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Da-Wen Sun, Series Editor



ENGINEERING ASPECTS OF THERMAL FOOD PROCESSING

Edited by
RICARDO SIMPSON



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ENGINEERING ASPECTS OF THERMAL FOOD PROCESSING

Contemporary Food Engineering

Series Editor

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*This book is dedicated to my wife, Anita; family,
José Ignacio, María Jesús, Enrique; my beloved mother,
Carmen; and to the memory of my father, Jorge.*

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Series Preface

Food engineering is a multidisciplinary field of applied physical sciences combined with a knowledge of product properties. Food engineers provide technological knowledge essential to the cost-effective production and commercialization of food products and services. In particular, food engineers develop and design processes and equipment in order to convert raw agricultural materials and ingredients into safe, convenient, and nutritious consumer food products. However, food engineering topics are continuously undergoing changes to meet diverse consumer demands, and the subject is being rapidly developed to reflect the market needs.

For the development of the field of food engineering, one of the many challenges is to employ modern tools and knowledge, such as computational materials science and nanotechnology, to develop new products and processes. Simultaneously, improving food quality, safety, and security remain critical issues in food engineering study. New packaging materials and techniques are being developed to provide a higher level of protection to foods and novel preservation technologies are emerging to enhance food security and defense. Additionally, process control and automation are among the top priorities identified in food engineering. Advanced monitoring and control systems have been developed to facilitate automation and flexible food manufacturing. Furthermore, energy saving and minimization of environmental problems continue to be important food engineering issues and significant progress is being made in waste management, efficient utilization of energy, and the reduction of effluents and emissions in food production.

The *Contemporary Food Engineering* series consists of edited books and attempts to address some of the recent developments in food engineering. Advances in classical unit operations in engineering applied to food manufacturing are covered as well as such topics as progress in the transport and storage of liquid and solid foods; heating, chilling, and freezing of foods; mass transfer in foods; chemical and biochemical aspects of food engineering and the use of kinetic analysis; dehydration, thermal processing, nonthermal processing, extrusion, liquid food concentration, membrane processes, and applications of membranes in food processing; shelf life, electronic indicators in inventory management, and sustainable technologies in food processing; and packaging, cleaning, and sanitation. These books are intended for use by professional food scientists, academics researching food engineering problems, and graduate level students.

These books have been edited by leading engineers and scientists from many parts of the world. All the editors were asked to present their books so as to address market needs and pinpoint cutting-edge technologies in food engineering. Furthermore, all contributions have been written by internationally renowned experts who have both academic and professional credentials. All authors have attempted to

provide critical, comprehensive, and readily accessible information on the art and science of a relevant topic in each chapter, with reference lists provided for further information. Therefore, each book can serve as an essential reference source to students and researchers at universities and research institutions.

Da-Wen Sun
Series Editor

Preface

In the last 10 years, there has been a remarkable growth in research in the field of thermal processing, which indicates that the process is thriving and expanding all over the world. This book has been written with the intention of revising and updating the physical and engineering aspects of thermal processing of packaged foods.

Each chapter has been contributed by a renowned authority on a particular process and in this way the book covers all aspects of thermal processing. The book consists of four parts: (I) Fundamentals and New Processes, (II) Modeling and Simulation, (III) Optimization, and (IV) Online Control and Automation.

Part I consists of six chapters. Dr. Donald Holdsworth has written an outstanding introduction emphasizing the increased use of new packaging materials, including retortable pouches, and the use of containers made from other plastic composite materials. Dr. Silva and Dr. Gibbs have contributed the most complete and up-to-date chapter on pasteurization including a detailed account of the importance of *sous vide* processing. Chapter 3 has been written by top researchers from Unilever and deals with aseptic processing, a field which has expanded and developed in the last decade due to customer demand for better quality products. Chapter 4 is devoted to new and emerging technologies. This chapter is the result of collaboration among selected authors from academia and the industry. Traditional methods have been successful; however, limitations in heat transfer mean that this technology is not capable of providing convenient and high quality products. To overcome these limitations, methods using electromagnetism have been investigated and developed. The first part concludes with two excellent chapters on high-pressure processing by Dr. Gustavo Barbosa-Cánovas and coworkers. Chapter 5 discusses the principles behind four modeling approaches—analytical, numerical, macroscopic, and artificial neural networks—that can be used to predict temperatures in a high-pressure system. Chapter 6 highlights some applications of each modeling approach to high-pressure/low-temperature systems and high-pressure/high-temperature conditions reported in the literature.

Part II also consists of six chapters. Starting with this part, we have included two viewpoints on the crucial topic of thermal inactivation of microbial cells and bacterial spores. Due to the relevance of this subject in thermal food processing, we have asked the most prominent authors to collaborate on this work. Chapter 7 was written by Dr. Micha Peleg and coworkers and Chapter 8 was written by Dr. Arthur Teixeira and Dr. Alfredo Rodriguez. As the processing of heat-preserved foods in flexible pouches has gained considerable commercial relevance worldwide in recent years, Dr. Amézquita from Unilever and Dr. Almonacid from Chile cover the most important aspects of retortable pouch processing and mathematical modeling in Chapter 9. Although thermal processing, or canning, has proven to be one of the most effective methods of preserving foods while ensuring the product remains safe from harmful bacteria, it also has strong effects on the sensory characteristics of the product, such as color, texture, and nutritional value. In Chapter 10, Dr. Ramaswamy

and Dr. Dwivedi discuss rotary processing and how it can be used to overcome this difficulty. The last two chapters of this part deal with mathematical modeling. Chapter 11 has been written by Dr. Michele Chiumenti and coworkers and focuses on the mathematical modeling of ohmic heating as an emerging food preservation technology currently used by the food industry. Chapter 12 includes a comprehensive review of computational fluid dynamics and has been written by the well-known professor Da-Wen Sun and coworkers.

Part III consists of four chapters. The whole concept is to understand that mathematical optimization is the key ingredient for computing optimal operating policies and building advanced decision support systems. Chapter 13 on optimization has been contributed by Dr. Julio Banga and his outstanding team. This chapter deals not only with global optimization in thermal processing, but several food processes such as thermal sterilization, contact cooking, and microwave processing that can also be analyzed to find optimal operating procedures computed via global optimization methods. Chapter 14 proposes a new economic evaluation procedure to optimize the system design and operation of multiple effect evaporators compared to the traditional chemical engineering approach based on total cost minimization. Chapter 15 describes the optimization of in-line aseptic processing and demonstrates that it is essential for successful commercial exploitation. Chapter 16 analyzes plant production productivity, although an important problem in food processing, it has received little attention in thermal processing. This type of optimization, scheduling to maximize efficiency of batch processing plants, has become well known and it is commonly practiced in many processing industries.

Part IV consists of two chapters. Chapter 17 describes a practical and efficient (nearly precise, yet safe) strategy for online correction of thermal process deviations during retort sterilization of canned foods. In Chapter 18, authors from academia (Dr. Osvaldo Campanella) and industry (Dr. Clara Rovedo, Dr. Jacques Bichier, and Dr. Frank Pandelaers) analyze and discuss manufacturers' businesses in today's competitive marketplace. For such purposes, manufacturers must face challenges of increasing productivity and product quality, while reducing operating costs and safety risks. Traditionally, plant automation has been the main tool to assist the manufacturer in meeting those challenges.

Series Editor



Professor Da-Wen Sun was born in southern China and is a world authority on food engineering research and education. His main research activities include cooling, drying, and refrigeration processes and systems; quality and safety of food products; bioprocess simulation and optimization; and computer vision technology. His innovative studies on vacuum cooling of cooked meats, pizza quality inspection by computer vision, and edible films for shelf life extension of fruits and vegetables have been widely reported in national and international media. Results of his work have been published in over 180 peer-reviewed journal papers and in more than 200 conference papers.

Professor Sun received his first class BSc honors and MSc in mechanical engineering, and his PhD in chemical engineering in China before working in various universities in Europe. He became the first Chinese national to be permanently employed in an Irish university when he was appointed college lecturer at the National University of Ireland, Dublin (University College Dublin), Ireland, in 1995, and was then continuously promoted in the shortest possible time to senior lecturer, associate professor, and full professor. Sun is now Professor of Food and Biosystems Engineering and the director of the Food Refrigeration and Computerized Food Technology Research Group at University College Dublin.

As a leading educator in food engineering, Sun has contributed significantly to the field of food engineering. He has trained many PhD students, who have made their own contributions to the industry and academia. He has also given lectures on advances in food engineering on a regular basis at academic institutions internationally and delivered keynote speeches at international conferences. As a recognized authority in food engineering, he has been conferred adjunct/visiting/consulting professorships from 10 top universities in China including Zhejiang University, Shanghai Jiaotong University, Harbin Institute of Technology, China Agricultural University, South China University of Technology, and Jiangnan University. In recognition of his significant contribution to food engineering worldwide and for his outstanding leadership in the field, the International Commission of Agricultural Engineering (CIGR) awarded him

the CIGR Merit Award in 2000 and again in 2006. The Institution of Mechanical Engineers based in the United Kingdom named him Food Engineer of the Year 2004. In 2008 he was awarded the CIGR Recognition Award in honor of his distinguished achievements in the top one percent of agricultural engineering scientists in the world.

Professor Sun is a fellow of the Institution of Agricultural Engineers and a fellow of Engineers Ireland. He has also received numerous awards for teaching and research excellence, including the President's Research Fellowship, and has received the President's Research Award from University College Dublin on two occasions. He is a member of the CIGR executive board and honorary vice president of CIGR; editor-in-chief of *Food and Bioprocess Technology*—an international journal (Springer); former editor of *Journal of Food Engineering* (Elsevier); series editor of the *Contemporary Food Engineering* book series (CRC Press/Taylor & Francis); and an editorial board member for the *Journal of Food Engineering* (Elsevier), *Journal of Food Process Engineering* (Blackwell), *Sensing and Instrumentation for Food Quality and Safety* (Springer), and the *Czech Journal of Food Sciences*. He is also a chartered engineer.

Editor

Ricardo Simpson is currently working as a full professor at the Chemical and Environmental Engineering Department, Universidad Técnica Federico Santa María, Chile. He holds a biochemical engineering degree from the P. Universidad Católica de Valparaíso (PUCV, 1980), an MS in food science and technology (1990) and a doctorate in food science (1993) from Oregon State University, and a diploma in economics from the Universidad de Chile (1981). He lectured at PUCV from 1984 to 1999 and became a full professor in 1998. He was also a member of the Food Technology Study Group of CONICYT (equivalent to NSF).

Ever since Dr. Simpson obtained his PhD in 1993, he has been a prolific contributor to the food industry, not only in Chile, but also internationally (e.g., Unilever). His contributions have been summarized in more than 140 conference presentations and more than 50 refereed publications (as author or coauthor), thus advancing the understanding of many aspects of food engineering. He has also done extensive collaborative work with the Chilean food processing industry. He is one of the leading experts in the world in thermal processing of foods, having helped establish and improve food engineering programs at universities in Chile, Peru, and Argentina. He has presented short courses for the food industry in Costa Rica, Chile, Peru, and Argentina on energy conservation, thermal processing, and mathematical modeling applied to the food industry and also on project management. He has coplanned and codirected an international congress, the IV Ibero-American Congress in Food Engineering, and a national congress on food science and technology, both held in Valparaíso, Chile, in 1995 and 2003, respectively. He also planned and directed the national congress on mass and heat transfer held in Valparaíso, Chile, in 1996. He was vice president of the organizing committee of ICEF 10 (International Congress on Engineering and Food) held in Viña del Mar in April 2008. In recent years, he has published an average of eight refereed articles per year and has delivered several invited talks to international audiences. He has made outstanding contributions to engineering programs in education, research, development, consulting, and technology transfer that have resulted in improved food production, quality of life, and education for people living in Chile and Latin America. Recently, he completed a 4-month stay at Unilever's Food Research Center in Vlaardingen, and he was appreciated by the management for his work at its laboratory.

Dr. Simpson has consolidated his expertise as one of the leading experts in Latin America in thermal processing research (commercial sterilization of low-acid canned foods) in the last 3 years, and has been widely recognized in the international arena. Since 2002, he has published several manuscripts, patents, and book chapters on this field.

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Part I

Fundamentals and New Processes

1 Principles of Thermal Processing: Sterilization

S. Donald Holdsworth

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1.1 INTRODUCTION

The objective of thermal processing of food products, which involves heating and cooling, is to produce a shelf-stable product, which is free from pathogenic organisms and will not produce food spoilage. The primary necessity is to destroy microorganisms capable of growing in the product and to prevent further spoilage by suitable packaging. In the conventional canning process, which uses a wide range of packaging materials, including tinplate, aluminum, glass, plastics, and composites, the filled and sealed containers are subjected to a heating and cooling regime. Alternatively, continuous flow heat exchangers can be used and the product packaged under aseptic conditions. The heating and cooling regime is known as the *process* and this chapter is concerned with the determination and validation of a process for a specific product, packed in a particular container size and heat processed in a given type of pressurized retort. The heating medium may involve steam, steam/air mixtures, or hot water, and the cooling medium is primarily water. The technology of canning is not discussed here but is detailed in numerous texts (see, e.g., Lopez, 1987; Fellows, 1990; Rees and Bettison, 1991; Brennan et al., 1992; Larousse and Brown, 1997; Ramaswamy and Singh, 1997). Current developments in the technology of in-container sterilization are fully discussed by Richardson (2001, 2004).

The operation of inactivating microorganism is generally referred to as *sterilization*, although it is not the same as the medical operation which involves the complete removal of microbial species. There is generally no need to remove thermophilic organisms, which have no public health significance and the process is described as *commercial sterilization*. The only requirement is that the products are not stored at a temperature in excess of 32°C, when the microorganisms will germinate causing product spoilage. This also requires products to be cooled rapidly after processing. If the ambient temperature of storage exceeds this temperature, e.g., hot climate countries, then it will be necessary to submit the product to a more severe process.

An important factor on deciding the severity of a process is the pH of the product, which may vary from neutrality pH 7 to acidic about pH 2.8. The food poisoning microorganism *Clostridium botulinum* and many other types of sporing and nonsporing bacteria are inhibited from growth at pH 4.5 (or slightly higher <4.7). Consequently this figure is often taken as the dividing line between the requirements of a mild process, e.g., pasteurization, 100°C and a more severe process, often known as a *botulinum process* involving temperatures of 118°C–125°C. The main classes of acidic products are fruit-based and preacidified products and these are discussed in Chapter 2.

It is possible to identify at least four groups of products:

- Group 1: Low-acid products (pH 5.0 and above)—meat products, marine products, milk, some soups, and most vegetables
- Group 2: Medium-acid products (pH 4.5–5.0)—meat and vegetable mixtures, specialty products, including pasta, soup, and pears
- Group 3: Acid products (pH 3.7–4.5)—tomatoes, pears, figs, pineapple, and other fruits
- Group 4: High-acid products (pH 3.7 and below)—pickles, grapefruit, citrus juices, and rhubarb

The bulk of food products are in the class requiring a sterilization process, e.g., meat, fish, and vegetables. This is a generalization and there are products which come on the dividing line, e.g., tomatoes and pears, and depend on the variety and maturity. For products in this pH region, it is necessary to conduct extensive trials to establish that food poisoning organisms are inhibited. Similarly for formulated products, it is necessary to examine the inhibitory effects of the ingredients.

Another factor that must be taken into account is the initial microbial loading of the product. This may be controlled by paying attention to handling and preparation procedures and hygiene conditions.

So far no consideration of the effect of heat processing on the food product has been mentioned; however, it is important to consider nutrient destruction, loss of vitamin potency, and overall quality deterioration. These will be affected but the duration and severity of the process, consequently, there is a need to determine an *optimum process* that delivers the necessary sterilization requirements and minimizes the quality degradation.

1.2 KINETICS OF THERMAL PROCESSING

1.2.1 MICROBIAL DESTRUCTION

The engineering design of a process requires a quantitative measure of the effect of temperature and duration time on the destruction of microorganisms. It is usually considered that microbial death can be represented by a first-order kinetic equation, i.e., the destruction rate is proportional to the concentration c of microorganisms (Equation 1.1)

$$-dc/dt = kc \quad (1.1)$$

where

t is the time

k is the reaction rate constant with units of reciprocal time

This can be integrated to give Equation 1.2 which expresses the concentration at any time t , where c_0 is the concentration at time zero.

$$c/c_0 = e^{-kt} \quad (1.2)$$

The value of k can be determined from the van't Hoff isochore equation (Equation 1.3).

$$k = Ae^{-E/RT} \quad (1.3)$$

This is usually known as the Arrhenius model for microbial inactivation, where A is the pre-exponential factor, E is the activation energy, and R the universal gas constant. It is usual to specify a reference temperature and the corresponding k -value being k_{ref} .

The traditional approach to this is slightly different and is based on the number, N , of microorganisms at time t . Thus, if the logarithm of the number of spores is plotted against time a semilinear plot is obtained with a negative slope. This has an intercept N_0 and a slope of $-1/D$, where D is called the decimal reduction time of the microbial species, usually a highly heat-resistant spore. This can be represented by Equation 1.4.

$$\log N = \log N_0 - t/D \quad (1.4)$$

The two approaches are very similar in the temperature range around the figure of 120°C. The D -value is usually quoted in minutes and the k -value in seconds; hence $D = 2.3/60k$. Most spore survival curves are not linear but show *shoulders* and *tails* and many equations have been developed to deal with these curves and many theories discussed for their occurrence. A summary of some alternative models for microbial inactivation is given by Holdsworth and Simpson (2007), and also in Chapters 7 and 8.

The log D -value when plotted against temperature T usually shows a linear relation and in order to compare differing organism it is necessary to use a reference

temperature T_{ref} , e.g., 250°F or 121.1°C corresponding to D_{ref} . Using this to define the thermal death relationship results in Equation 1.5

$$\log(D/D_{\text{ref}}) = -(T - T_{\text{ref}})/z \quad (1.5)$$

where z is the temperature change necessary to change the D -value by 1 log-cycle, i.e., by a factor of 10.

Using the Arrhenius model outlined briefly above the z -value is given by Equation 1.6

$$z = 2.303RTT_{\text{ref}}/E \quad (1.6)$$

Having established the necessary kinetic functions, either k and E or D and z these can be used for determining the times and temperatures for a satisfactory process (see Section 1.3). Specific values for these factors have been determined for a wide range of microorganisms in a variety of media and food products (see Holdsworth and Simpson, 2007).

For *C. botulinum* spores, k can reliably be determined using $A = 2 \times 10^{60} \text{ s}^{-1}$ and $E = 310.11 \times 10^3 \text{ J mol}^{-1} \text{ K}^{-1}$ using the Arrhenius approach and $z = 10^\circ\text{C}$ and $D_{121.1} = 0.3 \times 60 \text{ s}$ using the conventional canning approach.

More complex models to represent the thermal death of microorganisms, especially the effect of pH, have been established. These models which have a linear form are shown in Equation 1.7

$$\ln k = C_0 + C_1 T^{-1} + C_2 \text{pH} + C_3 \text{pH}^2 \quad (1.7)$$

For *C. botulinum*, in spaghetti/tomato sauce, the values of the constants were $C_0 = 105.23$, $C_1 = -3.704 \times 10^4$, $C_2 = -2.3967$, and $C_3 = 0.1695$ (Davey et al., 1995, 2001).

1.2.2 KINETICS OF FOOD QUALITY DESTRUCTION

The effect of heat on the constituents of foods is generally deleterious to the overall quality. These include the degradation of vitamins, the softening of texture, loss of color, development of off-flavors, and destruction of enzymes. Some of these are desirable, e.g., enzyme inactivation fruits and vegetables and softening of texture in meat and fish products. All these reactions, chemical or physical, have different kinetics to microbial inactivation. Bacterial spores have z -values between 7°C and 12°C, whereas other constituents have values up to 50°C. This means that if high processing temperatures are used for short times (usually referred to as high temperature short time [HTST]-processes) there will be less destruction of thermolabile components and conversely for longer processes there will be greater loss. Milk is a typical example of a highly thermolabile product and consequently benefits from HTST processing. This is usually achieved in a continuous flow process followed by aseptic packaging.

1.3 PROCESS DETERMINATION

1.3.1 HEAT PENETRATION F - AND J -FACTORS

Establishing times and temperatures for processing of packaged foods depends on evaluating the amount of heat the product has received. The uniformity of heating will depend on the consistency of the product, for example, food products which are thick tend the heat by conduction whereas fluid products heat by convection. This means that in conduction-type packs the outer layers will heat more rapidly than the center of the container, whereas fluid products will heat more uniformly. The main objective is to ensure that the slowest heating part of the food in a container receives the minimum process necessary to achieve a sterilized product. It is for this reason that in-container heat penetration experiments are performed to establish the necessary process. This is achieved by placing a thermocouple at the point of slowest heating (often referred to as the critical point) and observing the temperature–time profile. This is plotted in the form of a log temperature/linear time plot and is known as a heat penetration curve. The slope of this curve gives the rate of heat penetration f -value (f_h for heating and f_c for cooling) and the intercept gives the j -value (with similar designations). These two values are the most important factors for describing the process characteristics. The f -value depends on the thermal diffusivity of the product and the container dimensions and can be determined by calculation (Ball and Olson, 1957; Holdsworth and Simpson, 2007). The j -value is known as the lag factor and its value depends on the position inside the container. While this applies to conduction packs, convection packs have much lower f -values, i.e., heat much more rapidly, and are related to the ratio of the can surface area to the volume of the container.

The simple division of heating regimes into conduction and convection is an idealized situation. In practice there are systems which heat by convection initially and as the product thickens conduction heating characteristics are shown and vice versa. The graphs from this type of behavior are known as broken-heating curves. Usually there is a distinct break between the two sections of the graph and the corresponding values for heating f_1 and f_2 can be determined.

1.3.2 CRITERIA FOR ADEQUACY OF PROCESSING F - AND C -VALUES

The most important factor in food product sterilization is to be able to quantify the effect of the heating and cooling regime and establish that a given process is able to give a safe product. The universally agreed method of evaluating a process is based on the heat penetration curve and the use of lethal rates.

A measure of the lethal effect of heat on microorganism inactivation is the lethal rate L in minutes (Equation 1.8)

$$L = 10^{(T - T_{\text{ref}})/z} \quad (1.8)$$

The basis of process evaluation is that lethalties are additive and the total lethality can be determined by converting the heat penetration curve into a lethality–time curve and integrating the area under the curve. The total lethality for the process is known as the F -value and can be determined from Equation 1.9

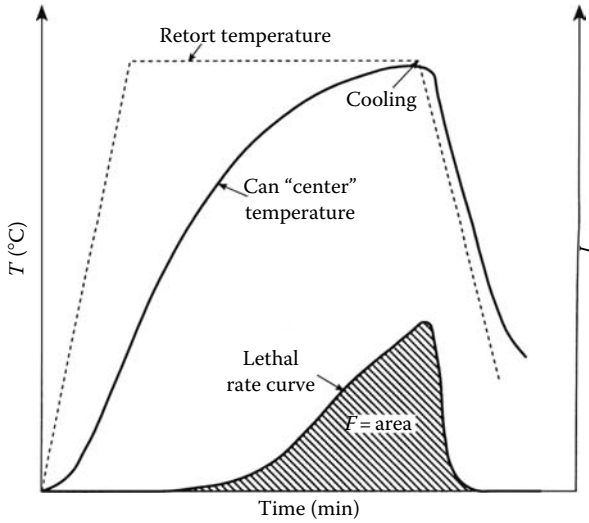


FIGURE 1.1 Graph of temperature (T) and lethal rate (L) against time.

$$F_{T_{\text{ref}}}^z = \int_0^t L dt = 1 \quad (1.9)$$

This is illustrated in Figure 1.1.

When the reference temperature is 250°F or 121.1°C, the F -value is designated as F_0 (known as F nought). The corresponding values of z are 18°F or 10°C. However for processes not involving a botulinum process, e.g., heat destruction of yeast spores in beer the F -value would have adscripts indicating the z -value and the reference temperature, e.g., $F_{212}^{12.5}$ or $F_{100}^{7.0}$.

It is often more convenient for pasteurization studies to use the pasteurization unit (PU) which is defined in the same way as a lethal rate; however, there is no agreed reference temperature and consequently this must be stated in every case, along with the appropriate z -value.

For continuous flow sterilizers used in aseptic processing operations the microbial destruction N/N_0 can be estimated from Equation 1.10

$$\frac{N}{N_0} = (2\pi/Q_v) \int_0^r r v x dr \quad (1.10)$$

where

$$x = 10^{-(l/v)D_{\text{ref}}}$$

Q_v is the volumetric flow rate

r is the radius of the tube

v is the fluid velocity

l is the length of tube

Solutions of this equation for various systems are summarized in Holdsworth (1992) and Lewis and Heppel (2000).

While it is convenient to determine the F -value from the area under the curve or by addition of the lethalties at equal time intervals, it is possible to use the theoretical equations for the temperatures and times derived from analytical equations (see Holdsworth and Simpson, 2007). A number of computer programs are available for calculating process, the most recent having been developed by Simpson and now available on a computer disk (see Holdsworth and Simpson, 2007).

By analogy it is possible to define a C -value which will give a measure of the deterioration of any chemical or physical property of the food provides appropriate z_c -values are available (see Equation 1.11) where

$$C = \int_0^t 10^{(T-T_{\text{ref}})/z} dt \quad (1.11)$$

The original concept was developed by Mansfield (1962, 1974) and was first applied to determining the degree of cooking of a product.

While heat penetration studies are based at the point of slowest heating, C -values at this point are not relevant. This has led to the use of C_s -value which is a mass-average value for the whole of the container (see Equation 1.12)

$$C_s = D_{\text{ref}} \log(c/c_0) \quad (1.12)$$

where c is the concentration of the heat vulnerable component at times 0 and t . The reference temperature for cooking studies is usually taken as 100°C.

The value for C_s may be obtained using Equation 1.13.

$$c/c_0 = \frac{1}{V} \int_0^V 10^{-C_c/D_{\text{ref}}} dV \quad (1.13)$$

where

$$C_c = \frac{1}{V} \int_0^t 10^{(T-T_{\text{ref}})/z_c} dt \quad (1.14)$$

For a more complete study of this subject, see Tucker and Holdsworth (1991) and Holdsworth and Simpson (2007).

1.4 OPTIMIZATION OF STERILIZATION AND COOKING

The fact that chemical and microbiological destruction kinetics differ leads to an important requirement for optimized processes (see Chapter 16). The current trend is to try to preserve the nutrients and flavors of food products by using techniques which reduce the heating load on the product and consequently increase the quality.

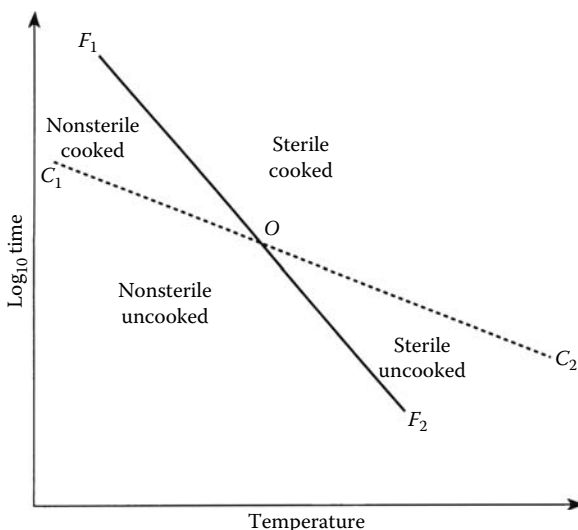


FIGURE 1.2 Diagram of idealized \log_{10} time versus temperature for microbial inactivation (F_1OF_2) and cooking (C_1OC_2) of food product, instantaneously heated.

The differing kinetic factors for nutrients and chemical species result in the need for optimization of heating conditions (see Section 1.2.2). The results of the incompatibility can be seen in Figure 1.2, where a log time versus temperature graph for an idealized situation of instantaneous heating of a food product to a given F -value is shown. Considering the sterilization line F_1OF_2 , all time–temperatures on the right of the line will represent processing conditions which result in sterile product whereas all conditions to the left will be nonsterile. When the corresponding cooking line is plotted, the graph shows four product regions only two of which represent conditions which will result in a sterilized product. Processing at relatively high temperatures for a relatively short time will therefore result in maximum nutrient retention.

Various methods of calculating the process required to achieve optimization are discussed in Chapters 13, 15, and 16 (see also Tucker and Holdsworth, 1991; and Holdsworth, 2004).

1.5 ESTABLISHING SAFE CRITERIA HEAT-PROCESSED FOODS

For low-acid foods ($\text{pH} \geq 4.5$) the most important criteria for ensuring the safety of heat-processed foods is that a minimum process must reduce the probability of survival of spores of *C. botulinum* to less than one spore in 10^{12} containers. A minimum process is usually taken as one which achieves an $F_0 = 3$ min; in practice, processes are usually higher than this (6–10 being typical) either for controlling spoilage organisms or achieving the correct degree of texture softening.

The most definitive sources for assessing whether a process is suitable for a particular product are those produced by the National Food Processors' Association in the United States (NFPA, 1971, 1982), which apply to low-acid products. However, Brown

(1991) has discussed a number of other criteria arising from European countries, in particular the statutory requirements for the processing of milk and milk products. Special regulations apply to the canning of cured meats where a salt or sodium nitrite is added, and other products which contain added microbial inhibitors.

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2 Principles of Thermal Processing: Pasteurization

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2.1 INTRODUCTION

Thermal pasteurization is a classical method of food preservation which extends the shelf life by inactivating vegetative cells of unwanted pathogenic and spoilage microorganisms with processing temperatures normally between 65°C and 95°C. This traditional physical process of food decontamination is still in common use today, being efficient, environmentally friendly, healthy, and inexpensive when compared with other technologies. As opposed to sterilization, the temperatures used are lower, allowing greater retention of the original properties of the raw food. A further step towards better quality can be achieved if pasteurization is used in combination with nonthermal food preservation methods such as the use of refrigerated distribution and storage (1°C–8°C), vacuum or modified atmosphere packaging, added preservatives, etc. This would allow the production of safe foods while minimizing the degradation of the “fresh” organoleptic and nutritive quality of the foods. Typical pasteurized foods include beverages such as milk, fruit juices, beer, low carbonated drinks, dairy products (e.g., cheese), meat and fish products (e.g., cured, cooked ham, hot-smoked fish), some sauces, pickles, and food ingredients. This chapter covers the pasteurization fundamentals, followed by a review focused on the heat resistance of relevant microbes in pasteurized foods, and finishes with a short section about the design of pasteurization processes for different types of foods.

2.2 FOOD PASTEURIZATION FUNDAMENTALS

This section presents the historical origin, definitions, and objectives of the pasteurization process. Also, the concept of quality and optimization of the process will be introduced, and finally, relevant equations used to model the inactivation of microbial and food-derived deteriorative enzymes, and the impact of the heat process on quality factors will be described and discussed.

2.2.1 HISTORICAL BACKGROUND AND DEFINITIONS OF FOOD PASTEURIZATION

The first investigations on pasteurization were carried out in 1765 by Spallanzani. He used a heat treatment to delay spoilage and preserve meat extract. From 1862 to 1864, Pasteur showed that temperatures of 50°C–60°C for a short time effectively eliminated spoilage microorganisms in wine. Pasteur (1876) also investigated beer spoilage. When milk producers adopted this process (Soxhlet, 1886; Davis, 1955; Westhoff, 1978), they were able to eliminate most of the foodborne illnesses. The main goal of pasteurization of low-acid chilled foods is the reduction of pathogens responsible for foodborne

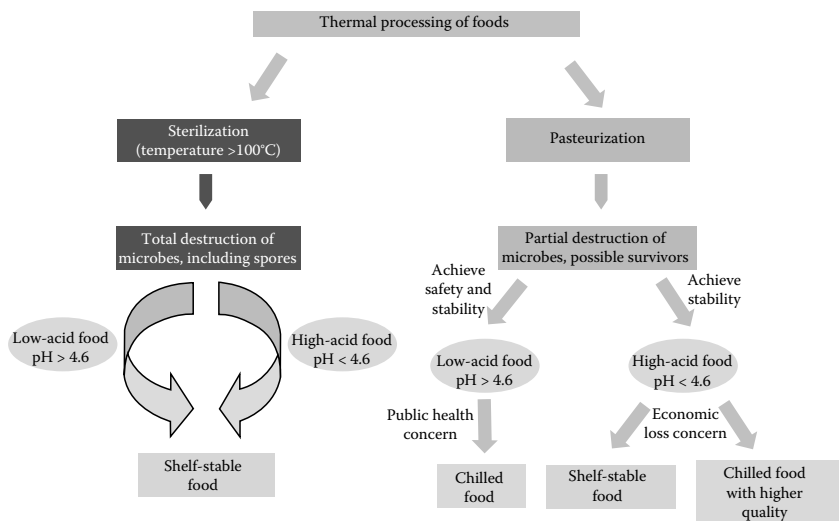


FIGURE 2.1 Thermal processing of foods.

illness and human disease, whereas in the case of high-acid foods, pasteurization is intended to avoid spoilage and economic losses (Figure 2.1).

2.2.1.1 Classical Definition of Food Pasteurization

The word “pasteurization” has its origin in the work of the French scientist Louis Pasteur, and refers to a mild heat treatment (50°C–90°C) used for food preservation, which aims to inactivate vegetative forms of pathogenic and spoilage microorganisms. Unlike sterilization, after pasteurization the food is not sterile since heat-resistant microbial spores are present (Lund, 1975a) (Figure 2.1). Therefore, other forms of preservation such as refrigeration (e.g., milk), atmosphere modification (e.g., vacuum packaging), addition of antimicrobial preservatives (e.g., salt, citric acid, benzoic acid, sorbic acid, sulphur dioxide, dimethyl dicarbonate, etc.), or combinations of the referred techniques are required for product stabilization and distribution. Exceptions are some processed foods that contain constituents or ingredients that are antimicrobial under certain conditions, not allowing microbial growth: fermented foods containing alcohol or acid (e.g., wine, beer, pickles), carbonated drinks (e.g., sodas), very sweet foods presenting $a_w < 0.65$ or soluble solids $> 70^\circ\text{Brix}$ (e.g., honey, jams, jellies, dried fruits, fruit concentrates), or salty foods (e.g. salted fish or meats). Other exceptions include the high-acid foods ($\text{pH} < 4.6$), which are stable at ambient conditions after a pasteurization process, because the acidic food environment is not conducive to the growth of harmful microorganisms and microbial spores in the pasteurized food. For these type of foods ($\text{pH} < 4.6$), a pasteurization process allows a long shelf-life (several months) at room temperature (Ramaswamy and Abbatemarco, 1996), and if refrigerated storage is used, a milder pasteurization may be applied and product quality is improved (Figure 2.1). If a food product has low acidity ($\text{pH} > 4.6$, e.g., milk), a shorter shelf-life (several days) is obtained after

pasteurization, but refrigerated storage is necessary to maintain product safety during storage, by restricting the growth of surviving pathogens (e.g., sporeformers) in the foods (Potter, 1986; Fellows, 1988; Adams and Moss, 1995).

2.2.1.2 Modern Definition of Food Pasteurization

Pasteurization was recently redefined by the U.S. Department of Agriculture as “any process, treatment, or combination thereof, that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage” (NACMCF, 2006). This definition therefore includes nonthermal pasteurization processes such as high pressure (HP). Thermal processing, which is the application of heat to foods, is the oldest method of pasteurization. More recently, the effects of nonthermal pasteurization, such as high intensity pulsed electric fields and HP on microorganisms and foods, have been investigated (Hite, 1899; Doevenspeck, 1961; Hülshager et al., 1981; Lehmann, 1996; Hendrickx and Knorr, 2001). Nevertheless, the efficacy of HP in terms of spore (Lee et al., 2006) and enzyme inactivation is limited (Raso and Barbosa-Cánovas, 2003; Van Buggenhout et al., 2006). In fact, HP was responsible for stimulating germination of *Talaromyces macrosporus* mold spores (Dijksterhuis and Teunissen, 2003). Thus, HP treatments followed by thermal processing have been proposed in order to inactivate the spores (Heinz and Knorr, 2001; Raso and Barbosa-Cánovas, 2003). With respect to combinations of heat and pressure for the destruction of *Clostridium botulinum* spores, concerns were expressed by Margosch et al. (2006) who demonstrated higher spore survival when using temperature and HP treatments simultaneously in comparison to the exclusive use of temperature.

2.2.2 DESIRABLE AND UNDESIRABLE CHANGES IN FOODS WITH THE APPLICATION OF HEAT: QUALITY OPTIMIZATION OF THE PROCESS

Thermal pasteurization, intended to inactivate pathogens and deteriorative microorganisms/enzymes, may also affect negatively the quality of foods (Lund, 1975b; Villota and Hawkes, 1986). Thus the heat application should be minimal and well balanced, being enough for food decontamination, while enabling maximum retention of the original food quality (Ramaswamy and Abbatemarco, 1996). The food color, aroma, flavor, and texture, readily perceived by food consumers, and nutritive/health value, are generally recognized as the major quality factors of foods, being used for quality optimization of the process. Knowing that various conditions of heating temperature (T) and time (t) lead to similar effects on microbial/enzymatic inactivation, a process that causes less impact on quality factors can be selected (Silva and Silva, 1997; Silva et al., 2003). Such optimization is possible because the thermal degradation kinetics of quality factors is much less temperature sensitive than the destruction of microorganisms (Teixeira et al., 1969; Holdsworth, 1985). The higher the pasteurization temperature applied, the shorter the time needed for the same microbial inactivation.

Most of the changes that occur in food as a result of pasteurization can be quantified with first-order kinetics (see Section 2.2.3.1), the z -value being a measure of the effect of temperature on the destruction rate of microbial/enzymatic/quality factors.

Approximate z -values of various thermally dependent factors were collected from the literature: vegetative bacteria, $z = 3.5^{\circ}\text{C}$ – 9.4°C (Table 2.3); fungal ascospores, $z = 5.2^{\circ}\text{C}$ – 9.2°C (Table 2.5); bacterial spores, $z = 4.2^{\circ}\text{C}$ – 15°C (Tables 2.1, 2.2, 2.4, and 2.6); enzymes, $z = 10^{\circ}\text{C}$ – 22°C (Table 2.7); flavor/odor, $z = 13^{\circ}\text{C}$ – 50°C (Ohlsson, 1980; Argáiz and López-Malo, 1995; Silva et al., 2000a); color, $z = 20^{\circ}\text{C}$ – 74°C (Sanchez et al, 1991; Silva and Silva, 1999); vitamin C, $z = 44^{\circ}\text{C}$ – 72°C (Silva and Silva, 1997); green olives texture, $z = 63^{\circ}\text{C}$ (Sanchez et al., 1991). The z -values of quality factors (13°C – 72°C) are, in general, higher than those found for microorganisms/enzymes (5°C – 19°C). Thus the use of high temperature for short time causes a larger increase in the rate of microbial/enzyme inactivation than in the degradation rate of quality factors (Lund, 1975b). This is the basis for high-temperature short-time processing (HTST; 71.7°C for 15 s) commonly used for milk pasteurization.

2.2.3 EQUATIONS FOR PROCESS DESIGN AND ASSESSMENT

Kinetic models are useful tools for the quantification of thermal inactivation of microorganisms or enzymes, and also quality changes. Classical equations to model isothermal microbial survivor curves will be presented in this section, although more recently some authors have demonstrated how thermal inactivation data can also be collected from nonisothermal experiments (Welt et al., 1997; Peleg and Pechina, 2000). Equations to assess process impact will also be presented. Finally, a brief overview of other models describing phenomena of “shoulders” and “tails” in microbial inactivation curves will be discussed.

2.2.3.1 Bigelow or First-Order Kinetic Models

Chemical reaction kinetics is used to describe the microbial/cell thermal inactivation. The change/deterioration of most food factors with isothermal time exposure follows zero- (Equation 2.1) or first-order (Equations 2.2 through 2.4) reaction kinetics (Villota and Hawkes, 1992). Simple first order can be described either by Equation 2.2 or, when dealing with microorganisms which also exhibit log-linear spore inactivation kinetics, by the Bigelow model (Equation 2.3; Bigelow and Esty, 1920; Teixeira, 1992). First-order reversible (or fractional) reaction kinetics (Equation 2.4) has also been observed for some quality factors.

$$\frac{F}{F_0} = -k_T \times t \quad (2.1)$$

$$\frac{F}{F_0} = e^{-k_T \times t} \quad (2.2)$$

$$\frac{F}{F_0} = 10^{-(t/D_T)} \quad (2.3)$$

$$\frac{F - F_{\infty}}{F_0 - F_{\infty}} = e^{-k_T \times t} \quad (2.4)$$

where

F is the microbial/enzyme/quality factor

F_0 and F_∞ are the values of the factor at time zero and infinite time, respectively

k_T is the reaction rate at temperature T (min^{-1})

D_T is the decimal reduction time at temperature T (min)

t is the time (min)

The temperature effect on the reaction rate constant, k_T , is described by the Arrhenius equation (Equation 2.5). The Bigelow model (Equation 2.6) can also be used for first-order kinetics (Saguy and Karel, 1980; Wells and Singh, 1988).

$$k_T = k_{T_{\text{ref}}} \times e^{-\left[\frac{E_a}{R} \times \left(\frac{1}{T+273.15} - \frac{1}{T_{\text{ref}}+273.15}\right)\right]} \quad (2.5)$$

where

$k_{T_{\text{ref}}}$ is the reaction rate at reference temperature (min^{-1})

T_{ref} is the reference temperature ($^{\circ}\text{C}$)

E_a is the activation energy (J/mol)

R is the universal gas constant (8.31434 J/mol/K)

$$D_T = D_{T_{\text{ref}}} \times 10^{\left(\frac{T_{\text{ref}}-T}{z}\right)} \quad (2.6)$$

where

$D_{T_{\text{ref}}}$ is the decimal reduction time at a reference temperature (min)

z is the number of degrees Celsius required to reduce D by a factor of 10 ($^{\circ}\text{C}$)

2.2.3.2 Nonlinear Survival Curves

Although log survivors vs time thermal inactivation kinetics is widely assumed to be linear, deviations from linearity (e.g., shoulder, tail, sigmoidal-like curves, biphasic curves, concave and convex curves) have been reported and remain unexplained, in particular with vegetative pathogens such as *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus* (Chiruta et al., 1997; Juneja et al., 1997; Juneja and Marks, 2005; Valdramidis et al., 2006; Buzrul and Alpas, 2007). The observation of tails in the inactivation of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in milk was explained by cell clumps rather than the existence of a more heat-resistant cell fraction (Klijn et al., 2001). With respect to microbial spore thermal inactivation, a log-linear behavior is commonly observed. A few exceptions have been reported in the literature, *Bacillus sporothermodurans* spores exhibited an upper concavity (Periago et al., 2004). Tails were also observed in the heat inactivation of prions (Periago et al., 2003). The added complexity of new models might not be worth using in terms of microbial survival predictions (Halder et al., 2007), in particular spore-forming microbes.

2.2.3.3 Process Design and Assessment

During the process, the integrated lethality at a single point within the food container, also known as pasteurization value (P), is the equivalent time of pasteurization at a certain temperature (T_{ref}) expressed in minutes (Equation 2.7) (Shapton, 1966):

$$P = \int_0^{PT} 10^{\left(\frac{T-T_{\text{ref}}}{z}\right)} dt \quad (2.7)$$

where

P is the pasteurization value (min)

PT is the total process time (min)

T_{ref} is the reference temperature for the pasteurization target ($^{\circ}\text{C}$)

z is the z -value for the pasteurization target ($^{\circ}\text{C}$)

Knowing the time–temperature history at a point in the container, Equation 2.8 can be used to calculate the microbial reduction or retention of quality parameters in foods, which follow first-order kinetics of change, at that single point during the process time.

$$\frac{F}{F_0} = 10^{\left(\frac{-1}{D_{\text{ref}}} \int_0^{PT} 10^{\left(\frac{T-T_{\text{ref}}}{z}\right)} dt\right)} \quad (2.8)$$

The Arrhenius model can be used to evaluate the temperature effect on the quality parameters with first-order reversible kinetics (fractional conversion model), such as flavor and aroma “cooked-notes” (Silva, 2000; Silva et al., 2000a). Equation 2.9 is used to calculate cooked-notes level at a single point at any time:

$$\frac{F}{F_0} = \frac{F_{\infty}}{F_0} + \frac{(F_0 - F_{\infty})}{F_0} \times \exp \left[-k_{T_{\text{ref}}} \int_0^{PT} e^{\left[\frac{E_a}{R} \times \left(\frac{1}{T+273.15} - \frac{1}{T_{\text{ref}}+273.15} \right) \right]} dt \right] \quad (2.9)$$

The P -value and microbial/quality factor change can also be determined in terms of container volume average (see Holdsworth and Simpson, 2007).

2.3 HEAT RESISTANCE OF MICROBES TARGETING THE PRODUCTION OF SAFE AND STABLE FOODS

The global incidence of foodborne disease is difficult to estimate. However, 1.8 million people were reported to have died in 2005 from diarrheal diseases. A high proportion of these cases can be attributed to contamination of food and in particular drinking water (WHO, 2007). Low-acid foods have been the cause of human diseases such as gastroenteritis and listeriosis. Common symptoms of foodborne illness include diarrhea, stomach cramps, fever, headache, vomiting, dehydration, and exhaustion. Proper thermal processing of foods can eliminate most of the causative agents of foodborne diseases. Although microbial spoilage of thermally processed foods can be caused by incipient spoilage (growth of bacteria before processing) and recontamination after processing (leakage), we will focus our attention on the survival and growth of thermophilic microorganisms (e.g., sporeformers) due to insufficient heat processing. Furthermore, the increasing demand for minimally processed foods by consumers has resulted in recent outbreaks of foodborne illnesses and fatalities in the wake of underpasteurized foods. Food spoilage with economic losses has been also observed.

The highest incidence of rapid spoilage of processed foods is caused by bacteria, followed by yeasts and molds (Sinell, 1980). Parasites (protozoa and worms), natural toxins, viruses, and prions can also be a problem if industry uses contaminated raw materials (FDA, 1992).

This section starts with a short introduction about microbial spores, followed by a review of the heat resistance of thermally driven microbiological and biochemical (enzymes) criteria to be considered as targets in designing pasteurization processes for low-acid chilled foods and high-acid shelf-stable foods. Thermal resistances listed from higher to lower values will be presented for the most significant microorganisms able to grow in chilled low-acid foods and shelf-stable high-acid foods.

2.3.1 SPORES: HEAT-RESISTANT MICROBIAL FORMS

A spore is a highly resistant dehydrated form of a dormant cell produced under conditions of environmental stress and as a result of “quorum sensing.” Molds and bacteria can produce spores, although mold spores are not as heat resistant as bacterial spores. Heat is the most efficient method for spore inactivation, and is presently the basis of a huge worldwide industry (Bigelow and Esty, 1920; Gould, 2006). Microbial spores are much more resistant to heat in comparison to their vegetative counterparts, generally being able to survive the pasteurization process. Spore heat resistance may also be affected by the food environment in which the organism is heated (Tables 2.1, 2.2, and 2.4 through 2.6). For instance, spores (and vegetative cells) become more heat resistant at low water activity (Murrel and Scott, 1966; Härnqvist and Snygg, 1972; Corry, 1976; King and Whiteland, 1990; Tournas and Traxler, 1994; Silva et al., 1999). If after pasteurization the storage temperature as well as the food characteristics (pH, water activity, food constituents) are favorable for a sufficient amount of time, surviving spores can germinate and grow to attain high numbers (e.g., 10^7 cells/g or mL). Subsequently, foodborne diseases and/or spoilage may occur.

The most dangerous sporeformers in low-acid chilled foods are the nonproteolytic strains of *Clostridium botulinum* (Gould, 1999; Carlin et al., 2000). Other human infections or intoxications from pasteurized (cooked) and chilled foods include spore-forming *Bacillus cereus* (Carlin et al., 2000). Unusual spoilage problems have been reported with *Alicyclobacillus acidoterrestris* in apple and orange juices (Brown, 2000) and other high-acid shelf-stable foods. There are a number of nonpathogenic sporeformers including facultative bacilli, butyric, thermophilic anaerobes, and molds that can cause significant economic losses to food producers. Control of spores during storage of pasteurized foods requires an understanding of both their heat resistance and outgrowth characteristics.

2.3.2 MICROBIAL HEAT RESISTANCE IN LOW-ACID PASTEURIZED CHILLED FOODS (pH > 4.6)

Minimally heated chill-stored foods have been increasing by 10% each year in market volume, since they are convenient (ready-to-eat and with longer shelf-life than fresh) and can better retain the original properties of the foods. For reasons of public safety, low-acid pasteurized foods are stored, transported, and sold under refrigerated conditions and with a limited shelf-life (Figure 2.1), to minimize the

outgrowth of pathogenic microbes in the foods during distribution. Beverages such as milk, certain fruit juices (e.g., tomato, pear, some tropical juices), dairy products (e.g., yoghurts, cheeses), poultry/meat/fish/vegetable products (e.g., cured, cooked ham), some shellfish (e.g., cockles), and some sauces are examples of low-acid pasteurized foods. Low-acid pasteurized and chilled foods also include refrigerated processed foods of extended durability (REPFED). Those are generally packaged under vacuum or modified atmospheres to ensure anaerobic conditions and submitted to mild heat treatments, being stored from a few days to several weeks depending on the food and severity of the heat process.

Following is a brief list of typical pathogens associated with foodborne diseases and outbreaks from improperly processed low-acid chilled foods. Nonproteolytic, psychrotrophic strains of *Clostridium botulinum* have been implicated in human botulism incidents caused by the following contaminated foods (Lindström et al., 2006): hot-smoked fish (Pace et al., 1967), canned tuna fish in oil (Mongiardo et al., 1985), canned truffle cream/canned asparagus (Therre, 1999), pasteurized vegetables in oil (Aureli et al., 1999), canned fish (Przybylska, 2003), and canned eggplant (Peredkov, 2004). Other examples of foodborne infections from raw and heated foods include *Bacillus cereus* (cooked rice and chilled foods containing vegetables), *Listeria monocytogenes* (milk, soft cheese, ice cream, cold-smoked fish, chilled processed meat products such as cooked poultry), *Escherichia coli* serotype O157:H7 (verotoxigenic *E. coli* VTEC; beef, cooked hamburgers, raw fruit juice, lettuce, game meat, cheese curd), *Salmonella enteritidis* (poultry and eggs), *Vibrio parahaemolyticus* (improperly cooked, or cooked, recontaminated fish and shellfish), *Vibrio cholerae* (water, ice, raw or underprocessed seafood), and foodborne trematodes from fish/seafood produced by aquaculture (FDA, 1992; Carlin et al., 2000; WHO, 2002; Keiser and Utzinger, 2005). Pasteurized milk and dairy products may also be contaminated with *Brucella*, thermophilic *Streptococcus* spp., and *Mycobacterium avium paratuberculosis* (MAP) (Grant, 2003), which can be infectious at low cell numbers, although they cannot grow at chill temperatures.

Psychrotrophic spoilage microbes such as lactic acid bacteria (LAB) (*Lactobacillus* spp., *Leuconostoc* spp., *Carnobacterium* spp.), molds (*Thamnidium* spp., *Penicillium* spp.), and yeasts (*Zygosaccharomyces* spp.) can occur in chilled low-acid foods during storage, in general due to postprocess contamination. These are very heat sensitive, for example, LAB $D_{63^{\circ}\text{C}}$ is 14 s in meat sausages (Franz and vonHoly, 1996) and $D_{60^{\circ}\text{C}}$ is 33 s in milk (De-Angelis et al., 2004).

2.3.2.1 Psychrotrophic Strains of *Clostridium botulinum*

Anaerobic spore-forming *Clostridium* species can be a problem in REPFED foods which are increasingly selected by consumers. These include the nonproteolytic psychrotrophic strains of *C. botulinum* (toxin types B, E, F) and the food-poisoning pathogen *Clostridium perfringens*, although the latter is not psychrotrophic. The mild pasteurization process applied to REPFED foods, followed by extended storage at chill temperatures, favors the survival and growth of psychrotrophic strains (Group II) of *C. botulinum* (Lindström et al., 2006). In spite of the low incidence of this intoxication, the mortality rate is high, if not treated immediately and properly. *C. botulinum* is of greatest concern on account of its spore's heat resistance (Table 2.1), being able to

TABLE 2.1
Heat Resistance of Psychrotrophic (Group II) Nonproteolytic Strains of *Clostridium botulinum* Spores

Food Product	Spore Inoculum, Botulinum Strains	T (°C)	D-Value (min)	z-Value (°C)	T Range (°C)	References
Crabmeat	Mixture of three strains: ‘Ham’, ‘Kapchunka’, 17B	88.9	13	8.6	88.9–94.4	Peterson et al. (1997)
		90.6	8.2			
		92.2	5.3			
		94.4	2.9			
Cod homogenate	ATCC 25765, ATCC 9564	75.0	54	8.6	75.0–92.0	Gaze and Brown (1990)
		80.0	18			
		85.0	4.0			
		90.0	1.1			
		92.0	0.60			
Turkey slurry	KAP B5	75.0	33	9.4	75.0–90.0	Juneja et al. (1995)
		90.0	0.80			
Carrot homogenate	ATCC 25765, ATCC 9564	75.0	19	9.8	75.0–92.0	Gaze and Brown (1990)
		80.0	4.2			
		85.0	1.6			
		90.0	0.36			
Turkey slurry	‘Alaska’	70.0	52	9.9	70.0–85.0	Juneja et al. (1995)
		85.0	1.2			
Whitefish paste	‘Alaska’, ‘Beluga’, 8E, ‘Iwanai’, ‘Tenno’	80.0	1.6–4.3	5.7–7.6	73.9–85.0	Crisley et al. (1968)
Blue crab	‘Alaska’, ‘Beluga’, crab G21-5, crab 25V-1, crab 25V-2	73.9	6.8–13	7.0–8.4	73.9–85.0	Lynt et al. (1977)
		76.6	2.4–4.1			
		79.4	1.1–1.7			
		82.2	0.49–0.74			
Oyster homogenate	‘Minnesota’, ‘Alaska’, crab G21-5, crab 25V-1, crab 25V-2	73.9	2.0–9.0	4.2–7.1	73.9–82.2	Chai and Liang (1992)
		82.2	0.080–			
			0.43			

T, temperature (°C)

survive mild heat treatments, including pasteurization, and requiring special storage conditions (Peck, 2006). The use of refrigerated storage can reduce or at least retard toxin production, given that this organism needs much longer storage periods to produce the lethal toxin: within 31 days at 3.3°C in beef stew (Schmidt et al., 1961); within 22 days at 8.0°C (Betts and Gaze, 1995); ≥55 days at 4.4°C, 8 days at 10°C, and 2 days at 24°C in crabmeat homogenates (Cockey and Tatro, 1974). Thus, to control human botulism in low-acid pasteurized foods the use of refrigerated storage ($T < 8^{\circ}\text{C}$) is required with a restricted shelf-life (Gould, 1999). Additional measures of safety with this risky class of foods include the use of added preservatives such as salt (>3.5%) and nitrites (>100 ppm) (e.g., cured meat products) (Graham et al., 1996). The unique use of such levels of salts is not sufficient to inhibit the mesophilic strains of *C. botulinum* (belonging to Group I, proteolytic, producing toxin types

A, B, and F) in pasteurized meat products, and these must be refrigerated ($<8^{\circ}\text{C}$) (Peterson et al., 1997).

Psychrotrophic strains of *C. botulinum* present different thermal resistances depending first on the heating menstruum (the food), and in some cases on the spores of a particular strain. Similar results were obtained with toxin type E and type B strains in cod and carrot homogenates (Gaze and Brown, 1990). Table 2.1 presents thermal resistance data obtained from the literature in decreasing order of resistance to heat. In summary, $D_{90^{\circ}\text{C}}$ varies from seconds to more than 8 min, $D_{85^{\circ}\text{C}}$ from a few seconds to 37 min, and $D_{80^{\circ}\text{C}}$ from a few minutes to 140 min. Crabmeat presented the highest D -value, 2.9 min at 94.4°C , using a mixture of 'Ham', 'Kapchunka', and 17B botulinum strains. Most of the authors published similar z -values, ranging between 7.2°C and 9.9°C . Oyster homogenate presented the lowest heat resistance for the five spore strains (two from outbreaks in 'Alaska' and 'Minnesota') of *C. botulinum* studied, the D - and z -values (4.2°C – 7.1°C) being the lowest recorded (Chai and Liang, 1992). The z -values were also low in whitefish paste (Crisley et al., 1968).

2.3.2.2 Other Pathogenic Spore-Forming Bacteria

Table 2.2 shows thermal resistance data of the spore-forming pathogens *Clostridium perfringens* and *Bacillus cereus*, which have been responsible for outbreaks in low-acid underpasteurized chilled foods. Studies with spores of six strains of *C. perfringens* demonstrated no growth at $T \leq 10^{\circ}\text{C}$, although spore germination and extended survival at low temperatures occurred (de-Jong et al., 2004). However, since this is the most common foodborne illness from a sporeformer, it was also considered in this review (Table 2.2). As a result of temperature abuse during distribution or storage of heated/cooked foods (e.g., meat products), *C. perfringens* may grow, especially in establishments where large quantities of foods are prepared several hours before serving (e.g., school cafeterias, hospitals, nursing homes, prisons, etc.) with concomitant difficulties in rapid chilling to below 10°C . Spores of *C. perfringens* are more heat resistant than those of nonproteolytic *C. botulinum*, for example, $D_{99^{\circ}\text{C}} = 23$ min in turkey, $D_{90^{\circ}\text{C}} = 31$ min in pork roll, and $D_{90^{\circ}\text{C}} = 14$ min in chicken breast. Some strains of *B. cereus* can grow at low temperatures ($T < 8^{\circ}\text{C}$) and can also present problems in underpasteurized refrigerated foods (Dufrenne et al., 1994, 1995; García-Armesto and Sutherland, 1997; Carlin et al., 2000; Choma et al., 2000). Eleven strains of isolated psychrotrophic strains of *B. cereus* able to grow at $\leq 7^{\circ}\text{C}$ in foods presented a $2.2 \text{ min} < D_{90^{\circ}\text{C}} < 9.2 \text{ min}$ in phosphate buffer (Dufrenne et al., 1995). Other published data gave $D_{90^{\circ}\text{C}}$ of 10 min and 4 min in pork roll (Byrne et al., 2006) and water (Fernández et al., 2001), respectively. Psychrotrophic strains of *B. cereus* seem to be more heat resistant than psychrotrophic strains of *C. botulinum* (Figure 2.2).

2.3.2.3 Non-Spore-Forming Psychrotrophic Pathogens

Table 2.3 shows the heat resistance of non-spore-forming pathogens able to grow at low temperatures ($T < 8^{\circ}\text{C}$). The pathogenic vegetative bacteria, *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Vibrio parahaemolyticus*, are also able to grow in foods with $\text{pH} > 4.6$ at temperatures lower than 6°C (Penfield and Campbell, 1990) but they are easily eliminated with a few seconds at 70°C or less (Table 2.3). Other non-spore-forming pathogens such as *Escherichia coli*, some strains of *Salmonella*, and *Aeromonas*

TABLE 2.2
Heat Resistance of *Clostridium perfringens* and *Bacillus cereus* in Low-Acid Foods (pH > 4.6)

Bacteria	Spore Inoculum	Food Product	T (°C)	D-Value (min)	z-Value (°C)	T Range (°C)	References
<i>Clostridium perfringens</i>	Three strains: NCTC 8238/9, ATCC 10288	Ground turkey	99.0	23	nr	nr	Juneja and Marmer (1996)
	Three strains: DSM 11784, NCTC 10614 (incidents), NCTC 08237	Pork luncheon roll	90.0 95.0 100.0	31 9.7 1.9	8.3	90.0–100.0	
	Three strains: NCTC 8238/9, ATCC 10288	Marinated chicken breast	90.0	14	nr	nr	Juneja et al. (2006)
	Three strains: DSM 4313 (incident), DSM 626, NCTC 07464	Pork luncheon roll	85.0 90.0 95.0	30 10 2.0	8.6	85.0–95.0	Byrne et al. (2006)
	Psychrotrophic strain INRA AVTZ415	Distilled water	85.0 90.0 95.0 100.0	16 3.9 1.0 0.24	8.2	85.0–100.0	Fernández et al. (2001)

T, temperature (°C); nr, not reported.

hydrophila (Palumbo et al., 1995; Schoeni et al., 1995; George, 2000; Papageorgiou et al., 2006) can also be a problem in chilled foods. However, pasteurization of a few seconds at 65°C or less should be sufficient to destroy these microorganisms (Table 2.3). In general, a few minutes at 60°C was enough to achieve one decimal reduction in *Listeria*, *E. coli*, *Salmonella*, and *Y. enterocolitica* populations in most of these foods (Figure 2.2). *A. hydrophila* presented the lowest heat resistance.

The potential for the growth of other vegetative bacterial pathogens such as *Campylobacter* spp., *V. cholerae*, *Shigella* spp., *Staphylococcus aureus*, *Enterococcus* spp. (FDA, 1992; Jay, 2000) in pasteurized/chilled foods is very low, since apart from being heat sensitive, food must be temperature abused during distribution to allow their growth.

Although the effect of MAP in humans is not known yet, this bacterium causes disease in cattle and as a precaution the design of pasteurization in milk and dairy products should consider this bacterium’s thermal resistance. *D*-values in milk at 63°C, 66°C, and 72°C are 15, 5.9, and <2.03 s, respectively, and *z*-value is 8.6°C (Pearce et al., 2001). Keswani and Frank (1998) obtained much higher *D*-values in milk (*D*_{63°C} = 1.6–2.5 min). *Coxiella burnetii* (the causative agent of ‘Q-fever’) has *D*-values of 4.14 min at 62.8°C and 2.21 sec at 71.7°C, *z*-value = 4.34°C in milk (Cerf and Condron, 2006).

Low-acid chilled foods									
D-value ≤ 3 min at									
50°C	55°C	60°C	65°C	75°C	80°C	85°C	90°C	95°C	
		<i>Listeria monocytogenes</i> <i>Escherichia coli</i> O157:H7 <i>Salmonella</i> spp.							<i>Clostridium botulinum</i>
<i>Aeromonas hydrophila</i>	<i>Yersinia enterocolitica</i> <i>Mycobacterium avium</i> Lactic acid bacteria		<i>Coxiella burnetii</i>						<i>Bacillus cereus</i>

High acid shelf stable foods									
D-value ≤ 3 min at									
60°C	65°C	70°C	75°C	80°C	85°C	90°C	95°C	100°C	
	<i>Saccharomyces cerevisiae</i>						<i>Alicyclobacillus acidoterrestris</i> <i>Bacillus</i> spp.		
Lactic acid bacteria	Some yeasts and moulds					<i>Neosartorya fischeri</i>			
				<i>Clostridium pasteurianum</i>		<i>Talaromyces flavus</i> <i>Eupenicillium javanicum</i> <i>Clostridium butyricum</i>	<i>Byssoschlamys nivea</i>		
				Polyphenoloxidase Peroxidase		Pectinesterase			

FIGURE 2.2 Minimum pasteurization temperature to achieve 1 logarithmic microbial reduction (1D) in a few minutes.

TABLE 2.3
Heat Resistance of Non-Spore-Forming Pathogenic Microbes
in Low-Acid Foods (pH > 4.6)

Bacteria	Food Product	T (°C)	D-Value (min)	z-Value (°C)	T Range (°C)	References
<i>Listeria monocytogenes</i>	Ground pork	55.0	47	5.9	55.0–70.0	Murphy et al. (2004a)
		70.0	0.085			
	Chicken gravy	50.0	119–195	5.2–6.1	50.0–65.0	Huang et al. (1992)
		65.0	0.19–0.48			
	Cooked lobster	51.6	97	5.0	51.6–62.7	Budiamoako et al. (1992)
		54.4	55			
		57.2	8.3			
		60.0	2.4			
		62.7	1.1			
	Rainbow trout roe	60.0	1.6	5.4	60.0–63.0	Miettinen et al. (2005)
		63.0	0.44			
	Liquid egg yolk	60.0	1.3	6.1	60.0–62.2	Schuman and Sheldon (1997)
		62.2	0.58			
	Liquid egg white	55.1	7.6	9.4	55.1–58.3	
		58.3	3.5			
	Vacuum-packed minced beef	50.0	36	4.2	50.0–60.0	Bolton et al. (2000)
		55.0	3.2			
		60.0	0.15			
<i>Escherichia coli</i> O157:H7	Ground pork	55.0	33	4.9	55.0–70.0	Murphy et al. (2004a)
		70.0	0.048			
	Fully cooked frank	55.0	25	5.1	55.0–70.0	Murphy et al. (2004b)
		70.0	0.038			
	Raw frank	55.0	21	5.1	55.0–70.0	
		70.0	0.031			
	Ground beef	55.0	21	6.0	55.0–65.0	Juneja et al. (1997)
		65.0	0.39			
	Ground meats: lamb, chicken, turkey, pork	55.0	11–12	6.5–6.9	55.0–65.0	Juneja et al. (1997); Juneja and Marmer (1999)
		65.0	0.29–0.38			
	Ground morcilla sausage	54.0	5.5	7.4	54.0–62.0	Oteiza et al. (2003)
		58.0	2.1			
		62.0	0.60			
	Ground meat beef, pork sausage, chicken, turkey	50.0	50–115	4.4–4.8	50.0–60.0	Ahmed et al. (1995)
		55.0	6.4–19			
		60.0	0.37–0.58			
<i>Salmonella</i> spp.	Green pea soup	60.0	10	5.7	60.0–71.1	Thomas et al. (1966)
		65.6	1.0			
	Ground pork	55.0	46	5.9	55.0–70.0	Murphy et al. (2004a)
		70.0	0.083			

TABLE 2.3 (continued)
Heat Resistance of Non-Spore-Forming Pathogenic Microbes
in Low-Acid Foods (pH > 4.6)

Bacteria	Food Product	T (°C)	D-Value (min)	z-Value (°C)	T Range (°C)	References
<i>Yersinia enterocolitica</i>	Chicken Thigh meat	55.0	12	6.9	55.0–62.5	Juneja (2007)
		60.0	3.2			
		62.5	0.84			
	Chicken breast meat	55.0	6.1	8.1	55.0–62.5	
		60.0	3.00			
		62.5	0.66			
	Liquid egg yolk	60.0	0.28	4.3	60.0–62.2	Shuman and Sheldon (1997)
		62.2	0.087			
	Liquid egg white	55.1	8.0	3.5	55.1–58.3	
		58.3	1.0			
	Vacuum-packed minced beef	50.0	21	nr	50.0–60.0	Bolton et al. (2000)
		55.0	1.1			
<i>Aeromonas hydrophila</i>		60.0	0.55			
	Whole and skim milks	62.8	0.17–0.18	nr	nr	Toora et al. (1992)
	Liquid whole egg	48.0	3.6–9.4	5.0–5.6	48.0–60.0	Schuman et al. (1997)
		60.0	0.026–0.040			

T, Temperature (°C); nr, not reported.

2.3.3 MICROBIAL AND ENDOGENOUS ENZYMES HEAT RESISTANCE
IN HIGH-ACID AND ACIDIFIED FOODS (pH < 4.6)

In high-acid and acidified foods, the main pasteurization goal is to avoid spoilage during distribution at room temperature, rather than avoiding outbreaks of public health concern (Figure 2.1). High-acid foods include most of the fruits, normally containing high levels of organic acids. The spoilage flora is mainly dependent on pH and soluble solids. The type of organic acids and other constituents of these foods such as polyphenols might also affect the potential spoilage microorganisms. Given the high acid content of this class of foods (pH < 4.6), the pathogens referred to in Section 2.3.2 (vegetative and spore cells) including the spore-forming *C. botulinum* are not able to grow. It is generally assumed that the higher the acidity of the food, the less probable is the germination and growth of bacterial spores, a pH < 4.6 being accepted as safe in terms of pathogenic sporeformers. However, various incidents in high-acid foods involving the spore-forming spoilage bacterium *Alicyclobacillus acidoterrestris* (Cerny et al., 1984; Jay, 2000) have been registered, since its optimum growth pH is between 3.5 and 4.5 for the type strain (Pinhatti et al., 1997) and optimum growth temperature is between 35°C and 53°C (Deinhard et al., 1987; Sinigaglia et al., 2003), depending on the strain.

Typical microbes associated with spoilage of high-acid and acidified shelf-stable foods are *A. acidoterrestris*, molds, yeasts, and some lactic acid bacteria (LAB).

Heat-resistant deteriorative enzymes such pectinesterase (PE), polyphenoloxidase (PPO), and peroxidase (PRO) may also degrade high-acid food quality during storage. Additionally, growth of spoilage spore-forming *Bacillus* and *Clostridium* has been registered in less acid foods ($3.7 < \text{pH} < 4.6$) such as tomato purée/juice, mango pulp/nectar, canned pear, and pear juice (Ikeyami et al., 1970; Shridar and Shankhapal, 1986). A review of the most thermally resistant pasteurization targets, such as microbial spores and enzymes, is presented.

2.3.3.1 *Alicyclobacillus acidoterrestris* Spores

A. acidoterrestris, is a thermoacidophilic, nonpathogen, and spore-forming bacterium identified in the 1980s (Deinhard et al., 1987; Wisotzkey et al., 1992), which has been associated with various spoilage incidents in shelf-stable apple and orange juices. The presence of ω -alicyclic fatty acids as the major natural cell membrane lipid component gave the name *Alicyclobacillus* to this genus (Wisotzkey et al., 1992). Since this microbe does not produce gas, spoilage is only detected by the consumer at the end of the food chain, resulting in consumer complaints, product withdrawal, and subsequent economic loss. Spoilage aromas and taste are related to the production of a bromophenol and guaiacol. A relatively low level of 10^5 – 10^6 cells/mL in apple and orange juices formed enough guaiacol (ppb) to produce sensory taint (Pettipher et al., 1997). Spoilage by *A. acidoterrestris* has been observed mainly in apple juice, but also in pear juice, orange juice, juice blends, and canned diced tomatoes (Cerny et al., 1984; Splittstoesser et al., 1994; Yamazaki et al., 1996; Pontius et al., 1998; Walls and Chuyate, 2000). Incidents were reported from all over the world (Germany, the United States, Japan, Australia, and the United Kingdom). A survey carried out by National Food Processors Association in the United States (Walls and Chuyate, 1998) in fifty seven companies, had shown that 35% of juice manufacturers had problems especially during warmer spring and summer seasons, possibly associated to *Alicyclobacillus*. Another incident with many complaints from consumers, referred to an iced tea ($\text{pH} = 2.7$) submitted to a thermal process of 95°C for 30 s, followed by hot-filling into cartons (Duong and Jensen, 2000). The slow cooling of the hot-filled tea or the high storage temperature may have allowed sufficient time for the spores to germinate and grow, causing taint problems. *A. acidoterrestris* spore germination and growth (to 10^6 cfu/mL) under acidic conditions was reported in orange juice stored at 44°C for 24 h (Pettipher et al., 1997), and also in apple, orange, and grapefruit juices stored at 30°C (Komitopoulou et al., 1999). Spore germination and growth was observed after 1–2 weeks in apple juice, orange juice, white grape juice, tomato juice, and pear juice incubated at 35°C (Walls and Chuyate, 2000). Red grape juice did not support growth (Splittstoesser et al., 1994), possibly due to the polyphenols. The increase of soluble solids from 12.5°Brix ($a_w = 0.992$) to 38.7°Brix ($a_w = 0.96$) inhibited growth of *A. acidoterrestris* spores (Sinigaglia et al., 2003).

The spores of *A. acidoterrestris* are very resistant to heat compared to the major spoilage microbes and enzymes typical in high-acid shelf-stable foods (Tables 2.4 through 2.7), presenting $4 \text{ min} < D_{90^\circ\text{C}} < 23 \text{ min}$, $1 \text{ min} < D_{95^\circ\text{C}} < 5 \text{ min}$ and $7^\circ\text{C} < z\text{-value} < 13^\circ\text{C}$. Much lower D -values were recorded in wine ($D_{85^\circ\text{C}} = 0.6 \text{ min}$) (Splittstoesser et al., 1997), potentially due to the alcohol or other constituents created by fermentation. Further conclusions about *A. acidoterrestris* spore thermal resistance are

TABLE 2.4
Heat Resistance of *Alicyclobacillus acidoterrestris* Spores in High-Acid Fruit Products (pH < 4.6)

Heating Medium	Spore Strain	pH	SS (°Brix)	T (°C)	D-Value (min)	z-Value (°C)	T Range (°C)	References
<i>Juices, nectars, fruit drinks, and wine</i>								
Orange juice drink	nr	4.1	5.3	95	5.3	9.5	nr	Baumgart et al. (1997)
Fruit drink	nr	3.5	4.8	95	5.2	10.8	nr	
Fruit nectar	nr	3.5	6.1	95	5.1	9.6	nr	
Apple juice	VF	3.5	11.4	85	56	7.7	85–95	Splittstoesser et al. (1994)
				90	23			
				95	2.8			
Grape juice	WAC	3.3	15.8	85	57	7.2	85–95	
				90	16			
				95	2.4			
Orange juice	Type	3.5	11.7	85	66	7.8	85–91	Silva et al. (1999)
				91	12			
Orange juice	DSM 2498; three isolated strains: 46; 70; 145.	3.2	9.0	85	50–95	7.2–11.3	85–95	Eiroa et al. (1999)
				90	10–21			
				95	2.5–8.7			
Orange juice	Z	3.9	nr	80	54	12.9	80–95	Komitopoulou et al. (1999)
				90	10			
				95	3.6			
Apple juice	Z(CRA 7182)	3.5	nr	80	41	12.2	80–95	
				90	7.4			
				95	2.3			
Cupuaçu extract	Type	3.6	11.3	85	18	9.0	85–97	Silva et al. (1999)
				91	5.4			
				95	2.8			
				97	0.57			
Grapefruit juice	Z	3.4	nr	80	38	11.6	80–95	Komitopoulou et al. (1999)
				90	6.0			
				95	1.9			
Berry juice	nr	3.5	nr	88	11	7.2	88–95	Walls (1997)
				91	3.8			
				95	1.0			
Wine	nr	nr	nr	75	33	10.5	75–85	Splittstoesser et al. (1997)
				85	0.57			
<i>Fruit concentrate</i>								
Black currant concentrate	Type	2.5	58.5	91	24	nr	nr	Silva et al. (1999)
Light black currant concentrate	Type	2.5	26.1	91	3.8	nr	nr	

SS, soluble solids (°Brix); T, temperature (°C); nr, not reported; *A. acidoterrestris* type strain, NCIMB 13137, GD3B, DSM 3922, ATCC 49025.

TABLE 2.5
Heat Resistance of Spoilage Fungal Ascospores in High-Acid Fruit Products (pH < 4.6)

Fungal Ascospores	Fruit Product	pH	SS (°Brix)	T (°C)	D-Value (min)	z-Value (°C)	T Range (°C)	References
<i>Byssoschlamys nivea</i>	Strawberry pulp	3.0	15.0	80.0	193	6.4	80–93	Aragão (1989)
				85.0	35			
				90.0	6.3			
				93.0	1.7			
<i>Neosartorya fischeri</i>	Pineapple concentrate	3.4	42.7	85.0	30	8.9	85–95	Tournas and Traxler (1994)
				90.0	7.6			
				95.0	2.3			
	Pineapple juice	3.4	12.6	85.0	20	9.2	85–95	
				90.0	4.8			
				95.0	1.7			
<i>Neosartorya fischeri LT025</i>	Apple juice	3.5	15.0	85.0	15	5.3	85–93	Gumerato (1995)
				88.0	4.7			
				90.0	2.6			
				93.0	0.43			
	Strawberry pulp	3.0	15.0	80.0	60	6.4	80–93	Aragão (1989)
				85.0	15			
90.0				2.6				
93.0				0.50				
<i>Talaromyces flavus</i>	Apple juice	3.7	11.6	87.8	7.8	5.2	nr	Scott and Bernard (1987)
				90.6	2.2			
	Strawberry pulp	3.0	15.0	75.0	54	8.2	75–90	Aragão (1989)
				80.0	18			
				85.0	3.3			
				90.0	0.90			
<i>Eukeniicillium javanicum</i>	Strawberry pulp	3.0	15.0	80.0	15	7.9	80–90	Aragão (1989)
				85.0	3.7			
				90.0	0.80			

SS, soluble solids (°Brix); T, temperature (°C); nr, not reported.

dependent on the spore strain and/or fruit product. As expected, when increasing the soluble solids from 26.1°Brix to 58.5°Brix in black currant concentrate, the $D_{91^{\circ}\text{C}}$ -values increased from 3.8 to 24.1 min (Silva et al., 1999). However, the growth of *A. acidoterrestris* is inhibited at high soluble solids concentration, for example, no growth was observed in apple concentrate between 30°Brix and 50°Brix (Walls and Chuyate, 2000) and white grape juice with more than 18°Brix (Splittstoesser et al., 1997).

2.3.3.2 Fungal Ascospores

Fungal growth in pasteurized foods, raw materials, and food ingredients should be avoided, since some of them are able to produce mycotoxins. Spores and vegetative