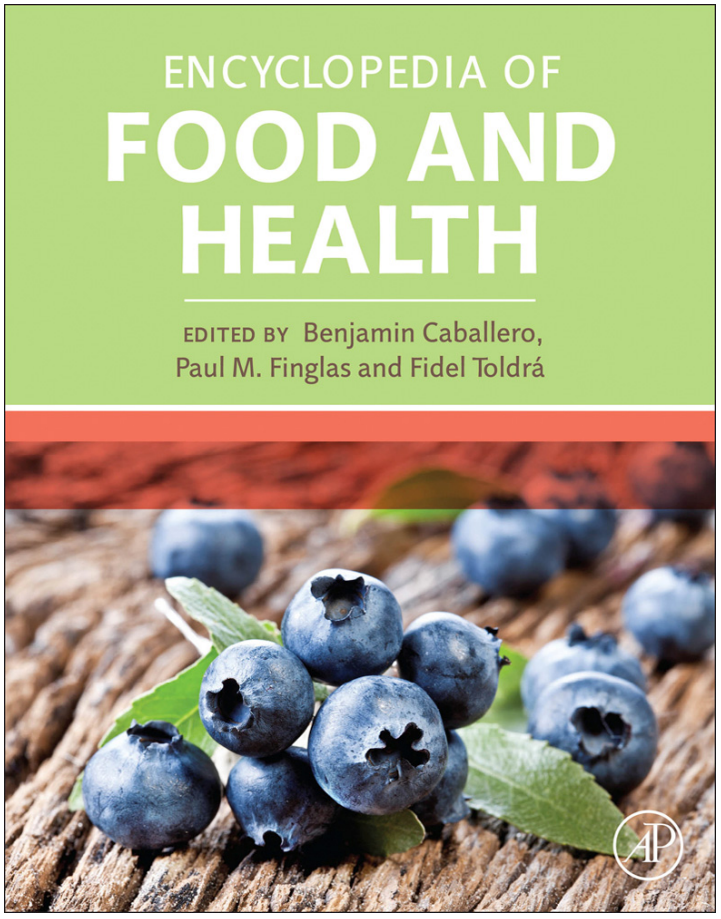

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Heat Treatment: Effect on Microbiological Changes and Shelf Life

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Introduction

Foods are complex ecosystems constituted by the environment and the microorganisms that live in it. The food environment consists of intrinsic factors such as pH, water activity, and nutritional components and extrinsic factors such as temperature and gaseous composition, among others. By manipulating these, factors microbial growth can be limited and enzymatic degradation can be avoided, achieving the food preservation.

Thermal treatment is one of the most important physical methods for increasing the shelf life of foods. It is a versatile and economical system that can be adapted to almost any foodstuff and package. It can also be combined with other physical or chemical stress factors to achieve the microbiological stability of foods, causing the least possible destruction of quality. Traditionally, foods have been sterilized in hermetically sealed tinplate cans or glass jars. The intensity of the heat treatment applied depends on the type of foodstuff, quality of raw materials employed, pH, transportation and storage conditions, and geographic retail area. If the treatment complies with 'commercial sterilization' standards and no recontamination is allowed, it is clear that foods preserved by sterilization are stable and safe from a microbiological point of view during their shelf life. To avoid recontamination, sterilization relies on the existence of a hermetic package and, in some cases, such as with aseptic packaging, on very strict additional hygiene measures.

Preservation of foods by heat is a relatively old technology that started in 1810 with Nicolas Appert's work. In the development of this technology, there were various steps or milestones. The science application to food preservation by heat really started 85 years after Appert published his work. During those years, millions of canned foods were produced but no significant scientific advance was reported. Until well into the twentieth century, the main form of preservation was heat treatment, although salting, drying, fermentation, cold smoking, and other preservation methods were also used.

The evolution of thermal processes depended mainly on the equipment development and also on knowledge of the biological mechanisms involved in those processes. Isaac Solomon, a canner from Baltimore, modified the technology in 1860. He added calcium chloride to the cooking water, increasing the boiling point to 115 °C, which made it possible to increase the rate of production and also increase the bactericidal efficacy of the process. But the canning industry was revolutionized by A. K. Shriver in 1874, when he invented the pressure cooker. In 1910, Peter Durand patented a process that was, in essence, Appert's procedure but using a metal container instead of glass jars. During the twentieth century, the evolution of thermal processes permitted the development of the aseptic packaging technology, hydrostatic sterilization, and high-temperature short-time (HTST) procedures. These and other developments were, in principle, engineering improvements aimed at more efficient heating of canned products.

At the same time, there were advances on the scientific side related to process calculations. The first mathematical equations for process calculations appeared in 1920 and 1923 and published by Bigelow and Ball, respectively. More recently, the increase of HTST applications has been related to the development of time–temperature integrators for thermal process control and evaluation.

Heat treatment impact on the safety and quality of a product depends on the rate of the heat-induced reactions, and the process time treatment can have a strong and negative impact on the quality of processed foods, which is not desirable, especially nowadays, when the consumer trend is to demand minimally processed products. For preservation purposes, measuring the impact of heat in terms of safety and quality is of the greatest importance in the design, control, evaluation, and optimization of the sterilization process.

Effect of Heat on Microorganisms

Heat treatment is the most efficient and widely used method for destroying microorganisms. However, resistance to heat depends on the type of microorganism. A major problem in discussing mechanisms of heat inactivation and injury is that heat brings about many changes in biological material and therefore identification of 'the event' that causes death or injury is difficult. Moreover, there are differences among strains of the same species, among species, and between spores and vegetative cells.

Effect of Heat on Vegetative Cells

Most vegetative cells are killed at the sterilization temperatures normally used in the food industry, over 110 °C. The most important vegetative cells related to the safety of heat-treated foods are *Salmonella*, *Listeria*, *Campylobacter*, and *E. coli*, microorganisms that can contaminate various foods including meat, milk, vegetables, fish, and eggs and that are a great problem if the products are not properly heat-treated and stored.

Heat resistance to pasteurization temperatures depends on the species and strain and is usually expressed in terms of *D*-values (the time needed to reduce microbial concentration by one $\log_{10}$). Microorganisms are more resistant to dry heat than to wet heat, although, in view of food composition, much attention should be paid to wet heat in food microbiology. Physiological state is also a factor that affects microorganisms' resistance, cells in stationary growth phase being more resistant than cells in exponential phase. Other factors, such as low pH, modification of a_w, and presence of organic acids, also affect microorganism thermal inactivation.

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In some cases, microorganisms can be sublethally injured by heat, making them more sensitive to adverse recovery conditions, such as organic acids, low pH, and low water activity. However, under these conditions, some microorganisms activate stress response mechanisms, and as a result, cross-resistance is often seen, leading, for instance, to greater heat resistance among the microbial population

Effect of Heat on Bacterial Endospores

To counteract unfavorable conditions, for instance, nutrient restriction, some microorganism species, especially *Bacillus* and *Clostridium*, activate a specific set of genes involved in sporulation. The bacterial endospore consists of a spore core with a membrane that will later form the vegetative cell. This core is surrounded by a cortex with a peptidoglycan structure and spore coat layers consisting of keratin on the outside. This morphology and the very low water content of the spore core play a crucial role in the high resistance (to heat and other stresses) of the bacterial endospore. When conditions become favorable, the spore starts to geminate and eventually develops into a new vegetative cell.

Since 1986, the year in which Cohn and Koch discovered the bacterial spore, its mechanisms of heat resistance and death have been studied in depth in an attempt to control inactivation processes. The high thermal resistance of spores has been attributed to the unequal distribution of water in the protoplast, providing stabilization to critical molecules such as DNA or RNA. However, this unequal distribution does not account for the extreme resistance of spores of some microorganisms, such as *Bacillus stearothermophilus*; consequently, it appears that there are other additional factors related to the high resistance of spores.

Heat can cause bacterial spore activation or induce deactivation and latency, damage, mutations, or complete inactivation. When the temperature is increased over the optimum for growth, the microorganism is inactivated or suffers some kind of deterioration, inability to initialize germination, or inability to duplicate critical molecules. It is possible that high temperatures affect the germination mechanism by damaging the trigger mechanism or cortex lytic enzymes. It has also been observed that some bacterial spores injured by heat are sensitive to antibiotics that under normal conditions would not affect them. This might suggest that heat affects the membrane, favoring the surface activity of antibiotics. Recent studies have indicated that physical damage of DNA does not take place, which means that in spores, the DNA is protected against heat and that the mechanism of thermal inactivation is something other than damage to those molecules.

There are many factors that can influence the heat resistance of bacterial endospores. The main factors affecting microorganisms can be grouped into four categories:

- Those affecting microorganisms during sporulation
- Those that affect microorganisms during storage of the spores that are produced
- Those that are a consequence of treatments given before, during, or after the treatment and also the nature of the medium
- Those affecting microorganisms during the recovery of survivors

Factors affecting microorganisms during the sporulation process determine the heat resistance of the spores. The presence of metal ions (Ca^{2+}, Mg^{2+}, or Mn^{2+}) in the sporulation medium is the main extrinsic factor contributing to an increase in the heat resistance of spores. If the presence of those ions decreases, the thermal resistance of the spores also decreases. A diminution of certain fatty acids during sporulation can increase the heat resistance of spores, and lack of glucose in the sporulation medium can reduce their heat resistance. The presence or absence of some amino acids makes the heat resistance of spores vary. One of the most important factors affecting the heat resistance of microorganisms during the thermal process is pH. Acidic pH values during the heating process reduce the heat resistance of spores, while at pH values close to neutrality, the thermal resistance does not vary substantially. There is an important interaction between pH and temperature; some studies have shown that the effect of pH diminishes as treatment temperature increases. Other factors that can influence the heat resistance of spores during the heating process are NaCl concentration, water activity (a_w), the presence of sugar or some germinant such as lysozyme that can help to increase the apparent heat resistance, and sublethal heating of spores prior to the thermal process.

Shelf Life

Shelf life is the time after manufacturing, processing, and packaging that a product remains acceptable under defined environmental conditions. It is a function of product characteristics such as composition, pH, water activity and fat content, the package, and the environment in which the product is transported, stored, and sold. It is established primarily not only in terms of microbial stability but also in terms of sensory acceptability.

Shelf life studies should be carried out in the following circumstances:

- Development of new products, processes or packaging, or reformulation or modification of existing ones
- Change of place of production or production team
- When there are no previous studies of shelf life

The starting point for determining shelf life is to obtain the necessary information about the food studied (chemical and physical characteristics, type of packaging, and storage, among other things) to determine the most important pathogenic microorganism that can grow in the product and to examine the relevant scientific literature. If the results of these studies guarantee that the pathogenic microorganism cannot grow in the food, it is not necessary to continue further studies. However, if the results are not clear on the possibility of growth of the organism, then it is necessary to conduct a series of additional studies that may include one or more of the following:

- Historical data for products manufactured
- Predictive microbiology
- Studies of specific laboratory life (durability studies and challenge tests)

Durability Studies

Durability studies assess the growth of a microorganism in a food during storage under reasonably foreseeable conditions without artificial inoculation, considering only natural contamination of the foodstuff. They are realistic and the evolution of the natural product contamination is studied. However, interpretation may be difficult because of the low prevalence of units contaminated with a certain microorganism, the low concentration of microorganisms present in the product under study, and/or the heterogeneity of the microbial distribution in the food. This would imply the need to conduct further studies and perform challenge tests.

Challenge Tests

In general, these tests are used only when other methods for assessing the food safety or its stability cannot be carried out or are not sufficiently clear about the possibility of pathogen growth. They provide information about the behavior of microorganisms inoculated in the food studied under certain conditions in a laboratory environment before storage. International committees have established protocols for conducting challenge tests. They consist essentially in inoculating the food with a defined concentration of the pathogenic microorganism and measuring the changes in the concentration levels during storage time, considering the worst case. This type of test should take into account the variability in food (using different batches) and specific food contamination (inoculation of isolates of food). However, it is difficult to imitate the level and heterogeneity of contamination and the physiological state of the bacteria.

Challenge tests can be performed with two different objectives:

(1) Assessment of growth potential (δ):

The growth potential (δ) is the difference between the $\log_{10}$ cfu g^{-1} at the end of the test and the $\log_{10}$ cfu g^{-1} at the beginning of the test. The experimental results may show a wide dispersion, notably because the lag phase is included. They depend on factors such as the strain(s) inoculated, the physiological state of the strain(s), the intrinsic properties of the food (pH, NaCl content, a_w, nutritional content, associated microflora, antimicrobial constituents, etc.), and the extrinsic properties (time–temperature profile, gas atmosphere, etc.) The main advantages of this method are (i) that it is relatively simple to implement and (ii) that the results can be used directly. Its drawback is the lack of flexibility in the interpretation: the results are only valid for the food studied in the conditions studied, so new experiments have to be performed each time there is a change.

(2) Estimation of growth parameters (μ_{max}):

The drawbacks of the previous approach can be solved by combining predictive microbiology models and challenge tests assessing μ_{max} (growth rates). This approach consists of a laboratory-based study that measures the growth rate of the pathogenic microorganism involved in an artificially contaminated food stored at an appropriate temperature. The temperature used for the experiment is not (necessarily) the one used for predictions since it is possible to predict growth at a temperature other than the one tested or in a time–temperature profile chosen to be representative of the foreseeable conditions of transportation, distribution, and storage. Once the test has been performed, the maximum growth rate is calculated from the growth curve. In the exponential growth phase, plotting the natural logarithm of the number of cells against time produces a straight line, and the slope of this line is μ_{max}. With this test, it is possible to estimate the microorganism concentration on a given day of the shelf life study if the initial concentration is known and/or to estimate the maximum allowable concentration of the microorganism that may be present in a food on the day of production in order to comply with legal limits at the end of its shelf life.

Thermal Process Calculation

Thermal process calculation is a fundamental step in ensuring the safety of thermally processed foods during their shelf life and their optimization. The calculation has two main components: (a) microorganism inactivation kinetics and (b) heat transfer mechanisms in food.

The inactivation kinetic of microorganisms is determined by using mathematical models that describe the rate of inactivation at a specific temperature and the dependence of the rate kinetic parameter on temperature. It has generally been accepted that when a homogeneous microbial suspension of microorganisms (spores or vegetative cells) is subjected to a constant temperature, microorganisms die at a constant rate. This means that, at a defined time, the same percentages of live microorganisms will die in the next time unit. According to these ideas, microbial inactivation by heat (spores and vegetative cells) follows two laws: (a) survivor law and (b) thermal destruction (TD) law.

Survivor Law

This law is based on the assumption that the destruction by wet heat of a pure bacterial culture at a constant temperature follows an exponential negative kinetic. This means that the number of viable cells is exponentially reduced during the time of exposure to a lethal temperature (Figure 1).

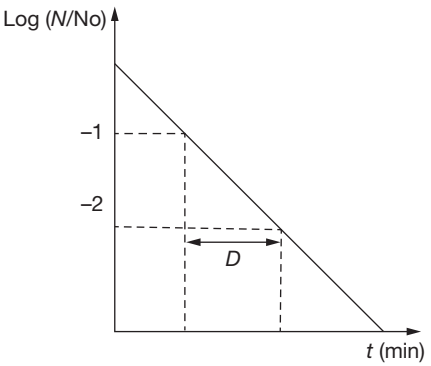

Figure 1 Survivor curve at constant temperature.

The parameter defining the destruction rate is named D_T, and it is defined as the treatment time, usually minutes or seconds, at a specific temperature that is required to reduce the bacterial population by 90%. This parameter is the best one for comparing the thermal resistance of different microorganisms, of the same or different species, at a given temperature, and of the same strain at different temperatures. It is also useful for evaluating the effect that environmental conditions have on the thermal resistance of microorganisms.

However, genetic or phenotypic heterogeneity of the cells in a population, mild or low temperature processes applied in food preservation, or any other stresses that may be applied to a microorganism population may result in nonlinearity. In these cases, shoulders appear, that is, the existence of a critical time before the microorganism is inactivated, or tails, showing a resistant population, or sigmoid curves, which are a combination of these two cases. A number of nonlinear models have been described in the literature, Weibull-like models, the biphasic model, normal distribution models, the logistic model, and Gompertz models being the ones most commonly used.

TD Law

When calculating sterilization or pasteurization processes, it is important to have knowledge of the variation of the rate constant with temperature.

The variation of D_T values with temperature was empirically modeled by Bigelow in 1921. This pioneer of thermal process calculations observed that by representing the logarithm of the rate constant versus temperature, a linear relationship was obtained for the temperature range studied. This curve was named the TD curve (**Figure 2**). The parameter that defines this TD relationship was called 'z,' and it indicated the change in treatment temperature (°C) required to achieve a tenfold increase or decrease in the D_T value.

Heat Penetration Curves and Process Calculation

The response of the product temperature to the temperature of the heating medium, for example, the steam retort temperature applied to a can, is governed by the physical laws of heat transfer and can also be expressed mathematically. Basically, there are two forms of temperature transfer: by conduction and by convection. The mechanism depends on the food; liquid food is usually heated by convection, while solid food is heated by conduction. The evolution of the temperature in the food can be measured by thermocouples installed in the package or in pipes containing the food, or mathematically by a computer model. The heat transport in foods is influenced by various factors including nature of the food, package, retort temperature, and the way in which the heat is applied. Heat penetration curves are obtained by plotting the evolution of food temperature versus treatment time. The parameters defining the heat penetration rate are deduced from heat penetration curves. These parameters, in addition to the microbial inactivation parameters, are used to calculate the thermal process properly.

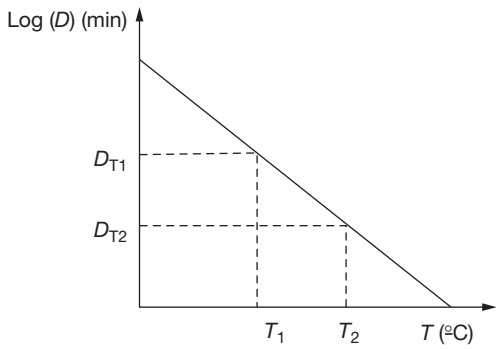

Figure 2 Thermal destruction curve.

There are two traditional ways to calculate the thermal process, the general method and the formula method first used by Ball in 1923 and improved in 1957 by Ball and Olson, known as Ball's formula method. The general method is more versatile and can be applied to almost any type of thermal process situation. However, this method is time-consuming and it soon gave way to the more convenient Ball's formula method. Ball's formula is very practical and rapid for estimating the sterilizing values of the process, although the accuracy of the calculation depends on how heat penetration parameters comply with certain restrictions established in the model.

Heat penetration curves and parameters can also be calculated mathematically, thus avoiding carrying out new experimental heat penetration assays when the retort or the size of the can is changed, for example. The models for carrying out these calculations are known as deterministic models, and they make use of a numerical solution by finite differences in the two-dimensional partial differential equation that describes conduction heat transfer in a finite cylinder.

The evolution of heat treatment procedures encouraged research on new models and tools to develop and validate thermal processes, not only from the point of view of safety but also from the point of view of retention of nutrients and sensory properties. Thus, the main objective of thermal process optimization is to maximize product quality while minimizing undesirable changes and cost. Optimization theory makes use of the different temperature sensitivities of microbial and quality factor destruction rates.

See also: *Clostridium botulinum*; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; *Clostridium*: Occurrence and Detection of *Clostridium perfringens*; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Heat Treatment: Principles and Techniques; Irradiation of Foods: Processing Technology and Effects on Nutrients: Effect of Ionizing Radiation on Food Components; *Listeria*: Detection; *Listeria*: Properties and Occurrences; Packaging: Aseptic Filling; Pasteurization: Effect on Sensory Quality and Nutrient Composition; Pasteurization: Principles and Applications; Preservation of Foods; Pulsed Electric Fields; *Salmonella*: Detection; *Salmonella*: Properties and Occurrence; Shigella; Spoilage: Bacterial Spoilage; Spoilage: Yeast Spoilage of Food and Beverages; *Staphylococcus*:

Staphylococcus: Occurrence and Properties; Sterilization of Foods; Storage Stability: Shelf Life Testing; Yeasts.

Further Reading

Bigelow WD (1921) The logarithmic nature of thermal death time curves. *Journal of Infectious Diseases* 29: 528–536.
Brul S, van Gerwen S, and Zwietering M (2007) *Modelling microorganisms in foods*. Cambridge/Boca Raton, FL: Woodhead Publishing Limited/CRC Press.
Gould GW (1989) *Mechanisms of action of food preservation procedures*. London: Elsevier Applied Sciences.
Hallström B, Skjöldebrand C, and Trägardh C (1988) *Heat transfer and food products*. London: Elsevier Applied Sciences.
Olson JC and Nottingham PM (1980) Temperature. In: Silliker JH, Elliot RP, Baird-Parker AC, Bryan FL, and Christian JHB (eds.) *Microbial ecology of foods. Factors affecting life and death of microorganisms*, vol. I. New York: Academic Press.
Richardson P (2001) *Thermal technology in food processing*. Boca Raton, FL/ Cambridge: CRC Press/Woodhead Publishing Limited.
Richardson P (2004) *Improving the thermal processing of foods*. Boca Raton, FL/ Cambridge: CRC Press/Woodhead Publishing Limited.
Smelt JPPM and Brul S (2014) Thermal inactivation of microorganisms. *Critical Reviews in Food Science and Nutrition* 54: 1371–1385.
Stumbo CR (1973) *Thermobacteriology in food processing*. New York, London: Academic Press Inc.
Sun DW (2012) *Thermal food processing: new technologies and quality issues*, 2nd ed. Boca Raton, FL: CRC Press.

Relevant Websites

http://www.campdenbri.co.uk/.
http://www.combase.cc/index.php/es/.
www.ift.com.
http://www.iftps.org/.
https://irisk.foodrisk.org/Default.aspx.
http://pmp.errc.ars.usda.gov/PMPOnline.aspx.