on influencing thermal destruction of *G. stearothermophilus* ATCC 10149 than pH values.

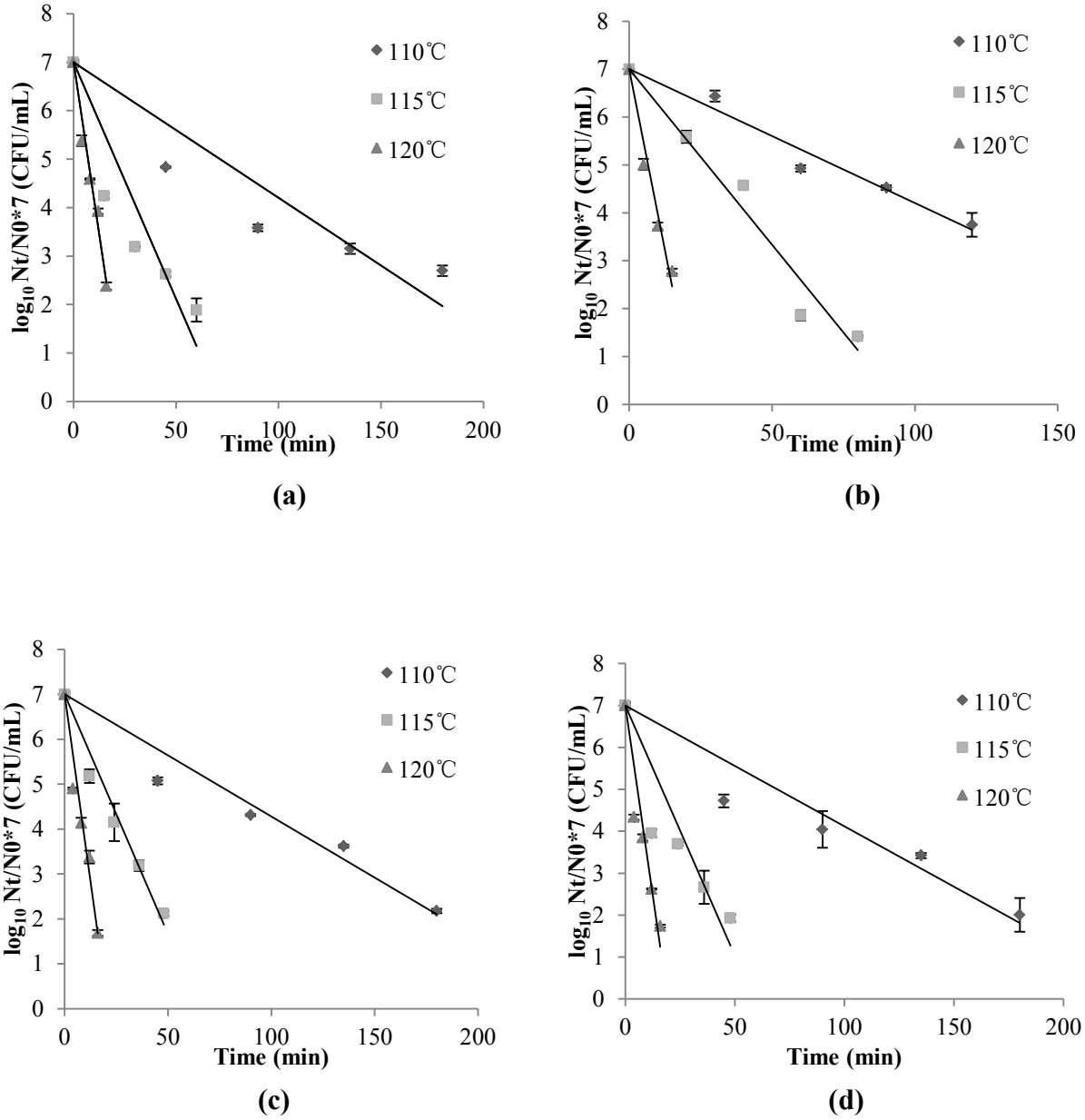
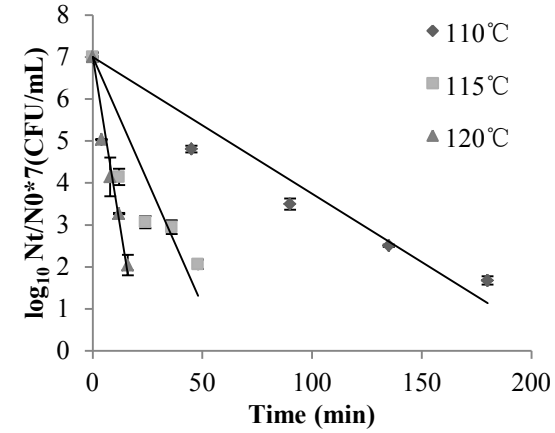
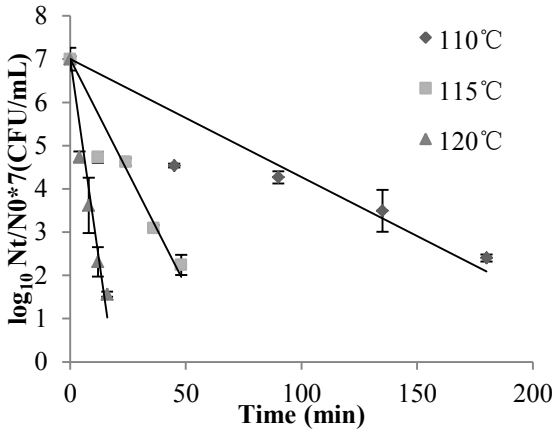


Figure 4.4 a-d. Survival curves of *G. stearothermophilus* in McIlvaine buffer at pH=8.0 (a), pH=7.0 (b), pH=6.0 (c), and pH=5.5 (d) respectively, at different temperatures, fitted to the first-order model



(e)



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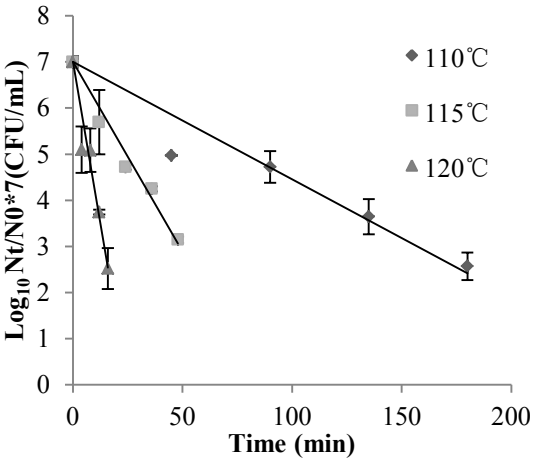

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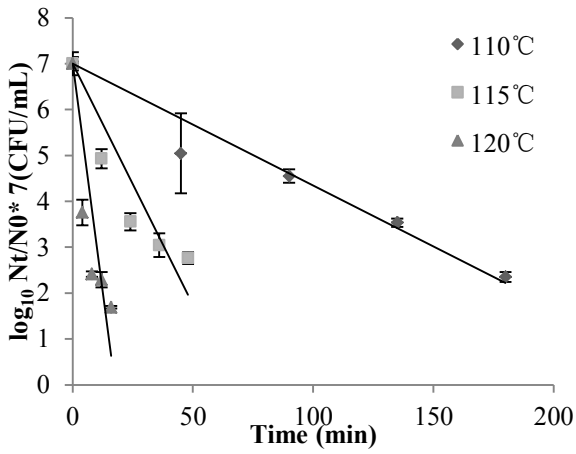


(h)

Figure 4.4 e-h. Survival curves of *G. stearothermophilus* in McIlvaine buffer at pH=5.0 (e), pH=4.5 (f), pH=4.0 (g), and pH=3.0 (h) respectively, at different temperatures, fitted to the first-order model

Table 4.6 D_T values (min) for thermal destruction of *G. stearothermophilus* ATCC 10149 in McIlvaine buffer at different pH values as the heating medium

pH	Temperature (℃)					
	110	R^2	115	R^2	120	R^2
8.0	44.47±1.342 ^{D}	0.982	13.20±0.552 ^{H}	0.888	3.95±0.061 ^{N}	0.982
7.0	43.73±2.505 ^{D}	0.957	15.63±0.111 ^{J}	0.921	3.96±0.066^{N}	0.974
6.0	43.30±0.631 ^{CD}	0.953	11.65±0.166 ^{F}	0.984	3.60±0.053 ^{L}	0.959
5.5	42.34±2.850 ^{CD}	0.939	12.61±0.519 ^{G}	0.865	3.76±0.047 ^{M}	0.921
5.0	35.03±0.385 ^{A}	0.964	10.85±0.269 ^{E}	0.854	3.75±0.182 ^{M}	0.970
4.5	36.74±2.299 ^{AB}	0.847	10.75±0.386 ^{E}	0.944	3.27±0.065 ^{K}	0.965
4.0	39.55±3.672 ^{BC}	0.933	12.20±0.420 ^{G}	0.971	3.88±0.180 ^{N}	0.942
3.0	40.84±3.835 ^{CD}	0.951	11.58±0.213 ^{F}	0.885	3.54±0.252 ^{L}	0.812

*Figures with the same letter are not significantly different (P>0.05)

Table 4.7 The Z values ($\bar{x}\pm s$), R^2 and the 95% confidence interval for the thermal destruction of *G. stearothermophilus* ATCC 10149 in McIlvaine buffer at different pH values

pH	Z value （℃）	R^2	95% confidence interval	
			lower limit (℃)	upper limit (℃)
8.0	9.51±0.161 ^{A}	0.999	9.25	9.77
7.0	9.59±0.262 ^{AB}	0.999	9.18	10.01
6.0	9.26±0.011 ^{A}	0.999	9.24	9.27
5.5	9.52±0.290 ^{A}	0.999	9.06	9.98
5.0	10.31±0.261 ^{C}	0.999	9.90	10.73
4.5	9.54±0.329 ^{AB}	0.998	9.01	10.06
4.0	9.94±0.233 ^{B}	0.999	9.93	10.31
3.0	9.59±0.342 ^{AB}	0.997	9.04	10.13

*Figures with the same letter are not significantly different (P>0.05)

4.5 Conclusions

In this study, the results show that *G. stearothermophilus* ATCC 10149 had less tolerance to heat in phosphate and McIlvaine buffers compared to spores in distilled water. Thus phosphate and citric acid can reduce heat resistance of *G. stearothermophilus*. However, when treatment temperature increased, effectiveness of substrate in medium on influencing thermal destruction of *G. stearothermophilus* was reduced. In addition, there were no apparent differences between phosphate and McIlvaine buffers on influencing thermal destruction of *G. stearothermophilus,* except at 110℃. Hence, the synergic effect of citric acid and phosphate on reducing heat sensitivity of *G. stearothermophilus* is only effective at lower temperatures. Despite of effect of substrate in heat medium, pH value can affect heat resistance of *G. stearothermophilus*. When pH value of heat medium was not pH 7.0, which is the optimum pH value for growth of *G. stearothermophilus*, the D values decreased. However, the effect was not apparent at 120℃. In addition, the Z values did not change when pH values decreased or increased. Therefore, temperature has greater effect on influencing thermal destruction of *G. stearothermophilus* ATCC 10149 than pH value and substrate of heat medium.

PREFACE TO CHAPTER 5

In previous two chapters, the first-order kinetics was used for characterizing thermal destruction behavior of *C. sporogenes* ATCC 7955 and *G. stearothermophilus* ATCC 10149. D value and Z value were applied for evaluating heat resistance of spores. Although the log-linear model is commonly used in previous literatures, deviations from the log-linear models have been noticed in the survival curves which also have been observed in some cases in this study for survival curves of *C. sporogenes* and *G. stearothermophilus*. Hence, alternative models for better fitting of thermal destruction behavior of spores was explored. Several non-linear inactivation models have been successfully used for such modeling, such as Weibull model, modified Gompertz model and log-logistic model. The aim of this study is to compare goodness of fit and predictive ability between the first-order kinetics and the Weibull model for thermal destruction of *C. sporogenes* and *G. stearothermophilus*. Data from previous chapters was used in this chapter.

Part of this research is prepared to publish in scientific journal. The research was carried out by the candidate under the supervision of Dr. H.S. Ramaswamy.

CHAPTER 5

ALTERNATE MODELS FOR THE THERMAL DESTRUCTION OF *CLOSTRIDIUM SPOROGENES* AND *GEOBACILLUS STEAROTHERMOPHILUS*

5.1 Abstract

Even though the first-order linear kinetics is frequently used for predicting microbial inactivation deviations from this log-linear behavior has been frequently observed. Destruction curves have been shown some times to have a shoulder or a tail when plotted on semi-log format. Alternative models for analyzing experimental data were desirable to see if they could better describe the survival data. In this study, the Weibull model was used as an additional model for the thermal destruction behavior of *Clostridium sporogenes* ATCC 7955 and *Geobacillus stearothermophilus* ATCC 10149. The model comparison between the Weibull model and the first-order linear kinetics was made by using the residual plots and scale-location plots. Curvature was found in some survival curves. Overall, upward curvature was found for *C. sporogenes* at lower treatment temperatures and downward curvature at higher treatment temperatures. Upward curves were found for most of the survival curves of *G. stearothermophilus*. Nonetheless, the Weibull model did not show any better fitness on predicting thermal destruction behavior of both spores than the first-order linear kinetics.

5.2 Introduction

In order to analyze thermal resistance of microorganisms, survival curves are utilized. Meanwhile, microbial inactivation kinetic models are used to determine the trend of destruction of survival curves. Generally, first-order kinetic is widely accepted and practiced (van Boekel, 2002). It is assumed that all cells or spores have same resistance to heat. Thus, the inactivation trend is semi-logarithmic linear reduction at a certain temperature treatment. Decimal reduction time (D value), which is time at certain temperature for reducing 10 fold of target treated microbial number, is the parameter to present heat resistance of microorganisms in this model. Experimental data show that D value is high dependent on temperature. Temperature sensitivity indicator (Z value) is introduced for presenting a temperature interval that could induce 10-fold increase or decrease of D-value (Peleg, 2000; Ramaswamy and Marcotte, 2005; van Boekel, 2002; Xiong et al., 1999 a). The equations of D value and Z value are showed in equation 5.1 and 5.2 respectively.

$$D = \frac{t_2 - t_1}{[log(N_1) - log\ (N_2)]} \tag{5.1}$$

where N_1 and N_2 represent the survivor number following heating for t_1 and t_2 time respectively. The unit of D value is minute.

$$Z_T = \frac{(T_2 - T_1)}{[\log(D_1) - \log(D_2)]} \tag{5.2}$$

where D_1 and D_2 are D-values at temperature T_1 and T_2 respectively. The unit of Z value is ℃.

However, it is a chance of a quantum of heat influence on cells death rate (Anderson et al., 1996; Peleg and Cole, 1998). Deviation of the first order kinetics had been frequently reported since this model does not fit for non-linear curves.

In fact, one microbial community contains different subpopulations with their own inactivation kinetics. It is one of the reasons that could explain appearance of non-linear survival

curves (van Boekel, 2002). Previous literatures reported several models for non-linear survival curve, including Weibull model, modified Gompertz model, log-logistic model and so on (Xiong et al., 1999 a). The Weibull model is a combination of accelerated failure-time model and parametric distribution. For convenience, this model is based on the assumption that possibility of individual cells or spores die by treatment disperses according to Weibull distribution, while the survival curves are in cumulative form for the distribution of lethal events (Chen, 2007). It is assumed that microorganisms do not have identical resistance and the differences are permanent (Cole et al., 1993).

$$\log \frac{N}{N_0} = -bt^p \tag{5.3}$$

where b and p are the two parameters of distribution; b is a scale parameter, which is a characteristic time and p is the shape parameter. When p >1 indicates that the survival curve is concave upward, while p < 1 indicates concave downward. It could indicates linear survival curve when p = 1, which is the same as first order kinetics. These two parameters could be used to calculate mean, variance and coefficient of skewness of survival curves (Peleg and Cole, 1998).

By viewing results from Chapter 3 and 4, curvature was observed in the survival curves. As the curvature may influence thermal death time, it is necessary to compare performance of log-linear model with non-log-linear model (Peleg and Cole, 1998; van Boekel, 2002). The objectives of this study were 1) determine the thermal inactivation kinetics of *C. sporogenes* ATCC 7955 and *G. stearothermophilus* ATCC 10149 by using Weibull model, 2) understand effect of buffer substrate and pH values on influencing heat resistance of the two spores by using Weibull model, and 3) compare log-linear model with Weibull model by applying residual analysis. The data for this study were taken from Chapter 3 and 4.

5.3 Materials and methods

5.3.1 Weibull model

Survival ratio, S(t), is defined as the ratio between the number of survivors after an exposure time t, N(t), and the initial number N(0) at t = 0min (Peleg, 2000).

$$S(t) = \frac{N_t}{N_0} \tag{5.4}$$

Weibull model is showed by equation 5.5 and 5.6.

$$S(t) = \exp\left[-\left(\frac{t}{\alpha}\right)^{\beta}\right] \tag{5.5}$$

$$\log S(t) = -\frac{1}{2.303} \times \left(\frac{t}{\alpha}\right)^{\beta} \tag{5.6}$$

where α (time) is the scale parameter, β is the dimensionless shape parameter.

Solving the equation 5.4 for Weibull model, equation 5.7 can be obtained.

$$\ln\left[-ln\left(\frac{N_t}{N_o}\right)\right] = \beta lnt - \beta ln\alpha \tag{5.7}$$

The plot of $\ln\left[-ln\left(\frac{N_t}{N_o}\right)\right]$ versus ln(t) is named as the hazard plot. It is a linear curve with a slope equals β and an intercept equals$-\beta ln\alpha$. After getting β and α, the reliable life t_R can be calculated. It is defined as the 90% percentile of the failure time distribution, which is analogous to D value in the first-order logarithmic kinetic (van Boekel, 2002).

$$t_R = \alpha \times (-ln0.1)^{\frac{1}{\beta}} = \alpha \times 2.303^{\frac{1}{\beta}} \tag{5.8}$$

For different decimal reduction, equation 5.8 can be presented as equation 5.9:

$$t_d = \alpha \times \left[-\ln(10^{-d})^{\frac{1}{\beta}}\right] \tag{5.9}$$

where d is the number of decimal reduction. For instance, 12D reduction is commonly used in commercial sterilization, $t_{12} = \alpha(27.631)^{\frac{1}{\beta}}$.

α is the characteristic time at which the survival function logS(t) = exp (-1). It is defined as the mean of the distribution describing the death time t_C of the microbial population. In addition, α is highly depended on β. The value of β can represent the shape of survival curve. When β<1, the survival curve is concave upward, and α increases with time. The remaining microorganisms have less probability of dying and become adaptable to heat. When β>1, the survival curve is concave downward, and α decreases with time. The remaining microorganisms have higher heat sensitivity. Cumulative damage of heat cause greater lethality. When β=1, the survival curve is linear, and α is constant. It represents that the probability of dying does not depend on time, which is the same as the first order kinetic. In addition, α and β are assumed to depend on treatment conditions according to the language of reliability engineering. (van Boekel, 2002)

5.3.2 Evaluating model performance

5.3.2.1 Residual plots

The difference between the observed data and the predicted value is defined as residuals.

$$r_i = O_i - F_i \tag{5.10}$$

where r is the residual of data number i. O_i is the observed value and F_i is the predicted value, or fitted value for data number i. The residual plots were obtained by plotting residuals versus the fitted values. It is useful for checking model assumption. The residual plots were obtained by using Microsoft Excel program.

5.3.2.2 Scale-location plots

The Scale-location plot was obtained by plotting the square root of absolute value of residuals versus the fitted values. If any distinct patterns could be observed in the plot, the model could be assumed that not suitable for analyzing the data (Maindonald and Braun, 2006). Microsoft Excel program was used for plotting the scale-location plot.

5.4. Results and discussion

5.4.1 Thermal destruction of *C. sporogenes* ATCC 7955

5.4.1.1 Survivor curves and model parameters

Data in this study were taken from Chapter 3 of this thesis. Figure 5.1 (a) shows the survival curves of *C. sporogenes* ATCC 7955 in distilled water as heating medium. Figure 5.1 (b) shows the survival curves of *C. sporogenes* ATCC 7955 in phosphate buffer at pH 7 as heating medium. Figure 5.1 (c to j) show the survival curves of *C. sporogenes* ATCC 7955 in McIlvaine buffer at different pH as heating medium. The hazard plots were obtained by plotting –lnS(t) versus ln(t). According to slope and intercept of the hazard plots, parameter β and α can be obtained. Table 5.1 summarizes parameters α, β, the R^2 value and t_d for the Weibull model of analyzing data of *C. sporogenes* ATCC 7955.

5.4.1.2 Parameter β

The values of parameter β for *C. sporogenes* ranged from 0.819 to 1.103 in distilled water, 0.528 to 1.097 in phosphate buffer at pH 7, and 0.542 to 3.469 in McIlvaine buffer at pH 7.0. For *C. sporogenes* heated in McIlvaine buffer at different pH, the parameter β ranged from 0.456 to 3.468. Studies of how external conditions influence the parameter β were also done. Figure A (Appendix I) shows plots of β versus treatment temperature for *C. sporogenes* heated in different heat medium. The plots show that β increased when treatment temperature increased for *C. sporogenes* in phosphate buffer at pH 7.0 and McIlvaine buffer at pH 8.0, 7.0, 6.0, 5.5, and 4.0. In McIlvaine buffer at pH 5.0, the parameter β decreased when temperature increased. For spores in distilled water and McIlvaine buffer at pH 4.5 and 3.0, β showed dependent on temperature according to quadratic model. However, no unitive trend of β was obtained. In addition, the quantity of treatment temperature was quite small for getting conclusion of how temperature influences β. Further studies are needed. Figure B (Appendix II) shows plots of β versus pH value for *C. sporogenes* heated in McIlvaine buffer at different treatment temperature. At 110℃,

β increased when pH decreased from 7.0 to 4.5 and decreased when pH decreased from 4.5 to 3.0. There was no distinct changing pattern of β at 115℃. At 120℃, greatest value of β appeared at pH 7.0. β decreased when pH value decreased from 7.0 to 3.0.

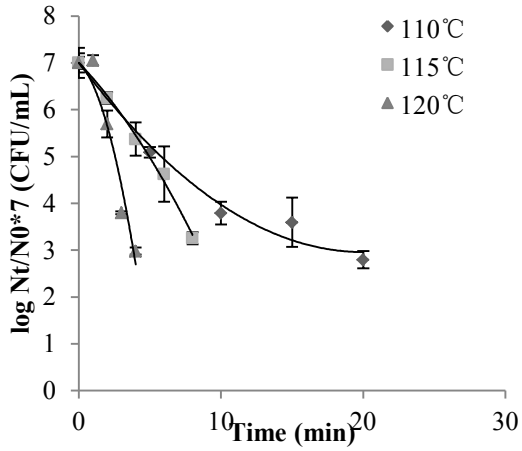
5.4.1.3 Parameter α

Figure C (Appendix III) shows plots of lnα for *C. sporogenes* versus treatment temperature in different heating medium. Figure D (Appendix IV) shows plots of lnα versus pH value for *C. sporogenes* heated in McIlvaine buffer at different treatment temperature. The results showed that parameter α did not have unitive and distinct dependence on temperature or pH value. As α is highly depend on β, the result is reasonable.

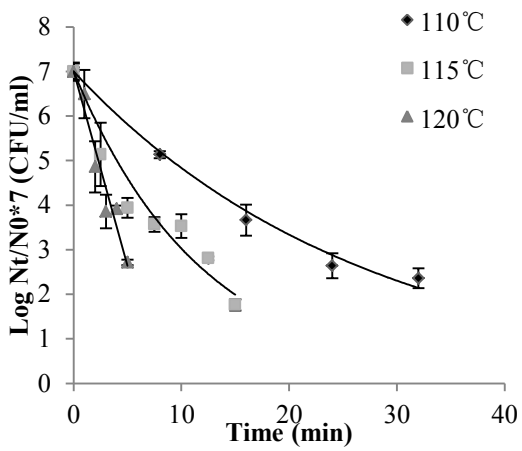
5.4.1.4 Reliable life for decimal reduction t_d

Since 12D reduction of *C. botulinum* is commonly applied as standard of commercial sterilization, t_{12} was used for determining the heat resistance of *C. sporogenes*. It is the time needed to reach 12-log reduction of spores. Greater value indicated greater heat resistance of spores. According to Table 5.1, t_{12} was decreased when heat temperature increased in all cases. *C. sporogenes* had greater t_{12} in phosphate buffer and McIlvainve buffer than in distilled water. It is same as we got in Chapter 3. In terms of pH value on influencing value of t_{12} for spores in McIlvaine buffer, only at 110℃ the results showed that t_{12} decreased when pH value was not neutral. No absolute trend of degradation or aggrandizement was observed of t_{12} when pH value changed at 115℃. At 120℃, the lowest t_{12} was obtained at pH 7. When pH decreased from 7.0 to 3.0, t_{12} increased with fluctuation.

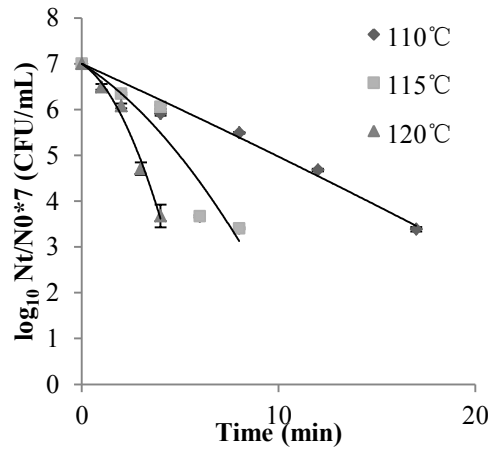
Table 5.2 shows the comparison between t_{12} and 12D value obtained by first-order kinetics. The differences between t_{12} and 12D ranged from 76.6 to -10.9 min. Overall, t_{12} was greater than 12D when β<1, and t_{12} was lower than 12D when β>1.



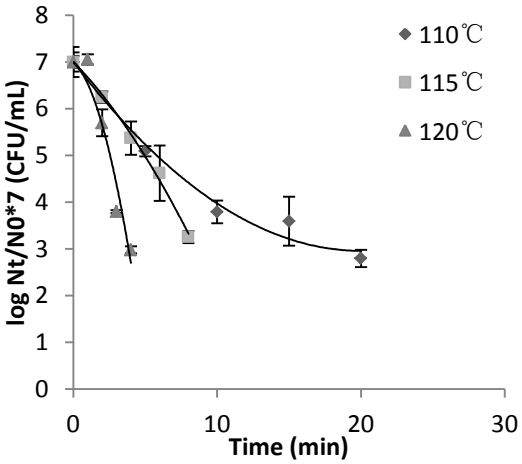
(a) Distilled water



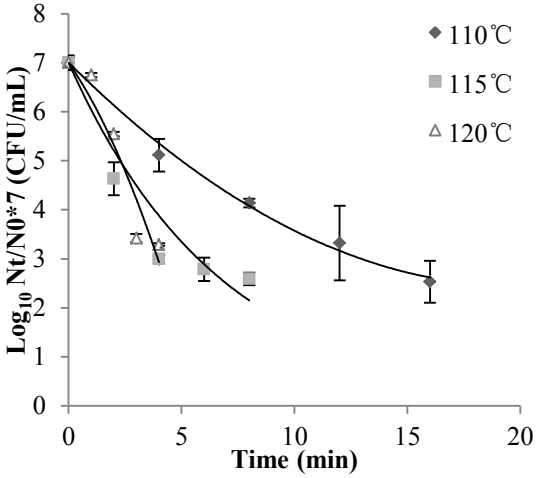
(b) Phosphate buffer at pH 7.0



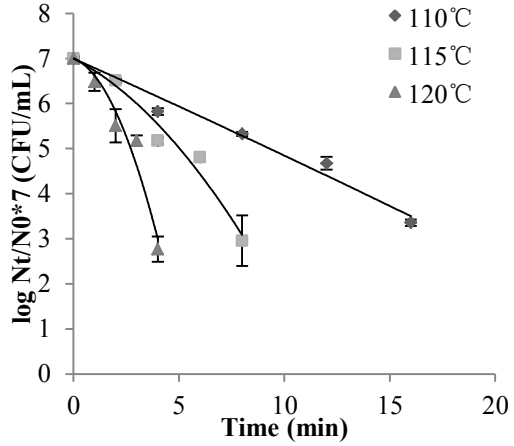
(c) McIlvaine buffer at pH 8.0



(d) McIlvaine buffer at pH 7.0

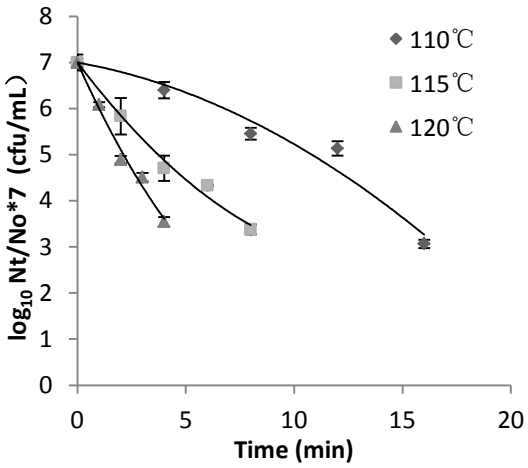


(e) McIlvaine buffer at pH 6.0

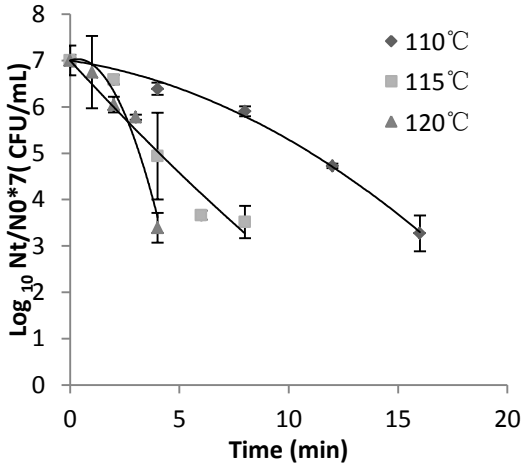


(f) McIlvaine buffer at pH 5.5

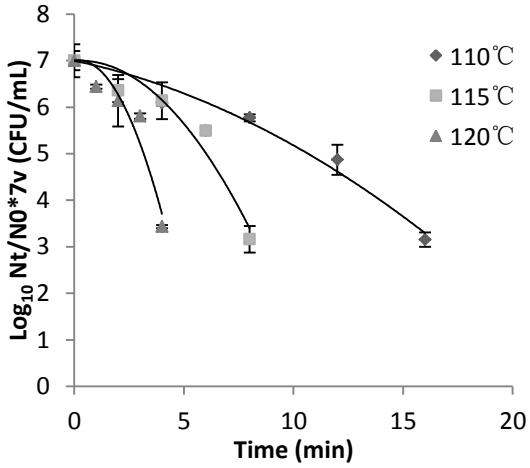
Figure 5.1 a-f Survival curves of *C. sporogenes* ATCC 7955 heated in different heat medium by using the Weibull model



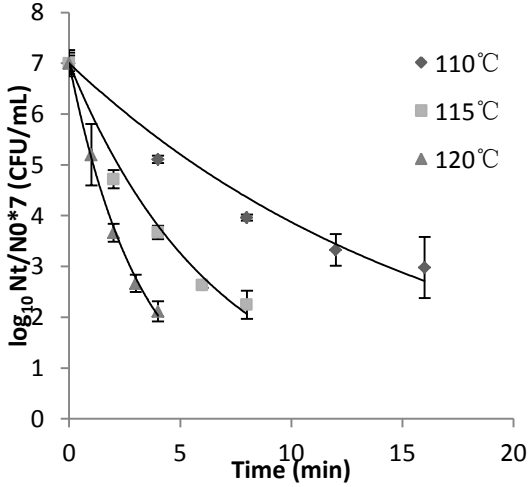
(g) McIlvaine buffer at pH 5.0



(h) McIlvaine buffer at pH 4.5



(i) McIlvaine buffer at pH 4.0



(j) McIlvaine buffer at pH 3.0

Figure 5.1 g-j Survival curves of *C. sporogenes* ATCC 7955 heated in different heat medium by using the Weibull model

Table 5.1 Weibull model parameters α, β, R^2 and t_{12} of *C. sporogenes* ATCC 7955 in different heat medium

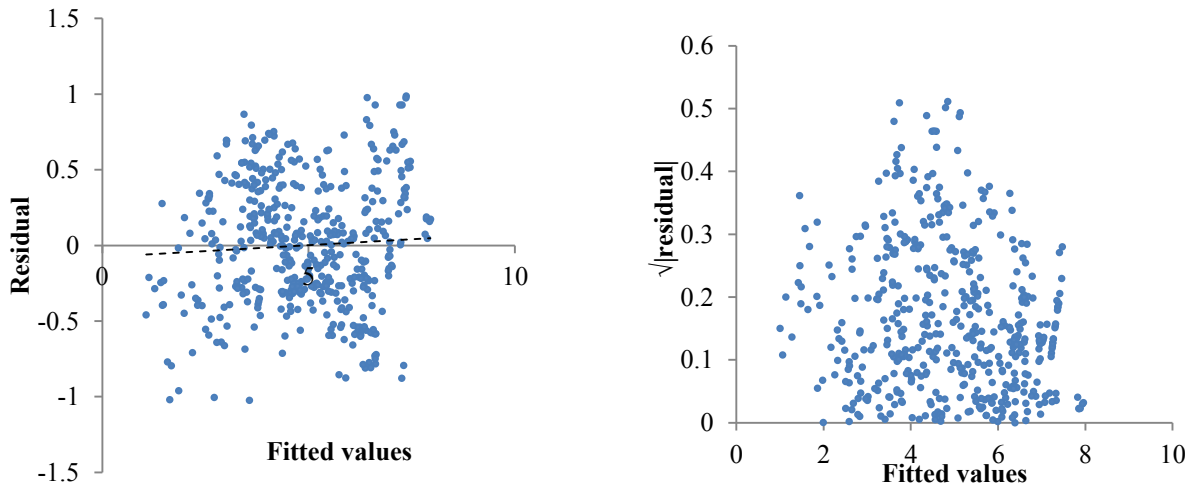
Matrix	T(℃)	α(min)	β	R^2	t_{12} (min)
Distilled water	110	3.161±0.152	0.887±0.018	0.984	133.4
	115	2.502±0.486	1.103±0.135	0.975	50.7
	120	0.353±0.127	0.819±0.116	0.976	20.3
Phosphate buffer at pH 7.0	110	0.851±0.170	0.683±0.047	0.970	109.9
	115	0.222±0.194	0.529±0.159	0.910	118.2
	120	0.603±0.445	1.097±0.446	0.926	12.4
McIlvaine buffer at pH 8.0	110	1.242±0.032	0.817±0.004	0.944	72.3
	115	1.777±0.101	1.351±0.046	0.870	20.7
	120	1.051±0.014	1.394±0.025	0.958	11.4
McIlvaine buffer at pH 7.0	110	0.303±0.077	0.542±0.049	0.927	137.7
	115	1.368±0.194	1.130±0.145	0.953	25.6
	120	1.812±0.263	3.468±0.888	0.920	4.7
McIlvaine buffer at pH 6.0	110	0.419±0.079	0.631±0.026	0.957	80.4
	115	0.064±0.043	0.456±0.072	0.874	93.3
	120	1.342±0.126	2.083±0.186	0.954	6.6
McIlvaine buffer at pH 5.5	110	1.138±0.057	0.774±0.018	0.954	83.2
	115	1.881±0.070	1.445±0.084	0.957	18.7
	120	1.004±0.278	1.461±0.267	0.943	9.7
McIlvaine buffer at pH 5.0	110	3.264±0.743	1.281±0.188	0.949	43.5
	115	0.532±0.301	0.811±0.193	0.965	31.9
	120	0.442±0.091	0.934±0.081	0.972	15.4
McIlvaine buffer at pH 4.5	110	3.590±0.212	1.303±0.038	0.961	45.8
	115	2.194±0.318	1.617±0.061	0.886	17.1
	120	1.010±0.197	1.256±0.330	0.694	14.2
McIlvaine buffer at pH 4.0	110	2.224±0.637	0.963±0.157	0.824	69.9
	115	1.971±0.652	1.235±0.255	0.785	29.0
	120	1.225±0.403	1.387±0.388	0.712	13.4
McIlvaine buffer at pH 3.0	110	0.472±0.138	0.625±0.0561	0.986	95.5
	115	0.151±0.0265	0.546±0.0221	0.984	65.8
	120	0.248±0.172	0.766±0.235	0.969	18.9

Table 5.2 Comparison of t_{12} and 12D for *C. sporogenes* ATCC 7955 in different heat medium

Matrix	T (℃)	t_{12} (min)	12D (min)	t_{12} – 12D (min)
Distilled water	110	133.4	120.0	13.4
	115	50.7	54.9	-4.2
	120	20.3	15.7	4.6
Phosphate buffer at pH 7.0	110	109.9	78.5	31.4
	115	118.2	41.5	76.7
	120	12.4	15.0	-2.6
McIlvaine buffer at pH 8.0	110	72.3	52.3	20.0
	115	20.7	27.0	-6.3
	120	11.4	15.0	-3.6
McIlvaine buffer at pH 7.0	110	137.7	60.4	77.3
	115	25.6	29.3	-3.7
	120	4.7	12.6	-7.9
McIlvaine buffer at pH 6.0	110	80.4	45.4	35.0
	115	93.3	25.7	67.6
	120	6.6	13.3	-6.7
McIlvaine buffer at pH 5.5	110	83.2	54.0	29.2
	115	18.7	27.8	-9.1
	120	9.7	13.8	-4.1
McIlvaine buffer at pH 5.0	110	43.5	54.4	-10.9
	115	31.9	24.6	7.3
	120	15.4	14.5	0.9
McIlvaine buffer at pH 4.5	110	45.8	52.9	-7.12
	115	17.1	24.5	-7.38
	120	14.2	15.7	-1.52
McIlvaine buffer at pH 4.0	110	69.9	55.9	14.0
	115	29.0	28.2	0.8
	120	13.4	15.5	-2.1
McIlvaine buffer at pH 3.0	110	95.5	49.8	45.7
	115	65.8	26.6	39.2
	120	18.9	13.3	5.6

5.4.1.5 Model comparison

Figure 5.2 and 5.3 show the residual plot and scale-location plot for the first-order linear kinetics and the Weibull model respectively. The residual plot and scale-location plot for the first-order linear kinetics did not show any distinct pattern of distribution of residuals. While the residual plot for the Weibull model showed that residuals clustered in the first and forth quadrant and tailed to the third quadrant. It could be modeled as a second order polynomial equation. In addition, the scale-location plot revealed that variance of the estimates decreased when fitted value increased. Although residuals for the Weibull model are overall smaller than that for the first-order kinetics, the linear model is much easier to be implemented. Therefore, the first-order linear kinetics is better than the Weibull model on analyzing data of *C. sporogenes* ATCC 7955 in this study.



(a) **(b)**

Figure 5.2 The residual plots (a) and scale-location plot (b) for the first-order linear kinetic for *C. sporogenes* ATCC7955

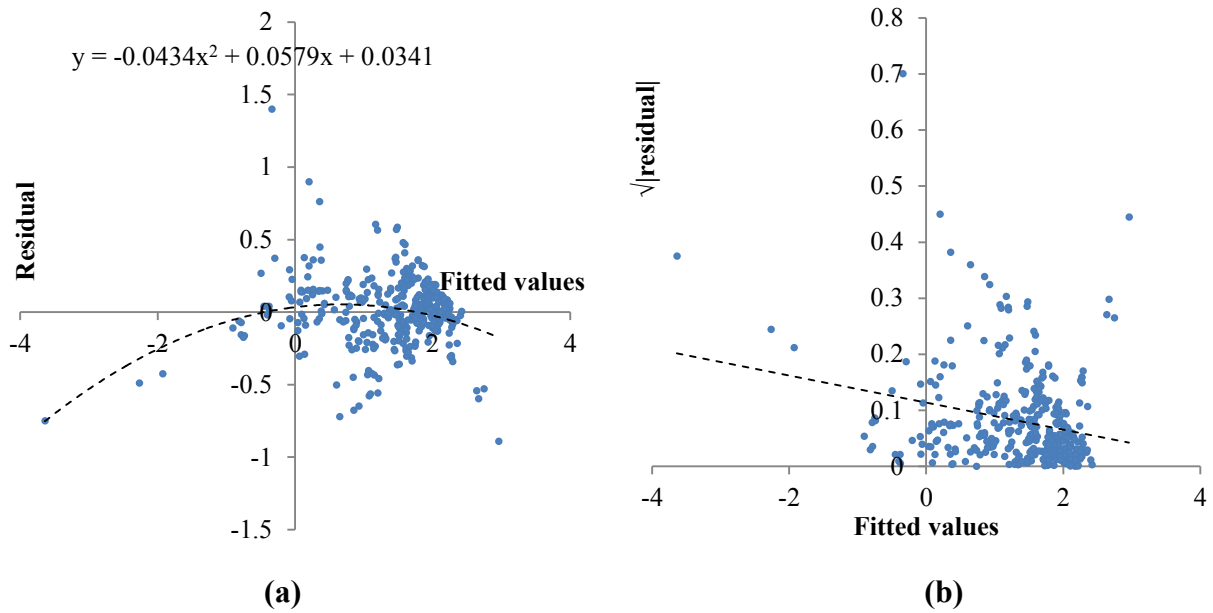
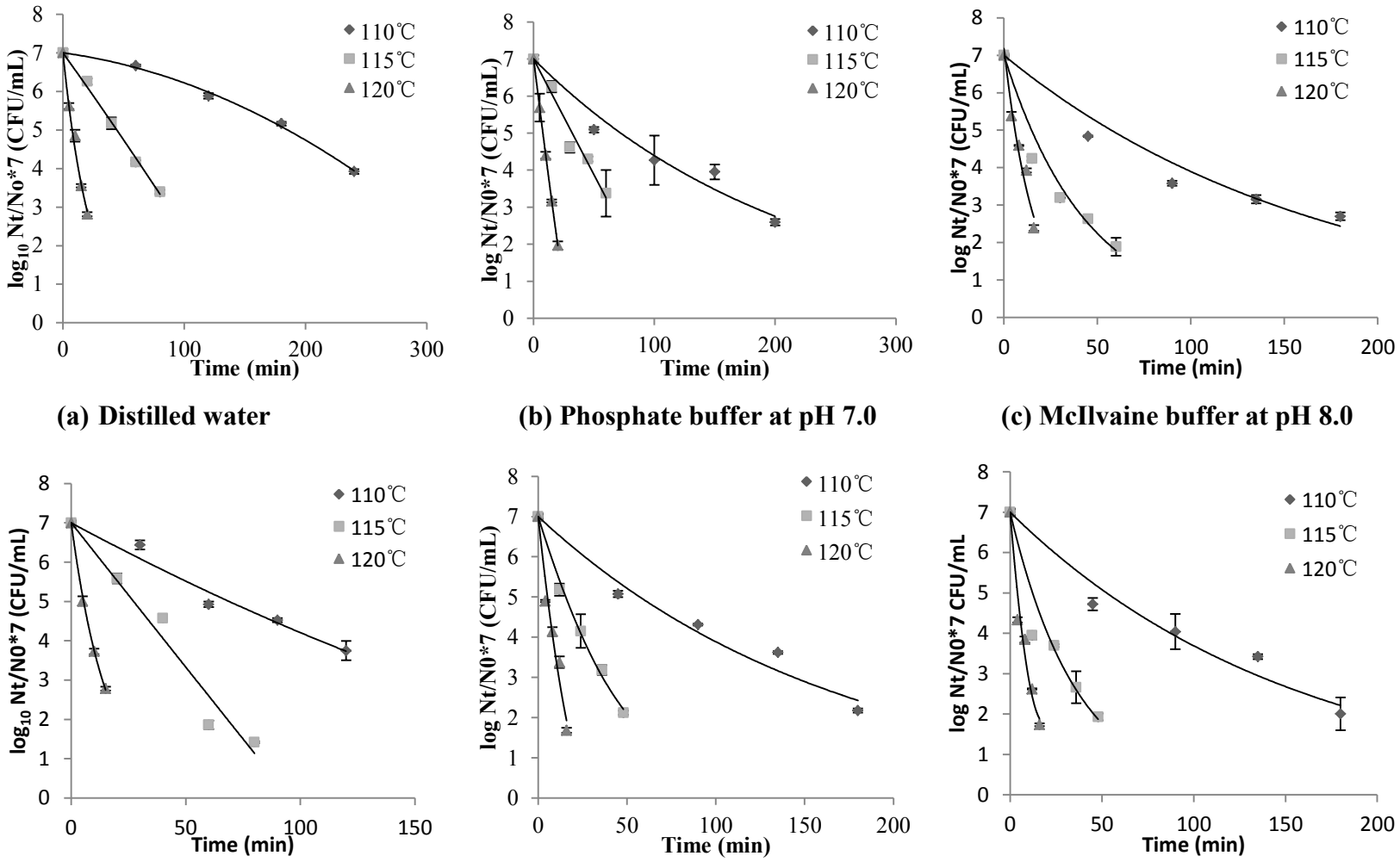


Figure 5.3 The residual plots (a) and scale-location plot (b) for the Weibull model for for *C. sporogenes* ATCC7955

5.4.2 Thermal destruction of *G. stearothermophilus* ATCC 10149

5.4.2.1 Survivor curves and model parameters

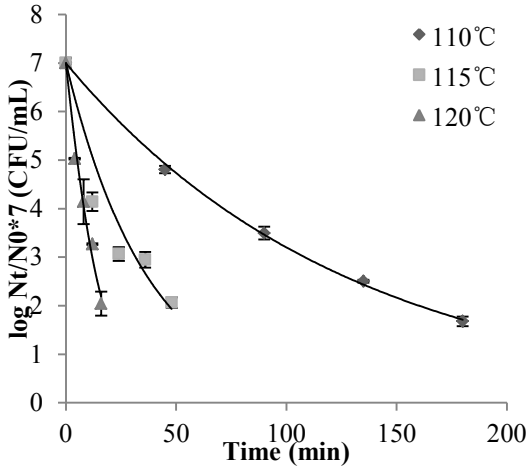
Data in this study were taken from Chapter 4 of this thesis. Figure 5.4 (a) shows the survival curves of *G. stearothermophilus* ATCC 10149 in distilled water as heating medium. Figure 5.4 (b) shows the survival curves of *G. stearothermophilus* ATCC 10149 in phosphate buffer at pH 7.0 as heating medium. Figure 5.4 (c to j) show the survival curves of *G. stearothermophilus* ATCC 10149 in McIlvaine buffer at different pH as heating medium. The hazard plots were obtained by plotting –lnS(t) versus ln(t). According to slope and intercept of the hazard plots, parameter β and α can be obtained. Table 5.3 summarizes parameters α, β, the R^2 value and t_d for the Weibull model of analyzing data of *G. stearothermophilus* ATCC 10149.



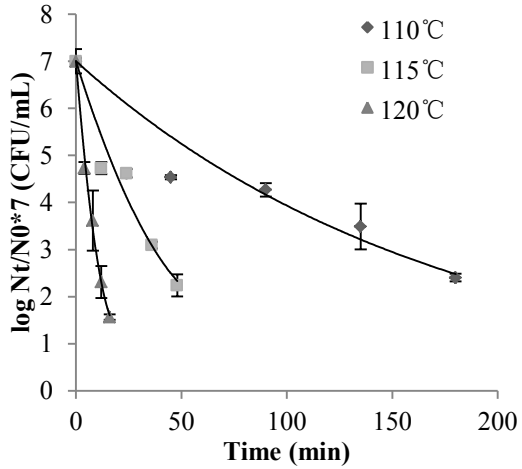
(a) Distilled water **(b) Phosphate buffer at pH 7.0** **(c) McIlvaine buffer at pH 8.0**

(d) McIlvaine buffer at pH 7.0 **(e) McIlvaine buffer at pH 6.0** **(f) McIlvaine buffer at pH 5.5**

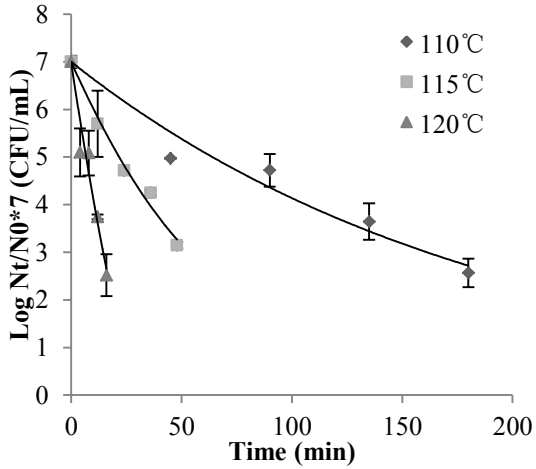
Figure 5.4 a-f Survival curves of *G. stearothermophilus* ATCC 10149 heated in different heat medium by using the Weibull model



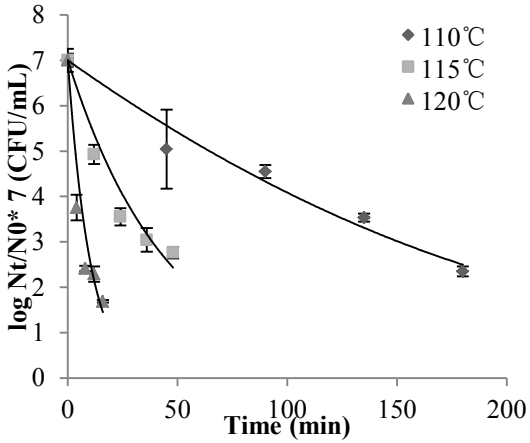
(g) McIlvaine buffer at pH 5.0



(h) McIlvaine buffer at pH 4.5



(i) McIlvaine buffer at pH 4.0



(j) McIlvaine buffer at pH 3.0

Figure 5.4 g-j Survival curves of *G. stearothermophilus* ATCC 10149 heated in different heat medium by using the Weibull model

Table 5.3 Weibull model parameters α, β, R^2 and t_5 of *G. stearothermophilus* ATCC 10149 in different heat medium

Matrix	T(℃)	α(min)	β	R^2	t_5 (min)
Distilled water	110	76.182±3.000	1.588±0.068	0.995	354.9
	115	11.927±1.156	1.144±0.056	0.992	100.9
	120	1.530±0.101	0.822±0..011	0.984	29.2
Phosphate buffer at pH 7.0	110	3.793±0.904	0.558±0.021	0.864	300.6
	115	8.236±0.603	1.113±0.041	0.920	73.9
	120	1.709±0.571	0.985±0.032	0.991	20.4
McIlvaine buffer at pH 8.0	110	1.675±0.145	0.494±0.015	0.967	235.5
	115	0.237±0.046	0.436±0.019	0.992	64.5
	120	0.732±0.077	0.715±0.026	0.957	22.3
McIlvaine buffer at pH 7.0	110	25.750±2.955	1.258±0.089	0.930	179.6
	115	7.921±0.726	1.063±0.048	0.954	78.9
	120	0.624±0.050	0.687±0.014	0.996	21.9
McIlvaine buffer at pH 6.0	110	5.695±0.376	0.628±0.010	0.954	278.9
	115	2.020±0.585	0.708±0.057	0.973	63.7
	120	0.413±0.031	0.631±0.017	0.935	19.9
McIlvaine buffer at pH 5.5	110	2.470±0.599	0.531±0.037	0.901	245.3
	115	0.147±0.074	0.378±0.039	0.866	94.2
	120	0.151±0.015	0.495±0.011	0.930	21.3
McIlvaine buffer at pH 5.0	110	3.495±0.193	0.641±0.006	0.998	158.1
	115	0.148±0.059	0.369±0.035	0.947	111.9
	120	0.474±0.015	0.649±0.012	0.940	20.4
McIlvaine buffer at pH 4.5	110	1.037±0.369	0.429±0.052	0.803	311.8
	115	0.726±0.074	0.552±0.008	0.818	60.9
	120	0.348±0.012	0.646±0.019	0.949	15.3
McIlvaine buffer at pH 4.0	110	3.347±0.819	0.555±0.062	0.834	272.1
	115	8.222±1.234	1.289±0.128	0.896	53.1
	120	0.624±0.472	0.639±0.157	0.749	28.5
McIlvaine buffer at pH 3.0	110	14.509±1.800	0.955±0.053	0.810	187.2
	115	0.636±0.136	0.524±0.022	0.944	66.9
	120	0.023±0.027	0.346±0.058	0.962	27.0

5.4.2.2 Parameter β

The values of parameter β for *G. stearothermophilus* ranged from 0.822 to 1.588 in distilled water, 0.558 to 1.113 in phosphate buffer at pH 7.0, and 0.687 to 1.258 in McIlvaine buffer at pH 7.0. For *G. stearothermophilus* heated in McIlvaine buffer at different pH, the β ranged from 0.346 to 1.289. Figure E (Appendix V) shows plots of β versus treatment temperature for *G. stearothermophilus* heated in different heat medium. For spores heated in distilled water, McIlvaine buffer at pH 7.0 and 3.0, β decreased when treatment temperature increased. While β increased with temperature increased in phosphate buffer at pH 7.0 and McIlvaine buffer at pH 8.0 and 4.5. For spores in McIlvaine buffer at pH 6.0, 5.5, 5.0 and 4.0, β showed depend on temperature according to quadratic model. Thus, no uniform effect of temperature on influencing β for *G. stearothermophilus* was observed. Figure F (Appendix VI) shows plots of β versus pH value for *G. stearothermophilus* heated in McIlvaine buffer at different treatment temperature. At 110℃, spores heated at pH 7 had greatest β. When pH was not neutral, β was lower than 1. For spores heated at 115℃, the greatest β appeared at pH 4.0. And only for spores at pH 7.0 and 4.0, β was greater than 1. At 120℃, β was lower than 1 in all cases. The values of β decreased when pH values decreased.

5.4.2.3 Parameter α

Figure G (AppendixVII) shows plots of lnα for *G. stearothermophilus* versus treatment temperature in different heating medium. The results revealed that lnα decreased with higher treatment temperature, except value of lnα for spores at 115℃ increased in McIlvaine buffer at pH 4. Figure H (Appendix VIII) shows plots of lnα versus pH value for *G. stearothermophilus* heated in McIlvaine buffer at different treatment temperature. No apparent change trend of lnα observed when pH value changed in all cases.

5.4.2.4 Reliable life for decimal reduction t_d

G. stearothermophilus is thermophile. It has greater heat resistance than *C. botulinum*.

Therefore, 5D of the spores was used for sterilization. In this study, t_5 was applied to determine the heat resistance of *G. stearothermophilus*. According to Table 5.3, t_5 decreased when heat temperature increased in all cases. *G. stearothermophilus* had greater t_5 in phosphate buffer and McIlvainve buffer than in distilled water. It is same as we got in Chapter 4. However, no obvious change trend of t_5 observed when pH value changed for spores heated in McIlvaine buffer at 110 and 115℃. At 120℃, value of t_5 slightly decreased when pH value decreased from 8.0 to 4.5. The results were totally different from results obtained by the first-order linear kinetics.

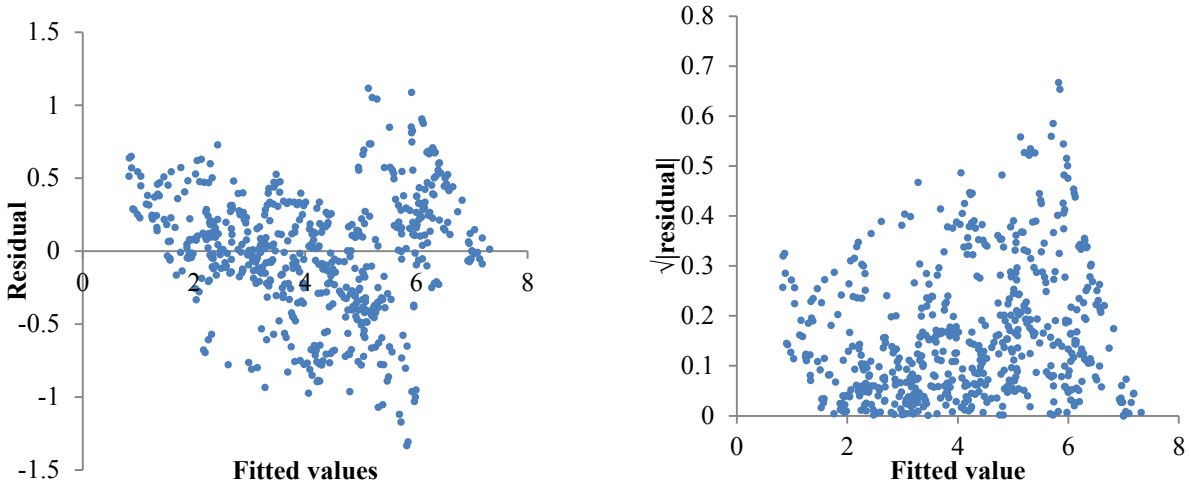
Table 5.4 shows the comparison between t_5 and 5D value obtained by first-order kinetics. The differences between t_5 and 5D ranged from 127.8 to -93.5 min. Overall, t_5 was greater than 12D when β<1, and t_5 was lower than 12D when β>1.

5.4.2.5 Model comparison

Figure 5.5 and 5.6 show the residual plot and scale-location plot for the first-order linear kinetics and the Weibull model respectively. The residual plot and scale-location plot for the first-order linear kinetics did not show any distinct pattern of distribution of residuals. For the residual plot of Weibull model, no distinct pattern was shown. However, the scale-location plot revealed that variance of the estimates decreased when fitted value increased. Therefore, the first-order linear kinetics is better than the Weibull model on analyzing data of *G. stearothermophilus* ATCC 10149 in this study.

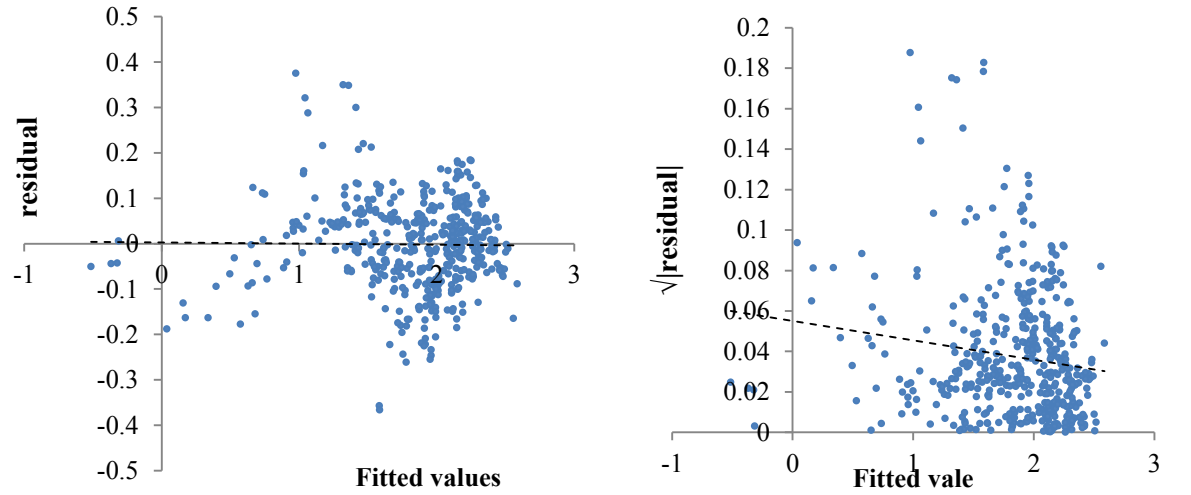
Table 5.4 Comparison of t_5 and 5D for *G. stearothermophilus* ATCC 10149 in different heat medium

Matrix	T (℃)	t_5 (min)	5D (min)	t_5 – 5D (min)
Distilled water	110	354.9	449.0	-94.1
	115	100.9	107.0	-6.1
	120	29.2	27.7	1.5
Phosphate buffer at pH 7.0	110	300.6	250.0	50.6
	115	73.9	81.5	-7.6
	120	20.4	20.5	-0.1
McIlvaine buffer at pH 8.0	110	235.5	223.0	12.5
	115	64.5	66.0	-1.5
	120	22.3	19.8	2.5
McIlvaine buffer at pH 7.0	110	179.6	219.0	-39.4
	115	78.9	78.0	0.9
	120	21.9	19.8	2.1
McIlvaine buffer at pH 6.0	110	278.9	217.0	61.9
	115	63.7	58.5	5.2
	120	19.9	18.0	1.9
McIlvaine buffer at pH 5.5	110	245.3	212.0	33.3
	115	94.2	63.0	31.2
	120	21.3	18.8	2.5
McIlvaine buffer at pH 5.0	110	158.1	175.0	-16.9
	115	111.9	54.5	57.4
	120	20.4	18.8	1.6
McIlvaine buffer at pH 4.5	110	311.8	184.0	127.8
	115	60.9	54.0	6.9
	120	15.3	16.4	-1.1
McIlvaine buffer at pH 4.0	110	272.1	198.0	74.1
	115	53.1	61.0	-7.9
	120	28.5	19.4	9.1
McIlvaine buffer at pH 3.0	110	187.3	204.0	-16.7
	115	66.9	58.0	8.9
	120	27.0	17.7	9.3



(a) (b)

Figure 5.5 The residual plots (a) and scale-location plot (b) for the first-order linear kinetic for *G. stearothermophilus* ATCC 10149



(a) (b)

Figure 5.6 The residual plots (a) and scale-location plot (b) for the Weibull model for *G. stearothermophilus* ATCC 10149

5.4.3 Comparison with other studies

The First-order linear kinetics is frequently used for analyzing inactivation of microorganisms, including thermal destruction, high pressure and pulsed electric fields inactivation. Nevertheless, survival curves with curvature were commonly found from several literatures. There are different types of survival curves: linear curves, concave curves, convex curves, curves with a shoulder, curves with a tailing, sigmoidal curves, and biphasic inactivation (Geeraerd et al., 2005; Xiong et al., 1999 a). In fact, the shape of the survival curve is depended on many factors, including category of organisms and the lethal agent intensity. It is acknowledgement that less intensity leads to upward concavity and harsher intensity leads downward concavity (Peleg, 2000; Stone et al., 2009). Same results were found in this study. For *C. sporogenes,* upward curves were found at lower treatment temperature, while at 120℃ nearly all cases were found to be downward curves. In terms of *G. stearothermophilus*, upward curves were frequently found. As *G. stearothermophilus* is thermophile, it is adaptable.

However, the first-order kinetics only fit for expressing linear curves. Hence, non-linear models were introduced for studying microbial inactivation as well, including the Weibull model, modified Gompertz model and log-logistic model (Xiong et al., 1999 a). The Weibull distribution can be used for indicating the behavior of systems or events having some degree of variability. Thus, for the Weibull model, probability of lethality is supposed to follow the Weibull distribution. This model is widely applied for microbial inactivation, enzymatic and chemical degradation kinetics. Previous research studies reported that the Weibull model had better fitness on representing survival curves than the first-order kinetics. It was reported that greater fitness was found for the Weibull model on analyzing thermal destruction of *Bacillus cereus* than the first-order kinetics (Fernandez et al., 1999). Similar results were found for the Weibull model on modeling pressure inactivation of foodborne pathogens in milk (Chen, 2007).

As the Weibull model is an empirical model, it is not accurate for all cases. It is not advice to utilize a model, which is fit for one bacterium in a system or in a food matrix, for predicting the inactivation kinetics in other system or matrix (Buzrul and Alpas, 2007). Hassan and

Ramaswamy (2011) reported that the Weibull model had no difference on predicting thermal inactivation of *C. sporogenes* and *G. stearothermophilus* in meat and carrot alginate purees compared to the first-order kinetics. In this study also, the Weibull model was not good for analyzing *C. sporogenes* and *G. stearothermophilus* in all cases, even though high fitness was obtained for some plots. What is more, the main disadvantage of the Weibull model is that difficult interpretation of parameters into biological significance (Stone et al., 2009).

5.5 Conclusions

In this study, the Weibull model was used to determine the thermal destruction kinetics of *C. sporogenes* ATCC 7955 and *G. stearothermophilus* ATCC 10149 in different heat medium. The results showed that spores had less heat resistance when treatment temperature increased in all cases. No obvious or uniform dependence of parameters α and β on exterior conditions, including temperature and pH values, were revealed. The reliable life for decimal reduction t_d was applied to tell the thermal resistance of spores. The results showed that pH values did not influence t_d apparently. However, according to the residual plots and scale-location plots, the Weibull model had worse fitness on predicting thermal destruction of neither *C. sporogenes* ATCC 7955 nor *G. stearothermophilus* ATCC 10149 compared to the first-order kinetics. Nevertheless, it is obvious that no all survival curves followed the first-order kinetics. Further studies of alternating non-linear models for *C. sporogenes* and *G. stearothermophilus* other than the Weibull model are needed.

CHAPTER 6

GENERAL CONCLUSIONS

This work evaluated the thermal destruction kinetics of *C. sporogenes* ATCC 7955 and *G. stearotherophilus* ATCC 10149 in different buffer media and at different pH values. Based on the results, substrate of buffer media and the pH value affected the heat resistance of spores. Both *C. sporogenes* and *G. stearotherphilus* had the highest heat resistance in distilled water. Spores in the McIvaine buffer had lower heat resistance than in phosphate buffer probably because of the synergic effect between phosphate salts and citric acid. In terms of pH value, the maximum resistance to thermal destruction occurred near the neutral pH value of 7.0 for both *C. sporogenes* and *G. stearothermophilus* possibly because this pH also happens to be the best for their growth. Heat sensitivity of spores was enhanced on both sides of the neutral pH value with D values decreasing with an increase in pH above 7.0 and a decrease in pH below 7.0. However, no major differences were observed in the heat resistance when pH values became more acidic. In addition, treatment temperature played the most important role in influencing heat resistance of spores. When treatment temperature increased, the effect of substrate or pH value on enhancing heat sensitivity of spores was reduced.

While initial modeling was based on the first order log-linear models, subsequently, the Weibull model was also used for characterizing the thermal destruction kinetics of *C. sporogenes* and *G. stearothermophilus*. Since obvious deviations were observed from the log-linear first order behavior, and Weibull model demonstrated better fit for some test data and showed some concavity and convexity, but did not show any specific trends. The R^2 values associated with both Weibull and the first-order log-linear kinetic were both quite high. The residual plots and scale-location plots revealed that the Weibull model was not any better to predict the experimental data as compared to the first-order kinetics. Hence, in current study, the first-order linear kinetic is better for predicting thermal destruction trend of *C. sporogenes* and *G. stearothermophilus* than the Weibull model.

References

Abel-Santos, E. (2014). Endospores, sporulation and germination. In *Molecular Medical Microbiology,Three-Volume Set.(Second Edition)*.Tang, Y. W., Sussman, M., Liu, D., Poxton, I., and Schwartzman, J. (Eds.). Academic Press, New York, NY,163-178.

Aguilar-Rosas, S. F., Ballinas-Casarrubias, M. L., Nevarez-Moorillon, G. V., Martin-Belloso, O., and Ortega-Rivas, E. (2007). Thermal and pulsed electric fields pasteurization of apple juice: effects on physicochemical properties and flavour compounds. *Journal of Food Engineering*, *83*(1), 41-46.

Ahmed, N. M., Conner, D. E., and Huffman, D. L. (1995). Heat resistance of *Escherichia Coli O157: H7* in meat and poultry as affected by product composition. *Journal of Food Science, 60*(3), 606-610.

Akterian, S. G., Fernandez, P. S., Hendrickx, M. E., Tobback, P. P., Periago, P. M., and Martinez, A. (1999). Risk analysis of the thermal sterilization process.: Analysis of factors affecting the thermal resistance of microorganisms. *International journal of food microbiology*, *47*(1), 51-57.

Amaha, M., and Sakaguchi, K. I. (1954). Effects of carbohydrates, proteins, and bacterial cells in the heating media on the heat resistance of *Clostridium sporogenes*. *Journal of Bacteriology, 68*(3), 338.

Anderson, W. A., McClure, P. J., Baird‐Parker, A. C., and Cole, M. B. (1996). The application of a log‐logistic model to describe the thermal inactivation of *Clostridium botulinum* 213B at temperatures below 121.1℃. *Journal of Applied Bacteriology, 80*(3), 283-290.

Awuah, G. B., Ramaswamy, H. S., and Economides, A. (2007). Thermal processing and quality: principles and overview. *Chemical Engineering and Processing: Process Intensification, 46*(6), 584-602.

Bahçeci, K. S., and Acar, J. (2007). Modeling the combined effects of pH, temperature and ascorbic acid concentration on the heat resistance of *Alicyclobacillus acidoterrestis*. *International journal of food microbiology*, *120*(3), 266-273.

Brown, J. L., Tran-Dinh, N., and Chapman, B. (2012). *Clostridium sporogenes* PA 3679 and its uses in the derivation of thermal processing schedules for low-acid shelf-stable foods and as a research model for proteolytic *Clostridium botulinum*. *Journal of Food Protection*, *75*(4), 779-792.

Buzrul, S., and Alpas, H. (2007). Modeling inactivation kinetics of food borne pathogens at a constant temperature. *LWT-Food Science and Technology*, *40*(4), 632-637.

Cameron, E. J., and Esty, J. R. (1926). The Examination of Spoiled Canned Foods: 2. Classification of Flat Sour Spoilage Organisms from Nonacid Foods. *The Journal of Infectious Diseases*, 89-105.

Cameron, M. S., Leonard, S. J., and Barrett, E. L. (1980). Effect of moderately acidic pH on heat resistance of *Clostridium sporogenes* spores in phosphate buffer and in buffered pea puree. *Applied and Environmental Microbiology, 39*(5), 943-949.

Carroll, K. J. (2003). On the use and utility of the Weibull model in the analysis of survival data. *Controlled clinical trials*, *24*(6), 682-701.

Carter, A. T., and Peck, M. W. (2015). Genomes, neurotoxins and biology of *Clostridium botulinum* Group I and Group II. *Research in microbiology, 166*(4), 303-317.

Casolari, A. (1994). About basic parameters of food sterilization technology. *Food microbiology, 11,* 75-84.

Chen, H. (2007). Use of linear, Weibull, and log-logistic functions to model pressure inactivation of seven foodborne pathogens in milk. *Food Microbiology, 24*(3), 197-204.

Chen, H., and Hoover, D. G. (2003). Pressure inactivation kinetics of *Yersinia enterocolitica* ATCC 35669. *International Journal of Food Microbiology, 87*(1), 161-171.

Cole, M. B., Davies, K. W., Munro, G., Holyoak, C. D., and Kilsby, D. C. (1993). A vitalistic model to describe the thermal inactivation of *Listeria monocytogenes*. *Journal of Industrial Microbiology, 12*(3-5), 232-239.

Coroller, L., Leguérinel, I., and Mafart, P. (2001). Effect of water activities of heating and recovery media on apparent heat resistance of *Bacillus cereus* spores. *Applied and*

Environmental Microbiology, 67(1), 317-322.

de W Blackburn, C., Curtis, L. M., Humpheson, L., Billon, C., and McClure, P. J. (1997). Development of thermal inactivation models for *Salmonella enteritidis* and *Escherichia coli O157: H7* with temperature, pH and NaCl as controlling factors. *International Journal of Food Microbiology, 38*(1), 31-44.

Deindoerfer, F. H. (1957). Calculation of heat sterilization times for fermentation media. *Applied microbiology*, *5*(4), 221.

Deindoerfer, F. H., and Humphrey, A. E. (1959). Analytical method for calculating heat sterilization times. *Applied microbiology*, *7*(4), 256.

Diao, M. M., André, S., and Membré, J. M. (2014). Meta-analysis of D-values of proteolytic *Clostridium botulinum* and its surrogate strain *Clostridium sporogenes* PA 3679. *International journal of food microbiology*, *174*, 23-30.

Fakhouri, M. O., and Ramaswamy, H. S. (1993). Temperature uniformity of microwave heated foods as influenced by product type and composition. *Food Research International*, *26*(2), 89-95.

Farber, J. M., and Pagotto, F. (1992). The effect of acid shock on the heat resistance of *Listeria monocytogenes*. *Letters in Applied Microbiology, 15*(5), 197-201.

Feeherry, F. E., Munsey, D. T., and Rowley, D. B. (1987). Thermal inactivation and injury of *Bacillus stearothermophilus* spores. *Applied and Environmental Microbiology*, *53*(2), 365-370.

Fellows, P.J.. (2000). Processing by application of heat. In *Food Processing Technology - Principles and Practice* (2nd Edition). Woodhead Publishing, Cambrige, UK, 229-384.

Fernandez, A., Salmeron, C., Fernández, P. S., and Martınez, A. (1999). Application of a frequency distribution model to describe the thermal inactivation of two strains of *Bacillus cereus*. *Trends in food science and technology*, *10*(4), 158-162.

Fernandez, P. S., Ocio, M. J., Rodrigo, F., Rodrigo, M., and Martinez, A. (1996). Mathematical model for the combined effect of temperature and pH on the thermal resistance of *Bacillus*

stearothermophilus and C*lostridium sporogenes* spores. *International journal of Food Microbiology, 32*(1), 225-233.

Finley, N., and Fields, M. L. (1962). Heat activation and heat-induced dormancy of *Bacillus stearothermophilus* spores. *Applied Microbiology, 10*(3), 231-236.

Gálvez, A., López, R. L., Pulido, R. P., and Burgos, M. J. G. (2014). Biopreservation of milk and dairy products. In *Food Biopreservation*. Springer, New York, NY, 49-69

Gama, J. J. T., and de Sylos, C. M. (2007). Effect of thermal pasteurization and concentration on carotenoid composition of Brazilian Valencia orange juice. *Food Chemistry*, *100*(4), 1686-1690.

Gardner, W.H. (1973). Acidulants in Food Processing. In *Handbook of Food Additives Volumn I (second edition*). Furia, T.E.(Ed.),CRC Press, New York, NY,225-270

Geeraerd, A. H., Valdramidis, V. P., and Van Impe, J. F. (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International journal of food microbiology*, *102*(1), 95-105.

Ghani, A. A., Farid, M. M., and Chen, X. D. (2002). Theoretical and experimental investigation of the thermal inactivation of *Bacillus stearothermophilus* in food pouches. *Journal of Food Engineering*, *51*(3), 221-228.

Ghani, A. A., Farid, M. M., Chen, X. D., and Richards, P. (2001). Thermal sterilization of canned food in a 3-D pouch using computational fluid dynamics. *Journal of Food Engineering, 48*(2), 147-156.

Gil, M. M., Brandao, T. R., and Silva, C. L. (2006). A modified Gompertz model to predict microbial inactivation under time-varying temperature conditions. *Journal of Food Engineering, 76*(1), 89-94.

Grant, I. R., Ball, H. J., and Rowe, M. T. (1998). Effect of high-temperature, short-time (HTST) pasteurization on milk containing low numbers of *Mycobacterium paratuberculosis*. *Letters in Applied Microbiology*, *26*(2), 166-170.

Hansen, N. H., and Riemann, H. (1963). Factors affecting the heat resistance of nonsporing

organisms. *Journal of Applied Bacteriology, 26*(3), 314-333.

Hassan, H. F., and Ramaswamy, H. S. (2011). Heat resistance of *G. stearothermophilus* and *C. sporogenes* in carrot and meat alginate purees. *Journal of Food Processing and Preservation*, *35*(3), 376-385.

Hutton, M. T., Koskinen, M. A., and Hanlin, J. H. (1991). Interacting effects of pH and NaCl on heat resistance of bacterial spores. *Journal of food science*, *56*(3), 821-822.

Iciek, J., Błaszczyk, I., and Papiewska, A. (2008). The effect of organic acid type on thermal inactivation of *Geobacillus stearothermophilus* spores. *Journal of food engineering*, *87*(1), 16-20.

Iciek, J., Papiewska, A., and Molska, M. (2006). Inactivation of *Bacillus stearothermophilus* spores during thermal processing. *Journal of Food Engineering*, *77*(3), 406-410.

Ingraham, J. L., and Stokes, J. L. (1959). Psychrophilic bacteria. *Bacteriological reviews*, *23*(3), 97.

Jansen, E., and Aschehoug, V. (1951). *Bacillus* as spoilage organisms in canned foods. *Journal of Food Science*, *16*(1‐6), 457-461.

Jay, J. M. (2000). High-temperature food preservation and characteristics of thermophilic microorganisms. In *Modern Food Microbiology (Sixth Edition)*, Aspen Publishers, Gaithersburg, MD.341-362.

Katzin, L. I., Sandholzer, L. A., and Strong, M. E. (1943). Application of the decimal reduction time principle to a study of the resistance of coliform bacteria to pasteurization. *Journal of Bacteriology,* 45(3), 265.

Kim, J. U. H. E. E., and Naylor, H. B. (1966). Spore production by *Bacillus stearothermophilus. Applied microbiology*, *14*(4), 690.

Kristjansson, J. K. and Stetter, K.O. (1992) Thermophilic bacteria. In *Thermophilic Bacteria*. Kristjansson, J. K. (Ed), CRC press, Boca Raton, FL.2-18.

Laroche, C., Fine, F., and Gervais, P. (2005). Water activity affects heat resistance of microorganisms in food powders. *International Journal of Food Microbiology, 97*(3), 307-315.

Lee, H. S., and Coates, G. A. (2003). Effect of thermal pasteurization on Valencia orange juice color and pigments. *LWT-Food Science and Technology*, *36*(1), 153-156.

Ling, B., Tang, J., Kong, F., Mitcham, E. J., and Wang, S. (2014). Kinetics of food quality changes during thermal processing: a review. *Food and Bioprocess Technology, 8*(2), 343-358.

Linton, R. H., Carter, W. H., Pierson, M. D., and Hackney, C. R. (1995). Use of a modified Gompertz equation to model nonlinear survival curves for *Listeria monocytogenes Scott A. Journal of Food Protection, 58*(9), 946-954.

López, M., González, I., Condon, S., and Bernardo, A. (1996). Effect of pH heating medium on the thermal resistance of *Bacillus stearothermophilus s*pores. *International Journal of Food Microbiology, 28*(3), 405-410.

Löwik, J. A. M., and Anema, P. J. (1972). Effect of pH on the heat resistance of *Clostridium sporogenes* spores in minced meat. *Journal of Applied Bacteriology*, *35*(1), 119-121.

Luechapattanaporn, K., Wang, Y., Wang, J., Al‐Holy, M., Kang, D. H., Tang, J., and Hallberg, L. M. (2004). Microbial safety in radio‐frequency processing of packaged foods. *Journal of Food Science*, *69*(7), 201-206.

Lund, B. M., and Peck, M. W. (2013). *Clostridium botulinum*. In *Guide to Foodborne Pathogens (Second Edition)*. Labbe, R.G.and Garcia, S. (Eds) Wiley-Blackwell, Oxford, UK, 91-111.

Lund, B. M., Graham, A. F., and Franklin, J. G. (1987). The effect of acid pH on the probability of growth of proteolytic strains of *Clostridium botulinum*. *International journal of food microbiology*, *4*(3), 215-226.

Lynch, D. J., and Potter, N. N. (1988). Effects of organic acids on thermal inactivation of *Bacillus stearothermophilus* and *Bacillus coagulans* spores in frankfurter emulsion slurry. *Journal of Food Protection*, *51*(6), 475-480.

Mafart, P., Couvert, O., and Leguérinel, I. (2001). Effect of pH on the heat resistance of spores: comparison of two models. *International journal of food microbiology*, *63*(1), 51-56.

Mafart, P., Couvert, O., Gaillard, S., and Leguérinel, I. (2002). On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. *International journal of food microbiology*, *72*(1), 107-113

Mah, J. H., Kang, D. H., and Tang, J. (2008). Effects of minerals on sporulation and heat resistance of *Clostridium sporogenes*. *International journal of food microbiology, 128*(2), 385-389.

Maindonald, J., and Braun, J. (2006). *Data analysis and graphics using R: an example-based approach (Second Edition).* Cambridge University Press, Cambridge, UK.

Matser, A. M., Krebbers, B., van den Berg, R. W., and Bartels, P. V. (2004). Advantages of high pressure sterilisation on quality of food products. *Trends in Food Science and Technology, 15*(2), 79-85.

Mottram, D. S. (2007). The Maillard reaction: source of flavour in thermally processed foods. In *Flavours and Fragrances*. Berger, R. G. (Ed) Springer, Berlin, GER. 269-283.

Murrell, W. G., and Scott, W. J. (1966). The heat resistance of bacterial spores at various water activities. *Journal of General Microbiology*, *43*(3), 411-425.

Naim, F., Zareifard, M. R., Zhu, S., Huizing, R. H., Grabowski, S., and Marcotte, M. (2008). Combined effects of heat, nisin and acidification on the inactivation of *Clostridium sporogenes* spores in carrot-alginate particles: from kinetics to process validation. *Food microbiology*, *25*(7), 936-941.

Nakayama, A. and Sumiko, S. (1980). Evidence of" flat sour" spoilage by obligate anaerobes in marketed canned drinks. *Bulletin of the Japanese Society of Scientific Fisheries*, *46*(9), 1117-1123.

Nazina, T. N., Tourova, T. P., Poltaraus, A. B., Novikova, E. V., Grigoryan, A. A., Ivanova, A. E., Lysenko, A.M., Perunyaka, V.V., Osipov, G.A., Belyaev, S.S. and Ivanov, M. V. (2001). Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus, Bacillus*

thermoleovorans, Bacillus kaustophilus, Bacillus thermodenitrificans to *Geobacillus* as the new combinations *G. stearothermophilus, G. th*. *International Journal of Systematic and Evolutionary Microbiology, 51*(2), 433-446.

Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J., and Setlow, P. (2000). Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiology and Molecular Biology Reviews, 64*(3), 548-572.

Ocio, M., Sánchez, T., Fernandez, P. S., Rodrigo, M., and Martinez, A. (1994). Thermal resistance characteristics of PA 3679 in the temperature range of 110–121 ℃ as affected by pH, type of acidulant and substrate. *International Journal of Food Microbiology, 22*(4), 239-247.

Paredes‐Sabja, D., Gonzalez, M., Sarker, M. R., and Torres, J. A. (2007). Combined effects of hydrostatic pressure, temperature, and pH on the inactivation of spores of *Clostridium perfringens* type A and *Clostridium sporogenes* in buffer solutions. *Journal of Food Science, 72*(6), M202-M206.

Patazca, E., Koutchma, T., and Ramaswamy, H. S. (2006). Inactivation Kinetics of *Geobacillus stearothermophilus* Spores in Water Using High‐pressure Processing at Elevated Temperatures. *Journal of Food Science*, *71*(3), M110-M116.

Peleg, M. (2000). Microbial survival curves—the reality of flat "shoulders" and absolute thermal death times. *Food Research International*, *33*(7), 531-538.

Peleg, M., and Cole, M. B. (1998). Reinterpretation of microbial survival curves. *Critical Reviews in Food Science*, *38*(5), 353-380.

Peleg, M., Normand, M. D., and Corradini, M. G. (2005) Generating microbial survival curves during thermal processing in real time. J*ournal of Applied Microbiology, 98*(2), 406-417.

Periago, P. M., Fernández, P. S., Salmerón, M. C., and Martınez, A. (1998). Predictive model to describe the combined effect of pH and NaCl on apparent heat resistance of *Bacillus stearothermophilus*. *International journal of food microbiology*, *44*(1), 21-30.

Pflug, I.J., Holcomb, R.G., and Gomez, M.M.(2001) Principle of the Thermal Destruction of Microorganisms, in Disinfection, Sterilization and Preservation (Fifth Edition), Block, S.S.(Ed.), Lippincott Williams and Wilkins, Philadelphia,P.A., pp. 79-134

Piyasena, P., Dussault, C., Koutchma, T., Ramaswamy, H. S., and Awuah, G. B. (2003) Radio frequency heating of foods: principles, applications and related properties—a review. *Critical Reviews in Food Science and Nutrition, 43*(6), 587-606.

Powers, J. J., and Niven Jr, C. F. (1976) Effect of acidification of canned tomatoes on quality and shelf life. *Critical* R*eviews in Food Science and Nutrition, 7*(4), 371-396.

Rahman, M.S. and Sablani, S.S. (2009) Water Activity Measurement Methods of Foods. In *Food properties handbook (Seconde Edition)*. Rahman, M. S. (Ed.). CRC press. Boca Raton, FL., pp 9-30

Ramaswamy, H. S., and Marcotte, M. (2005).Thermal processing. In *Food processing: principles and applications*. CRC Press. Boca Raton, FL, 68-168

Ramaswamy, H. S., Shao, Y., and Zhu, S. (2010). High-pressure destruction kinetics of *Clostridium sporogenes* ATCC 11437 spores in milk at elevated quasi-isothermal conditions. *Journal of Food Engineering*, *96*(2), 249-257.

Richardson, P. (2001), *Thermal Technologies in Food Processing*. Woodhead Publishing Limited, Cambdrige, UK.

Rodrigo, F., Rodrigo, C., Fernandez, P. S., Rodrigo, M., and Martınez, A. (1999). Effect of acidification and oil on the thermal resistance of *Bacillus stearothermophilus* spores heated in food substrate. *International Journal of Food Microbiology*, *52*(3), 197-201.

Rodrigo, M., Martinez, A., Sanchez, T., Peris, M. J., and Safon, J. (1993). Kinetics of *Clostridium sporogene*s PA3679 spore destruction using computer - controlled thermoresistometer. *Journal of Food Science, 58*(3), 649-652.

Russell, A. D. (2003). Lethal effects of heat on bacterial physiology and structure. *Science progress*, *86*(1-2), 115-137.

Sakai, N., and Hanzawa, T. (1994). Applications and advances in far-infrared heating in Japan.

Trends in Food Science and Technology, 5(11), 357-362.

Sala, F. J., Burgos, J., Condon, S., Lopez, P., and Raso, J. (1995). Effect of heat and ultrasound on microorganisms and enzymes. In *New methods of Food Preservation,* Gould, G.W. (Ed.) Springer Science+Business Media, Dordrecht, NL. 176-204.

Santos, M. S., and Zarzo, J. T. (1995). Glucono-δ-lactone and citric acid as acidulants for lowering the heat resistance of *Clostridium sporogenes* PA 3679 in HTST working conditions. *International Journal of Food Microbiology, 25*(2), 191-197.

Santos, M. S., and Zarzo, J. T. (1996). Evaluation of citric acid and GDL in the recovery at different pH levels of *Clostridium sporogenes* PA 3679 spores subjected to HTST treatment conditions. *International Journal of Food Microbiology, 29*(2), 241-254.

Seyhun, N., Ramaswamy, H., Sumnu, G., Sahin, S., and Ahmed, J. (2009). Comparison and modeling of microwave tempering and infrared assisted microwave tempering of frozen potato puree. *Journal of food engineering*, *92*(3), 339-344.

Shao, Y., and Ramaswamy, H. S. (2011). *Clostridium sporogenes*-ATCC 7955 spore destruction kinetics in milk under high pressure and elevated temperature treatment conditions. *Food and Bioprocess Technology, 4*(3), 458-468.

Shao, Y., Zhu, S., Ramaswamy, H., and Marcotte, M. (2010). Compression heating and temperature control for high-pressure destruction of bacterial spores: an experimental method for kinetics evaluation. *Food and Bioprocess Technology*, *3*(1), 71-78.

Silla, S. M., Nuñez, K. H., Casado, G. A., and Rodrigo, E. M. (1992). The effect of pH on the thermal resistance of *Clostridium sporogene*s (PA 3679) in asparagus puree acidified with citric acid and glucono-delta-lactone. *International Journal of Food Microbiology, 16*(4), 275-281.

Smelt, J. P. P. M., and Brul, S. (2014). Thermal inactivation of microorganisms. *Critical reviews in food science and nutrition*, *54*(10), 1371-1385.

Smelt, J. P. P. M., Raatjes, G. J. M., Crowther, J. S., and Verrips, C. T. (1982). Growth and toxin formation by *Clostridium botulinum* at low pH values. *Journal of Applied Bacteriology*, *52*(1), 75-82.

Smoot, L. A., and Pierson, M. D. (1979). Effect of oxidation‐reduction potential on the outgrowth and chemical inhibition of *Clostridium botulinum* 10755A spores. *Journal of Food Science, 44*(3), 700-704.

Stone, G., Chapman, B., and Lovell, D. (2009). Development of a log-quadratic model to describe microbial inactivation, illustrated by thermal inactivation of Clostridium botulinum. *Applied and environmental microbiology*, *75*(22), 6998-7005.

Stumbo, C. R. (1973). *Thermobacteriology in Food Processing*, Academic Press, New York, NY.

Taylor, R. H., Dunn, M. L., Ogden, L. V., Jefferies, L. K., Eggett, D. L., and Steele, F. M. (2013). Conditions associated with *Clostridium sporogenes* growth as a surrogate for *Clostridium botulinum* in nonthermally processed canned butter. *Journal of dairy science, 96*(5), 2754-2764.

Ting, P. T., and Freiman, A. (2004). The story of *Clostridium botulinum*: from food poisoning to Botox. *Clinical medicine*, *4*(3), 258-261.

Townsend, C. T., Yee, L., and Mercer, W. A. (1954). Inhibition of the growth of *Clostridium botulinum* by acidification. *Journal of Food Science*, *19*(1‐6), 536-542.

Tucker, G. and Featherstone, S. (2010) Processing systems. In *Essentials of Thermal Processing*, Wiley-Blackwell, Oxford, UK. 109-130.

van Boekel, M. A. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International journal of food microbiology*, *74*(1), 139-159.

Walker, H. W. (1964). Influence of buffers and pH on the thermal destruction of spores of *Bacillus megaterium* and *Bacillus polymyxaa*. *Journal of Food Science, 29*(3), 360-365.

Watanabe, T., Furukawa, S., Hirata, J., Koyama, T., Ogihara, H., and Yamasaki, M. (2003). Inactivation of *Geobacillus stearothermophilus* spores by high-pressure carbon dioxide treatment. *Applied and Environmental Microbiology, 69*(12), 7124-7129.

Wong, Y. C., Herald, T. J., and Hachmeister, K. A. (1996). Comparison between irradiated and thermally pasteurized liquid egg white on functional, physical, and microbiological properties. *Poultry Science*, *75*(6), 803-808.

Xiong, R., Xie, G., Edmondson, A. E., and Sheard, M. A. (1999 a). A mathematical model for bacterial inactivation. *International Journal of Food Microbiology, 46*(1), 45-55.

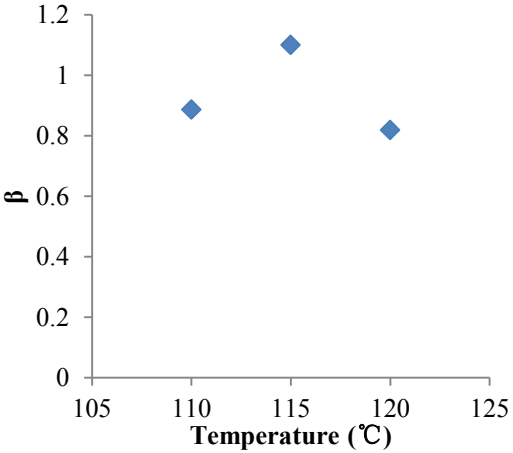
Xiong, R., Xie, G., Edmondson, A. S., Linton, R. H., and Sheard, M. A. (1999 b). Comparison of the Baranyi model with the modified Gompertz equation for modelling thermal inactivation of *Listeria monocytogenes Scott A*. *Food Microbiology, 16*(3), 269-279.

Yang, W. W., and Ponce, A. (2009). Rapid endospore viability assay of *Clostridium sporogenes* spores. *International Journal of Food Microbiology, 133*(3), 213-216.

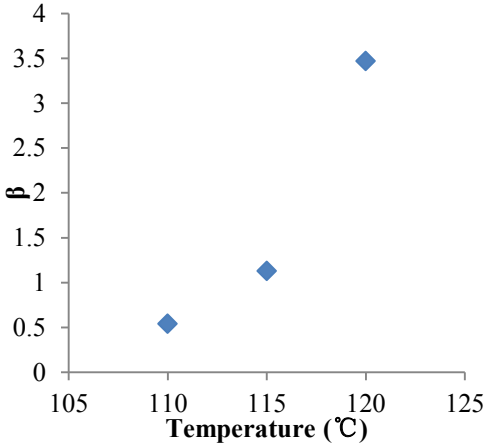
Yeom, H. W., Streaker, C. B., Zhang, Q. H., and Min, D. B. (2000). Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. *Journal of Agricultural and Food Chemistry*, *48*(10), 4597-4605.

Yeung, C. Y., Lee, H. C., Lin, S. P., Yang, Y. C., Huang, F. Y., and Chuang, C. K. (2006). Negative effect of heat sterilization on the free amino acid concentrations in infant formula. *European journal of clinical nutrition*, *60*(1), 136-141.

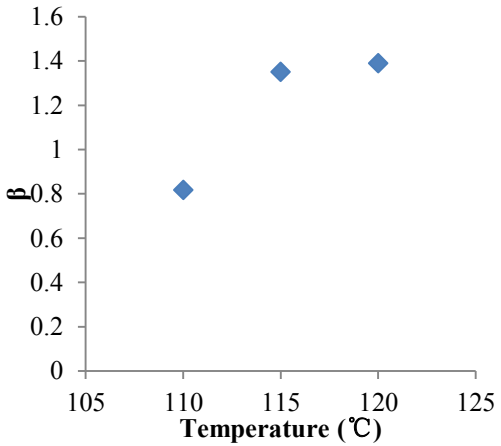
Appendix I Plots of β versus temperature (℃) for the Weibull model of *C. sporogenes* ATTC 7955



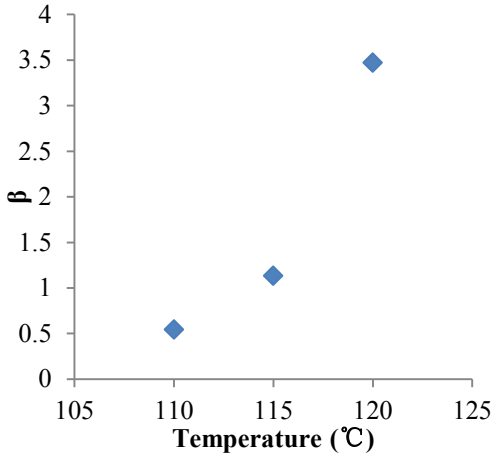
(a) Distilled water



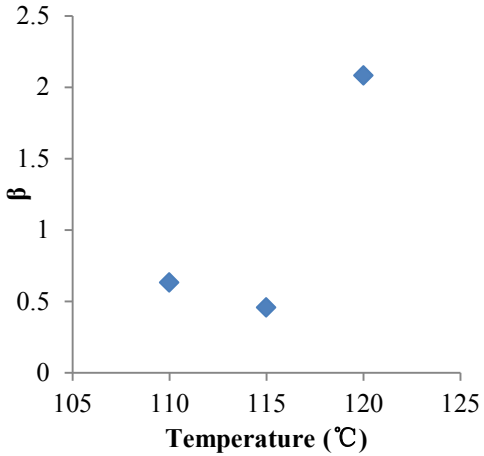
(b) Phosphate buffer at pH 7.0



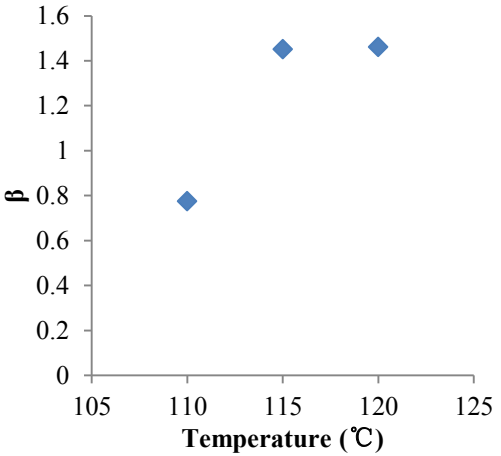
(c) McIlvaine buffer at pH 8.0



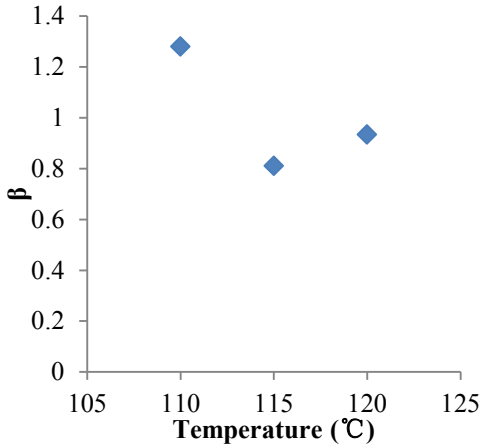
(d) McIlvaine buffer at pH 7.0



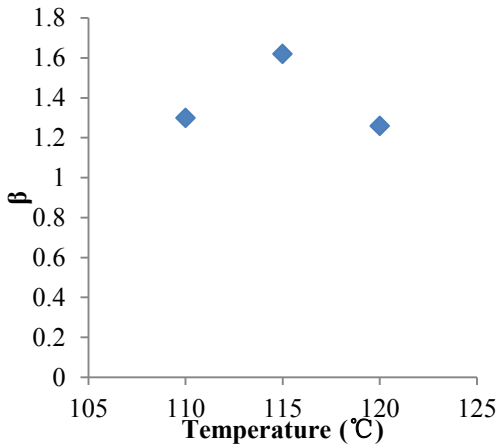
(e) McIlvaine buffer at pH 6.0



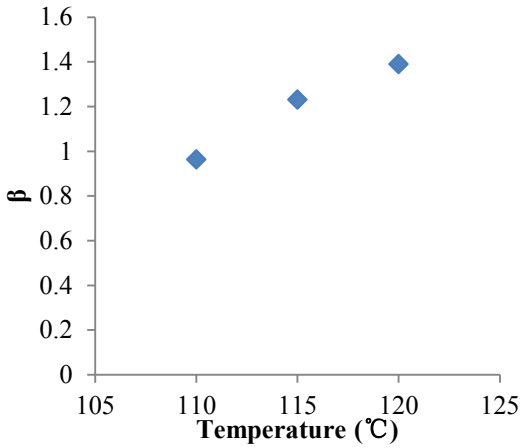
(f) McIlvaine buffer at pH 5.5



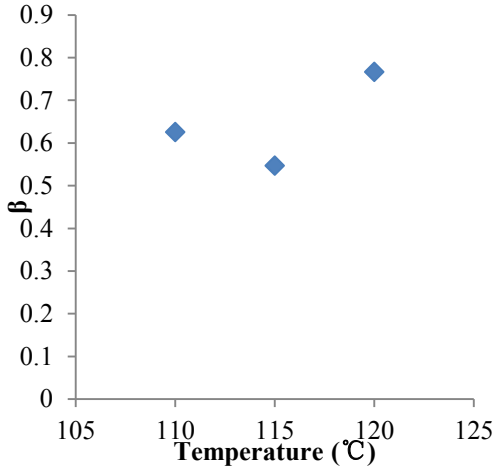
(g) McIlvaine buffer at pH 5.0



(h) McIlvaine buffer at pH 4.5



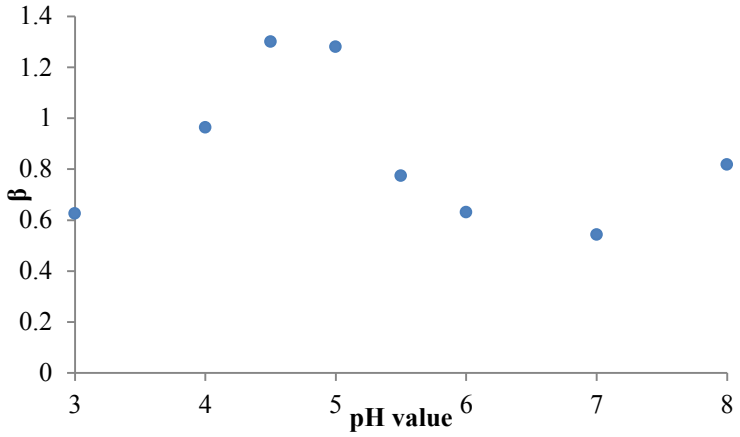
(i) McIlvaine buffer at pH 4.0



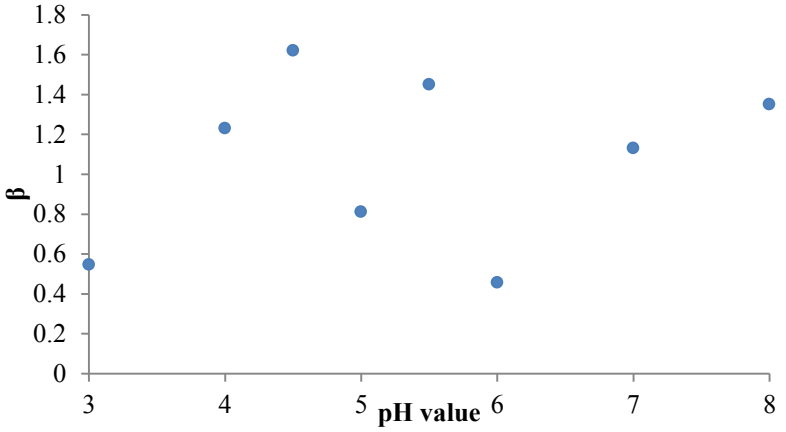
(j) McIlvaine buffer at pH 3.0

Figure A Plots of shape parameter β versus temperature (℃) for the Weibull model of thermal destruction of *C. sporogenes* ATTC 7955 in different heat medium

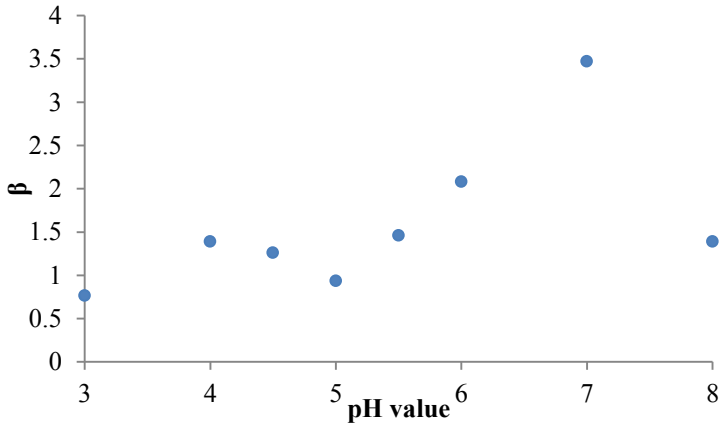
Appendix II Plots of β versus pH value for the Weibull model of *C. sporogenes* ATTC 7955



(a) 110℃



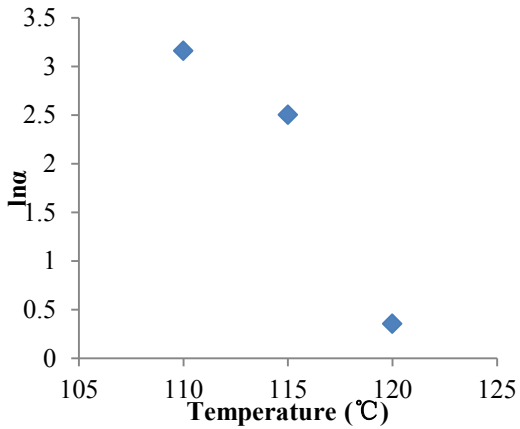
(b) 115℃



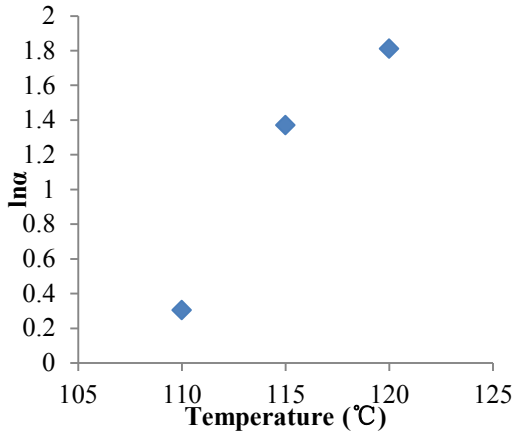
(c) 120℃

Figure B Plots of shape parameter β versus pH value for the Weibull model of thermal destruction of *C. sporogenes* ATTC 7955 in McIvaine buffer at different treatment temperature

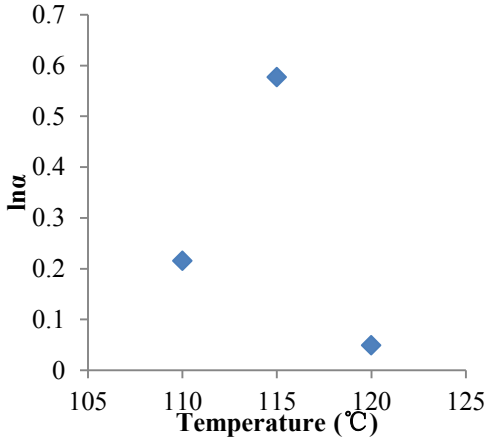
Appendix III Plots of lnα versus temperature (℃) for the Weibull model of *C. sporogenes* ATTC 7955



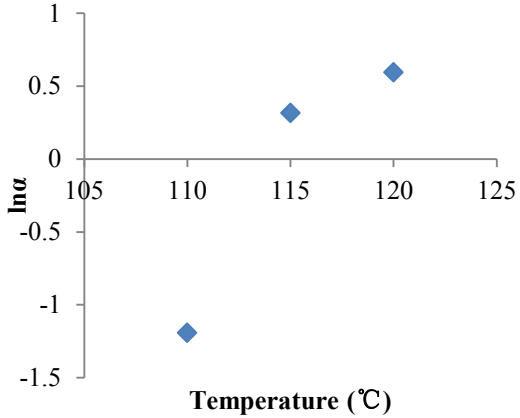
(a) Distilled water



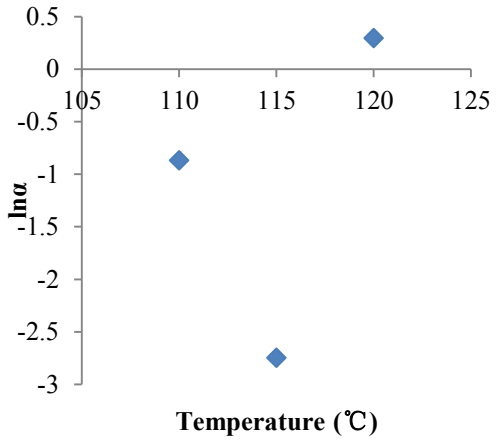
(b) Phosphate buffer at pH 7.0



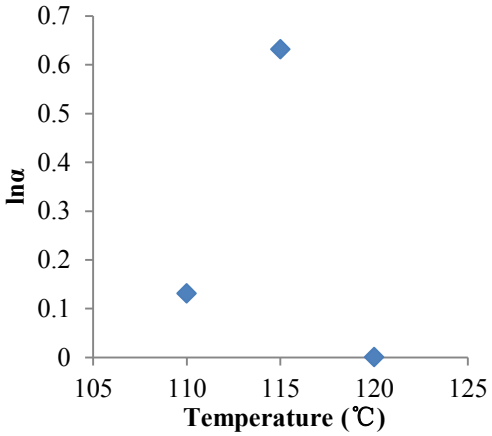
(c) McIlvaine buffer at pH 8.0



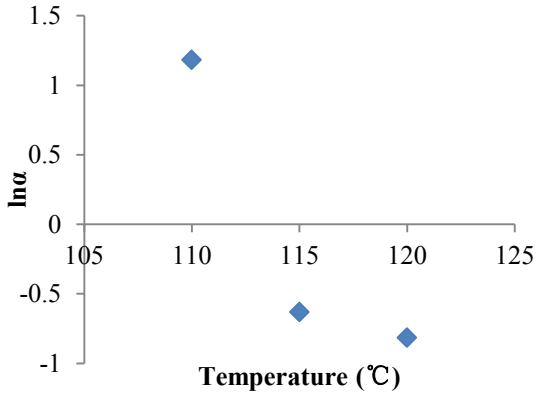
(d) McIlvaine buffer at pH 7.0



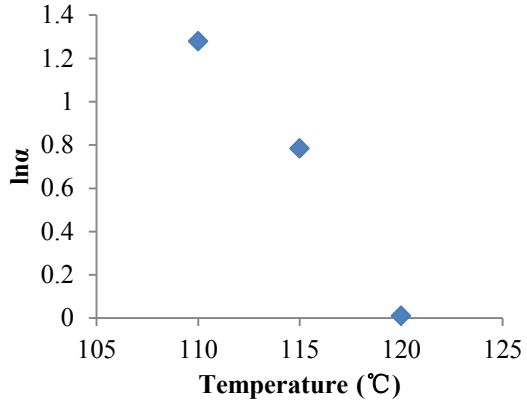
(e) McIlvaine buffer at pH 6.0



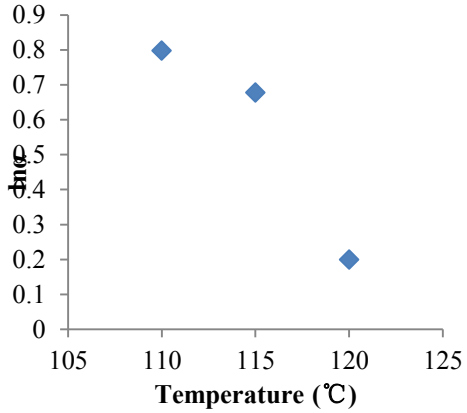
(f) McIlvaine buffer at pH 5.5



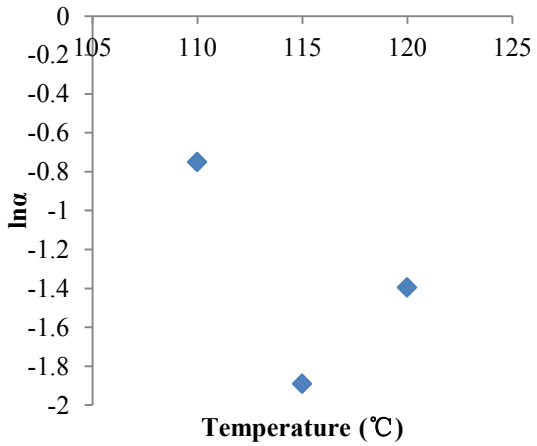
(g) McIlvaine buffer at pH 5.0



(h) McIlvaine buffer at pH 4.5



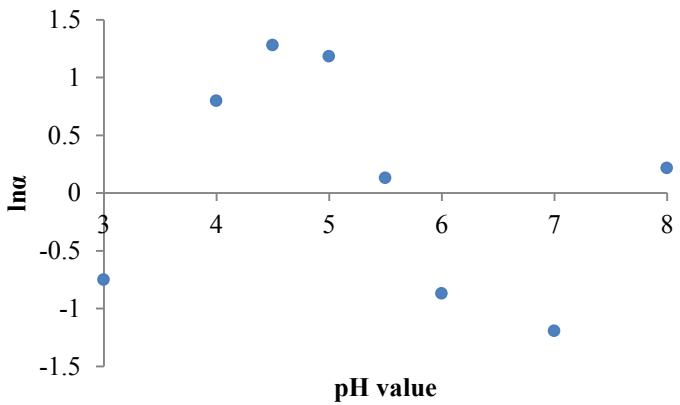
(i) McIlvaine buffer at pH 4.0



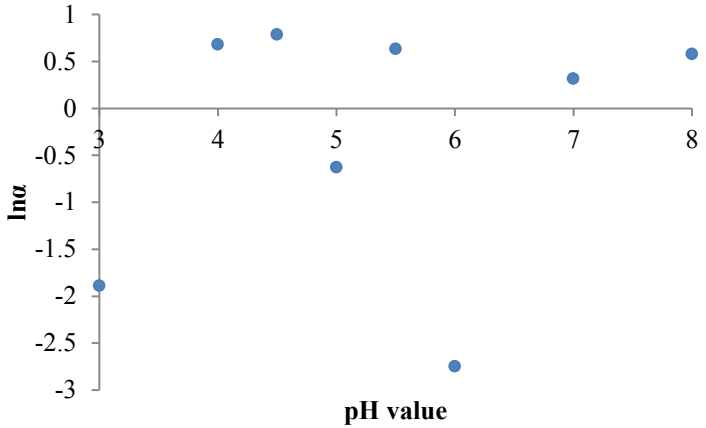
(j) McIlvaine buffer at pH 3.0

Figure C Plots of the natural logarithm of the scale parameter α versus temperature (℃) for the Weibull model of thermal destruction of *C. sporogenes* ATTC 7955 in different heat medium

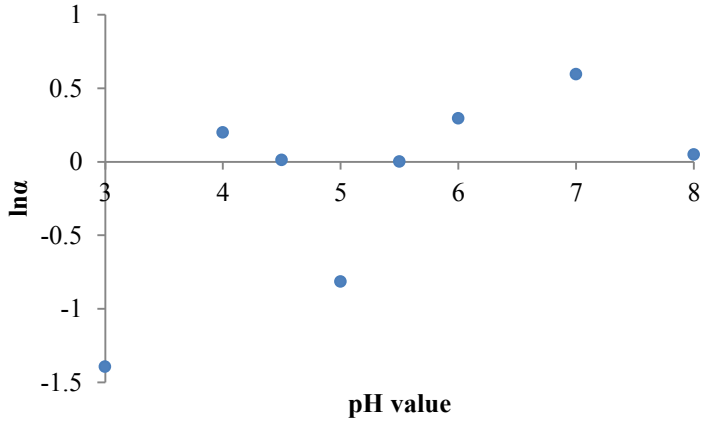
Appendix IV Plots of lnα versus pH value for the Weibull model of *C. sporogenes* ATTC 7955



(a) 110℃



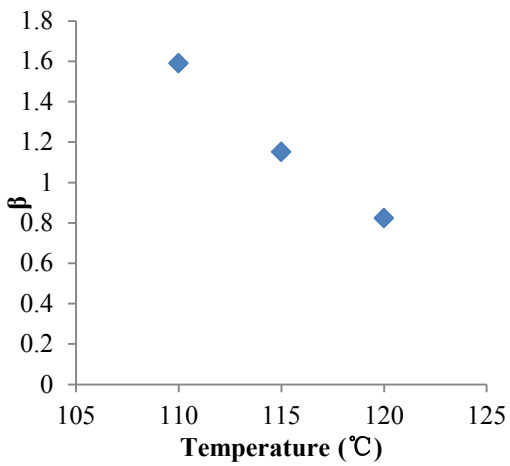
(b) 115℃



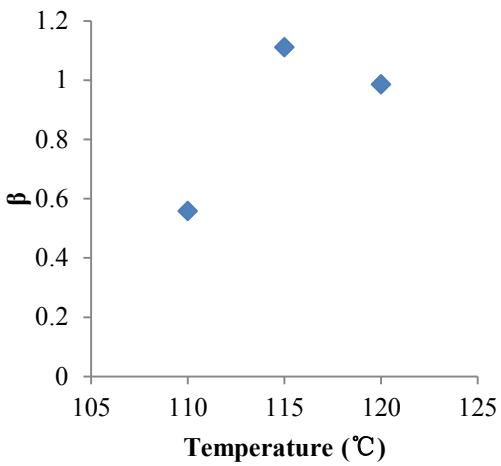
(c) 120℃

Figure D Plots of the natural logarithm of the scale parameter αversus pH value for the Weibull model of thermal destruction of *C. sporogenes* ATTC 7955 in McIvaine buffer at different treatment temperature

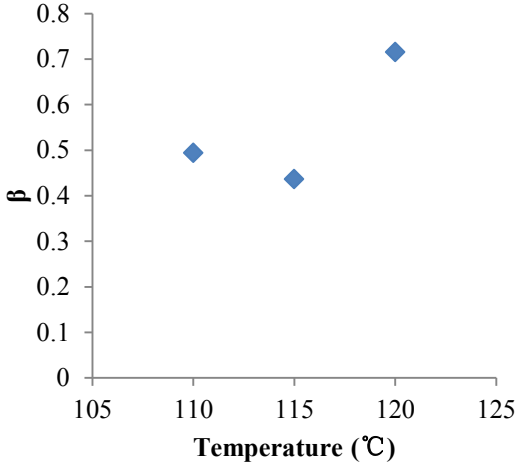
Appendix V Plots of β versus temperature (℃) for the Weibull model of *G. stearothermophilus* ATTC 10149



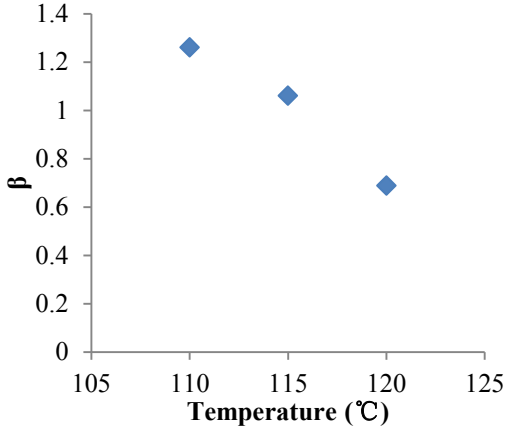
(a) Distilled water



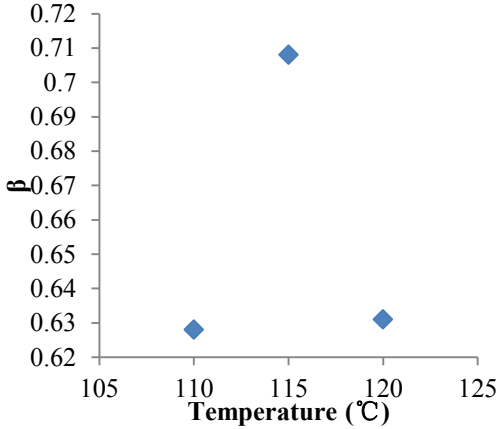
(b) Phosphate buffer at pH 7.0



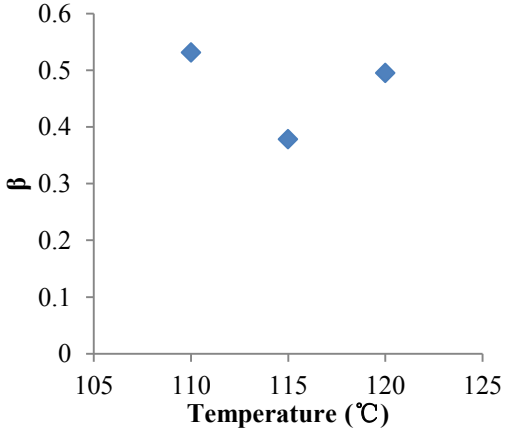
(c) McIlvaine buffer at pH 8.0



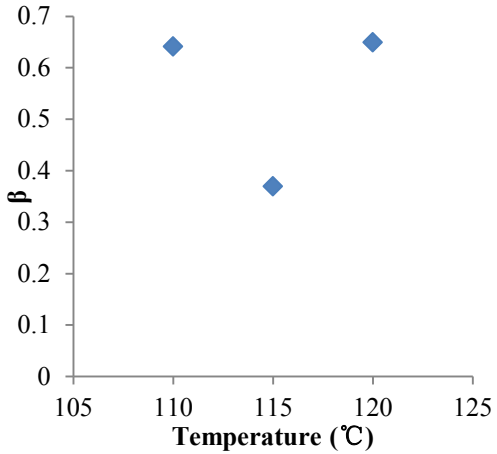
(d) McIlvaine buffer at pH 7.0



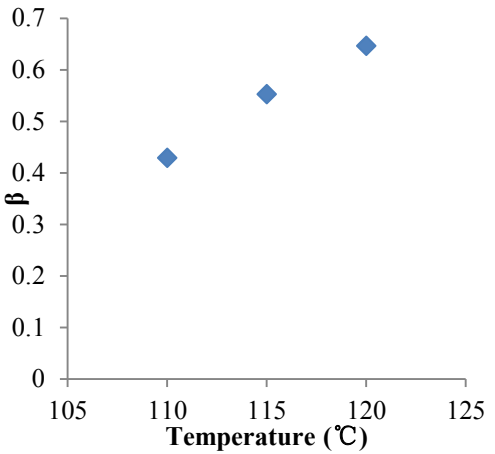
(e) McIlvaine buffer at pH 6.0



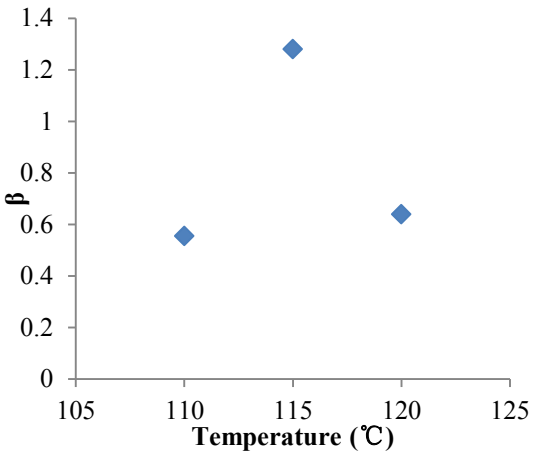
(f) McIlvaine buffer at pH 5.5



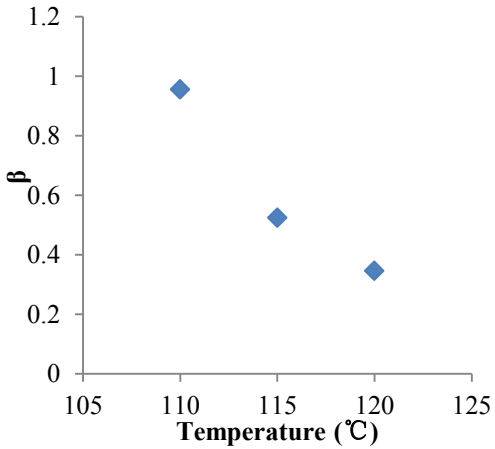
(g) McIlvaine buffer at pH 5.0



(h) McIlvaine buffer at pH 4.5



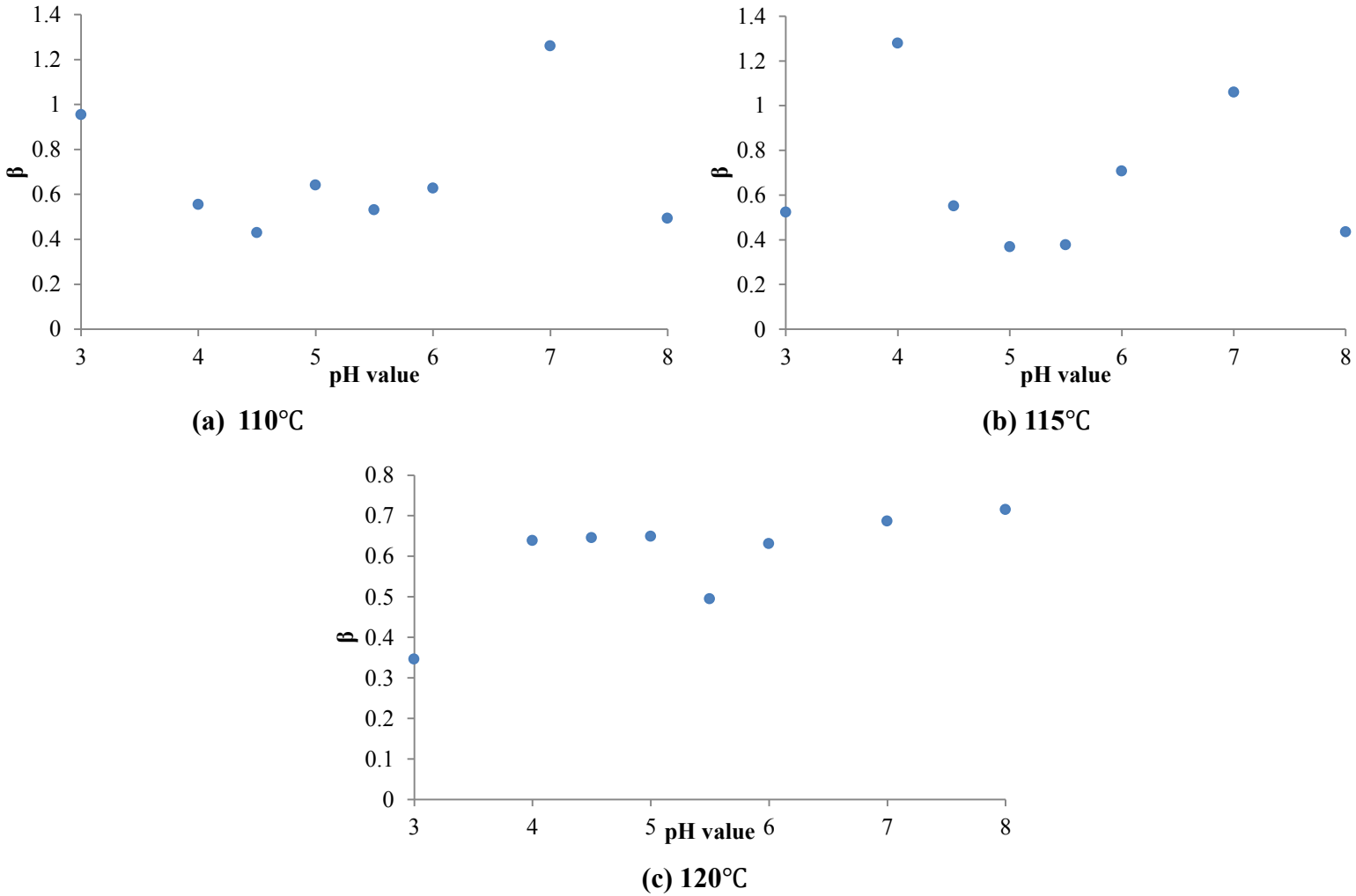
(i) McIlvaine buffer at pH 4.0



(j) McIlvaine buffer at pH 3.0

Figure E Plots of shape parameter β versus temperature (℃) for the Weibull model of thermal destruction of *G. stearothermophilus* ATTC 10149 in different heat medium

Appendix VI Plots of β versus pH value for the Weibull model of *G. stearothermophilus* ATTC 10149



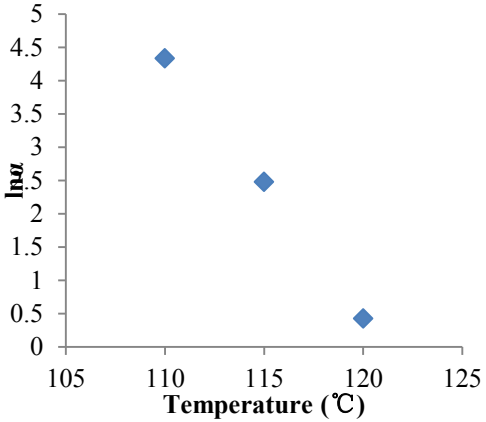
(a) 110℃

(b) 115℃

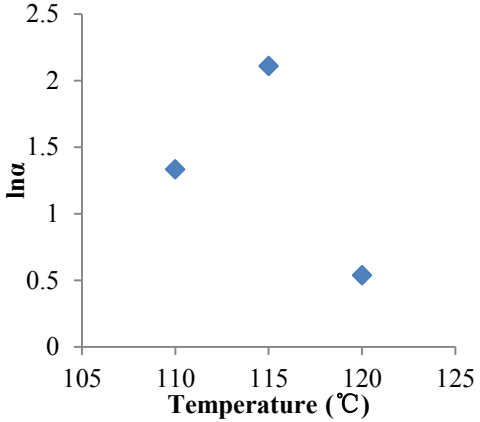
(c) 120℃

Figure F Plots of shape parameter β versus pH value for the Weibull model of thermal destruction of *G. stearothermophilus* ATTC 10149 in McIvaine buffer at different treatment temperature

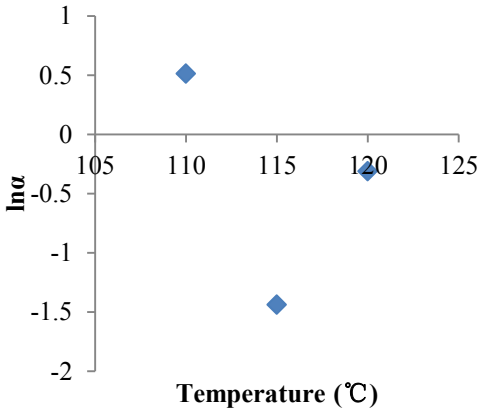
Appendix VII Plots of lnα versus temperature (℃) for the Weibull model of *G. stearothermophilus* ATTC 10149



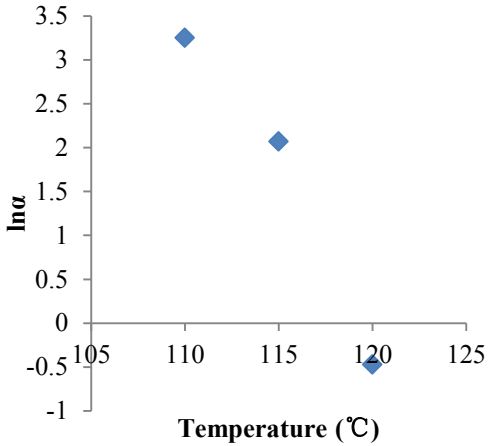
(a) Distilled water



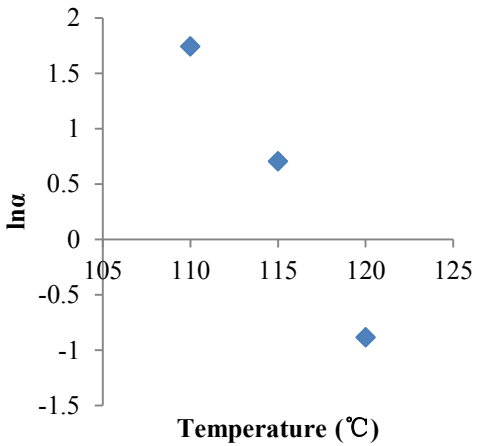
(b) Phosphate buffer at pH 7.0



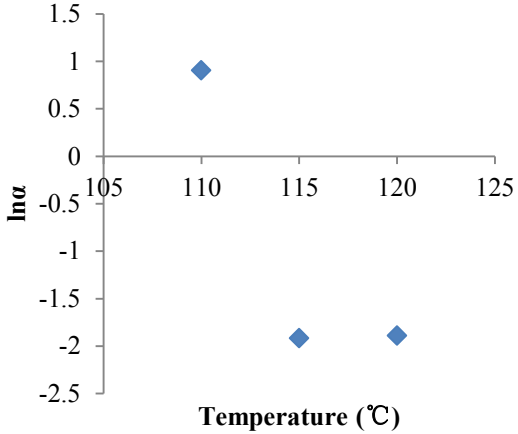
(c) McIlvaine buffer at pH 8.0



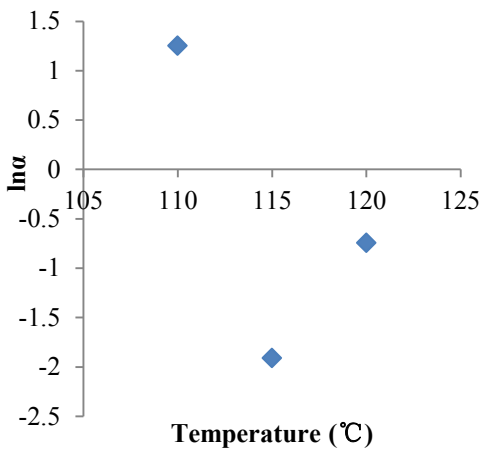
(d) McIlvaine buffer at pH 7.0



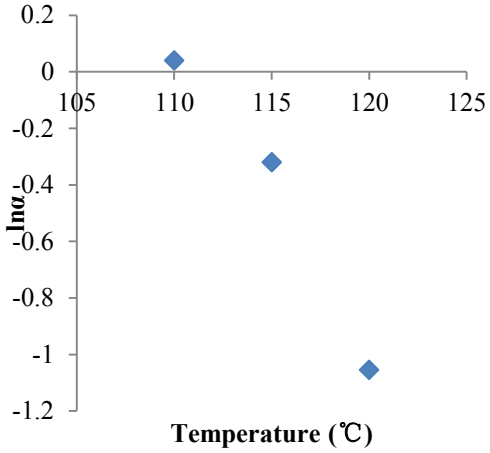
(e) McIlvaine buffer at pH 6.0



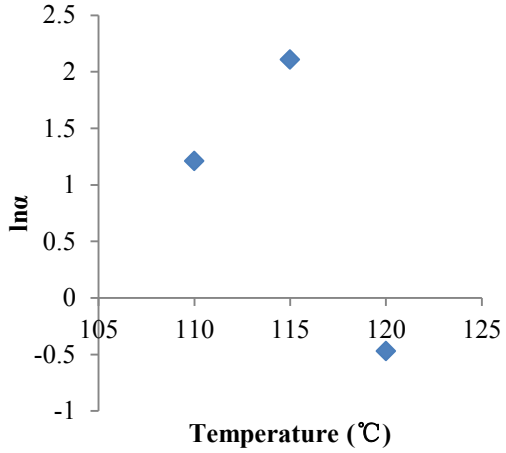
(f) McIlvaine buffer at pH 5.5



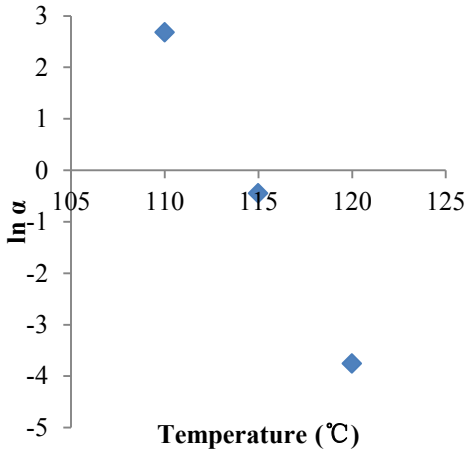
(g) McIlvaine buffer at pH 5.0



(h) McIlvaine buffer at pH 4.5



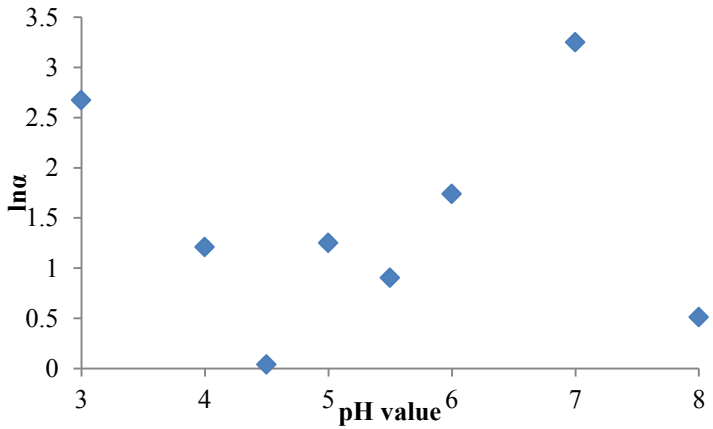
(i) McIlvaine buffer at pH 4.0



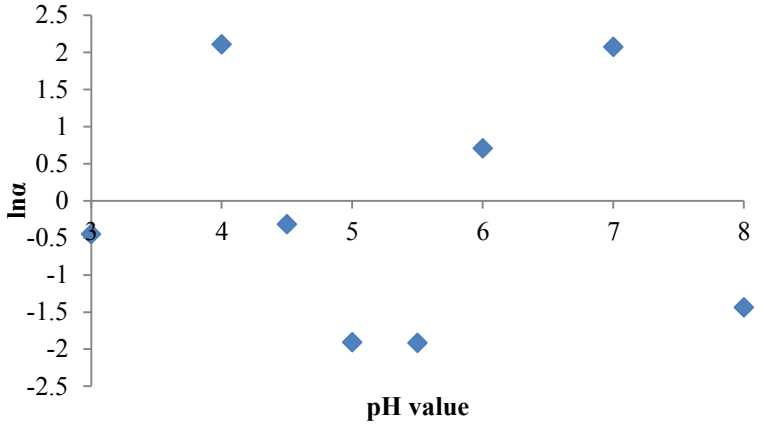
(j) McIlvaine buffer at pH 3.0

Figure G Plots of the natural logarithm of the scale parameter α versus temperature (℃) for the Weibull model of thermal destruction of *G. stearothermophilus* ATTC 10149 in different heat medium

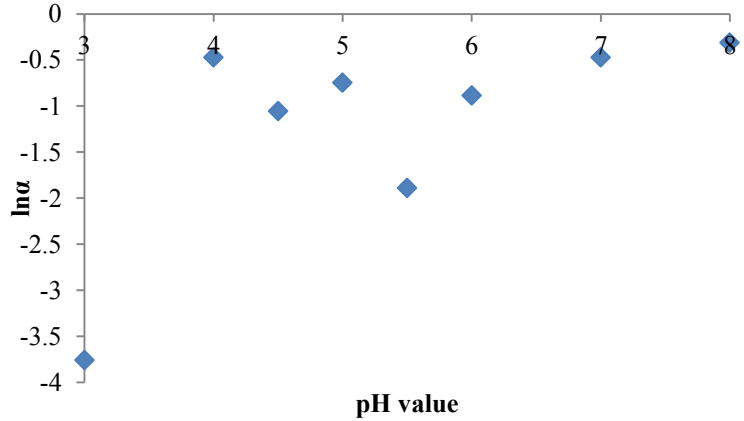
Appendix VIII Plots of lnα versus pH value for the Weibull model of *G. stearothermophilus* ATTC 10149



(a) 110℃



(b) 115℃



(c) 120℃

Figure H Plots of the natural logarithm of the scale parameter αversus pH value for the Weibull model of thermal destruction of *G. stearothermophilus* ATTC 10149 in McIvaine buffer at different treatment temperature