Technical Elements of New and Emerging Non-Thermal Food Technologies

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Technical Elements of New and Emerging Non-Thermal Food Technologies

Part I: Technical Elements

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PART I: TECHNICAL ELEMENTS

CHAPTER 1. INTRODUCTION

Consumer demands for high quality foods that are fresh tasting and nutritious have created considerable interest in the development of new food-processing techniques. Traditional food-processing technologies such as freezing, canning, and drying rely on heating or cooling operations. Although these technologies have helped to ensure a high level of food safety, the heating and cooling of foods may contribute to the degradation of various food quality attributes. The colour, flavor, and texture of foods processed solely by heating may be irreversibly altered. To ameliorate the undesirable thermal effects on foods, considerable effort has been made in commercial and academic circles to develop non-thermal technologies that rely on techniques other than heating or cooling operations.
During the past two decades, numerous papers have been published in the literature that describe research using these emerging technologies (1). Because these processing techniques have little or no thermal effects on foods, they are commonly referred to as **non-thermal preservation technologies**. Among these emerging technologies, the most promising ones for food application are high-pressure processing, use of pulsed-electric fields, and application of pulsed light. This review provides a technical description of each of these technologies, along with a discussion of their applications in food processing.

**CHAPTER 2. HIGH-PRESSURE PROCESSING**

High-pressure treatment of foods involves subjecting food materials to pressures as high as 9 000 times the atmospheric pressure. Pressure is applied uniformly throughout a food material, independent of its mass and time. Use of high pressure in food processing is an extension of a technology that is commonly employed in many other industrial processes, notably in the manufacturing of ceramics, diamonds, super-alloys, and sheet metal forming. Similarly, high isostatic pressures are routinely used in the manufacturing of polymeric compounds, such as for the synthesis of low-density polyethylene and in chemical reactors for the manufacturing of quartz crystals. Although commercial interest in the use of high-pressure technology in food processing has occurred only since the early 1990s, the effects of high pressure in inactivating microorganisms have been known for more than a century.

In 1899, one of the earliest investigations using high pressures in food processing involved the application of pressures in the range of 5 000 to 7 000 kg/cm² (see Table 1 for unit conversions) to reduce microbial levels in milk and meats (2). It was shown that a five- or six-log-cycle reduction in bacterial count was possible in milk when it was subjected to a high pressure of 6 800 kg/cm². Increased shelf life of meats was also observed when meat samples were pressurized to 5 400 kg/cm².

Around the turn of century, the effect of high pressure (6 000 kg/cm²) on coagulation of egg albumin was observed. Other studies showed that high-pressure processing was beneficial in extending the shelf life of processed fruits. These early studies demonstrated that application of high pressure had effects similar to the use of high temperature on proteins and microbial population in foods.

### 2.1 Equipment Considerations

The key components of a high-pressure system are the pressure vessel, pressurizing system, and ancillary components (Figure 1).
Figure 1. A typical high-pressure processing system for treating pre-packaged foods.

2.1.1 Pressure Vessel

A high-pressure vessel, in which products under treatment are subjected to pressure, is the key component of this technology. Pressure vessels are generally made of low-alloy steel and are routinely used in the ceramic and metal industries. However, in the case of food applications, a unique requirement for the high-pressure vessel is that it must undergo several thousand processing cycles per year to process large volumes of foods. The large number of required pressurized and depressurized cycles increases metal fatigue and reduces the life of the vessel. Furthermore, the vessel itself must be protected from any corrosion due either to the food material itself or to any liquids used for cleaning, and must be easy to clean.

2.1.2 Pressurizing Systems

Two types of pressurization systems defined as indirect and direct, are commonly employed in the industry.

In an indirect pressurization system, the pressurizing medium (e.g., water) is first pumped through an intensifier, into the pressure vessel. As shown in Figure 2, the intensifier is a high-pressure pump used to increase the pressure to desired levels. The intensifier is separate from the high-pressure vessel. This system requires high-pressure tubing and appropriate fittings to convey the pressurized medium to the pressure vessel.
Figure 2a. A 190 litre QUINTUS Press with external pumping system.
In a **direct pressurization system**, the pressure intensifier is located within the pressure vessel (Figure 3b). In this system, both pressure intensifier and the vessel are fabricated as a single unit, and the total size of the vessel can be quite large. A piston is used to deliver the high pressure to the product. This system requires heavy-duty seals that must withstand repeated opening and closure without leakage. A major limitation of this method is the need for efficient seals between the pressure vessel and the piston.

In the **wet bag configuration**, which is more suitable for food processing, a mould is first filled with the material outside the pressure vessel. The filled mould is then moved into the pressure vessel containing a pressure medium. With cold isostatic pressure, water is used as the pressure medium. In the **dry bag configuration**, the mould is fixed in place within the pressure vessel. The material to be treated is filled into the mould. The mould remains separated from the pressure medium by an elastomer tool.
In high-pressure processing, inert gases or water are the most commonly used pressure media. The relative incompressibility of water compared with gases makes it the preferred pressure medium in many applications. The decrease in volume of water is about 5 percent when its pressure is increased from 0 to 4 000 kg/cm² at 22° C. This volume reduction is much smaller compared with inert gases, where high volume reductions can make operations more hazardous. When water is used as a pressure medium when subjecting food materials to high pressure, there is instantaneous and uniform transmission of the pressure throughout the product being treated. Typically, small amounts of oil may be added into the water for anticorrosive and lubricant purposes.

2.2 Modes of Operation
A simplified mode of operation used in a high-pressure processing system is shown in Figure 4. In a high-pressure process, the pressure vessel is filled with a food product and pressurized for a desired time, following which it is depressurized.

Figure 4. A schematic flow diagram of high-pressure processing.
The time required to pressurize the vessel is influenced by the compressibility of the pressure medium and the nature of the food material. If water is used as the pressure-transmitting medium for most food materials, compressibility is similar to that of the pressure medium. Typically, the pressurization time for foods is independent of the quantity of food placed in the pressure vessel. The presence of air in the food increases the pressurization time, since air is considerably more compressible than water. After pressurization, the food is kept under high pressure for the required process time, which may be for several minutes. Upon completion of the pressure exposure, depressurization can be done quite rapidly.

2.2.1 Batch Processing Mode

High pressure processing in the batch processing mode offers several advantages in that different types of foods can be processed without cross-contamination, there is no need for clean up between runs, the equipment is relatively simple, and there is no risk of large quantities of foods becoming contaminated in case of equipment malfunction. Several pressure vessels may be operated in a controlled sequence to minimize any time lag associated with the time required for the pressurization of vessels.

Most of the high-pressure equipment currently used is operated in the batch mode. Since pressurizing and depressurizing steps can be rapidly accomplished, the low efficiency associated with batch processing may be minimized. Rapid pressurizing and depressurizing cycles also can cause metal fatigue and reduce the life of equipment. Above 4000 kg/cm², the weight of equipment increases significantly, as does its cost.

2.2.2 Semicontinuous Processing Mode

Another approach to high-pressure treatment of liquid foods is the use of a semicontinuous processing mode (3). This system involves a combination of multiple pressure vessels that are sequenced to provide a continuous flow. As shown in Figure 5, while one vessel is being pressurized, another may be in a decompression mode. This approach has been commercially used by companies such as the Wakayama plant in Japan, for the treatment of tangerine juice. In this process, three 50 litre pressure cells are sequenced to achieve a production rate of 4000 litre per hour (3). The pressure system, known as ACB high-pressure liquid processor (GEC Alsthom ACB, France) is equipped with a chamber having an internal volume of 4 litres. The compression process is done with water up to a maximum pressure of 400 MPa. Programmable pressure controllers are used to adjust pressurization and decompression rates. Appropriate temperature controls are used to maintain temperatures between -20° C and +80° C. This unit has been used in selected processing steps in wine production. The reduction in cost of a semicontinuous process is about 27 percent over a batch process for 500 litre per hour production (3).
Another continuous high-pressure system involves 5 metre long stainless-steel pipes that are wound like a coil with a pressure resistance of 700 MPa (4). An air-driven hydraulic pump is used to introduce liquid product into the pipes. With the outlet valve closed, the liquid is subjected to pressure. The coiled pipes are placed in thermostatically controlled water baths, in which the temperature is maintained between 5 and 80° C. The outlet valve is gradually opened to release the pressurized product in a continuous manner.

Other innovations in high-pressure system design include the use of pulsating high pressures (5). The pressure vessel is similar to those used in cold isostatic pressing. A unique feature of this new system is an air-driven pressure-increasing device that allows instantaneous change in pressure. Additionally, a pressure-reducing valve attached to the pressure vessel is useful in releasing pressurized water. By manipulating the pressure-reducing valve, desired pulsations are obtained. The pressure vessel is contained in a thermostatically controlled water bath. The investigators were able to achieve 500 MPa in 10 seconds. Reduced process times at high pressures were obtained when used in combination with higher temperatures. These studies emphasize the synergistic benefit of pressure and temperature in selected food applications.
The cost of high-pressure processing is dependent upon the combination of pressure, pressure holding time and temperature at which the product is processed (6). These variables must therefore be carefully selected. The cost per unit of production is lower for a single large production unit than for several small pressure units in parallel (6). This cost saving is possible because the capital cost of manufacturing a large pressure unit is lower than for manufacturing several small units.

2.3 Examples of Industrial-Scale High-Pressure Systems

The following two examples of industrial equipment for high-pressure applications are provided for illustration purposes. High-pressure equipment, manufactured by ABB Pressure Systems, AB, has been largely used for synthetic diamond manufacturing, sheet metal forming and for the extrusion of metal. Equipment developed specifically for food processing includes the QUINTUS Food Press (Figure 2). The pressure vessel is pre-stressed using a spring steel wire and remains in a pre-stressed state even under pressure. A replaceable liner is inserted into the cylinder for additional safety of operation. The press uses a retractable frame fabricated of pre-stressed wire winding in order to keep the top and bottom closures safely in place (Figure 2). The press is pressurized with an external pressure intensifier. Other designs involve a pump built into the press to obtain a wide range of pressure. As a total system, the QUINTUS Press may be incorporated in a bulk processing line, where the product is kept within large bulk containers during processing and storage, as shown in a conceptual drawing in Figure 6. As an alternative, food in retail-size packages, placed in a loading basket, may be processed under pressure and later transported directly for retail sales, as shown in Figure 7.
Figure 6. A bulk processing line for high-pressure treatment of foods contained in bulk packages. The contents are later transferred into retail packages.

Figure 7. High pressure processing of consumer packages.
2.4 Examples of Pilot-Scale High-Pressure Systems

A pilot-scale ultra-high-pressure food processor manufactured by Flow International Corporation (Kent, Washington) is shown in Figure 8. This unit was developed for use with pumpable products. The food is pumped into the pressure vessel, the pressure is raised and held for the required time and the food is discharged into filling containers by a computer-controlled process. An ultra-high-pressure pump (FLOW WaterNifeâ ) is used to pump the food into the chamber. In a multi-chamber system, filling, pressurizing and discharging operations can be appropriately sequenced to achieve maximum production rates.

![Figure 8. A high pressure processing system for pumpable foods.](image)

2.5 Commercial Applications of High-Pressure Technology in Food Processing

Some of the requirements for the suitability of a high-pressure system for food applications are as follows:

- Short cycle time for inactivating microorganisms and enzymes
- Safe to operate
- Easy to clean
- Accurate and reliable pressure control
- Low capital and operating costs.
Commercial application of high-pressure processing was first realized in Japan in 1992, when a Japanese company, Meidiya Foods, introduced jams processed with this new technology on the Japanese market. The products were well received by consumers. Since then, other products processed using this technology in Japan have included fruit juices, ice cream, Japanese unrefined rice wine and rice cakes containing herbs, such as *Yomogimochi* (7). These commercial applications of high pressure processing have also spurred interest in conducting research on high-pressure processing. More than 70 food companies and governmental institutions in Japan had acquired laboratory-scale equipment for testing as of 1992 (7).

During the last decade, numerous publications that describe the influence of pressure on various constituents and contaminants of foods such as spoilage microorganisms, food pathogens, enzymes, other food proteins and lipids have appeared in the literature. A diverse range of foods, including fruit juices, jams, vegetables, milk, yogurt, cheese, fish, pork and beef (Table 2), has been subjected to high-pressure treatments. In contrast to thermal treatment, high-pressure processing does not break covalent bonds in foods and thus preserves flavour. The effect of high pressures on enzymes is largely due to the denaturation of proteins. The effect of high-pressure processing on enzyme kinetics, other chemical reactions such as the Maillard reaction (which causes browning) and lipid oxidation (which leads to off-flavors in fat-containing foods) are the focus of current research.

Most studies indicate that the beneficial effects of high-pressure processing of foods are evident only when applied pressures exceed 400 MPa. Vegetative bacterial cells are inactivated by pressures between 400 and 600 MPa. Cell membranes of these organisms are damaged by high-pressure processing and they therefore cannot reproduce. Once damaged, the cells are unable to control the transport of water and ions across the membranes, leading to collapse of the cells. Under favorable conditions, however, the cells may repair themselves. Much higher pressures (pressures in excess of 800 Mpa) are required to inactivate bacterial spores. A pressure of 408 MPa for 2 minutes was sufficient to achieve a 6-log reduction of anaerobic plate count (APC), yeast and *E. coli* in apple juice (15).

Food materials may be subjected to high pressures either in bulk or as packaged foods. The advantages and disadvantages of these procedures have been discussed in the literature (16). When foods are first packaged and then pressurized, either as liquids or solids, there is no danger of post-processing contamination. However, packaged foods require more complex handling procedures. The filling efficiency in a pressure vessel is generally 50 to 70 percent, because of the geometrical shape of the packages. In addition, considerable time is required for loading, unloading, filling and venting of the vessel. Common packaging materials used are ethylene vinyl alcohol copolymer (EVOH) and polyvinyl alcohol (PVOH).

With bulk processing, the batch method is more suitable for pumpable foods, because the handling of these foods is simple. After processing, the product can be packaged using a variety of packaging materials such as glass or metal. The vessel is more fully utilized (up to 90 percent with food material) and a minimum amount of time is required for loading and unloading. Furthermore, the vessel does not require opening and closing. This method can also be made semicontinuous with the use of multiple pressure vessels operated in a desired sequence. Often there is a need for aseptic filling to avoid
post-processing contamination and all contact between food and equipment components must meet aseptic standards.

Another interesting application of high pressure is to store food materials at subfreezing temperatures under high pressure without actually freezing the food (17). The freezing point of water decreases with increasing pressure. Thus, a food under pressure may be kept at subfreezing temperature in an unfrozen state, minimizing the deleterious effects of ice crystal formation on food quality.

Because of the rapidity with which high pressure is transmitted throughout a food system, the size and geometry of the object being treated is not as critical as in the case of traditional thermal processing (17). In high-pressure processing, the need for size reduction may be eliminated, thus minimizing losses of nutrients and consequent environmental pollution. Similarly, high-pressure processing offers potential advantages due to low-temperature processing. A significant reduction in the leaching of cell constituents from potato cubes when blanched under pressure was achieved compared with traditional blanching (18). In high-pressure processing, the gelling phenomenon in proteins is different from that obtained with thermal treatment. This uniqueness provides new opportunities to create desired functionality of engineered foods. The increase of membrane permeability achieved with high pressure can be effectively used in controlling mass transfer in many food processes such as frying, blanching and dehydration (18).

As seen in Table 2, applications of high pressure processing include increasing the shelf life of foods and creating unique structural changes in foods that provide benefit for desired functions. Many of these changes influence the quality characteristics of foods. At present, there is a lack of sufficient data to describe completely the mechanisms and kinetics of reactions that influence the quality of foods when they are processed under high pressure. These are topics of current and future study.

CHAPTER 3. PULSED ELECTRIC FIELDS

As in the case of high-pressure processing, the beneficial effects of pulsed electric fields in reducing microbial levels in foods have been known for several decades. The pasteurizing effects of electric fields in foods were first observed in the early 1900s. According to Beattie and Lewis (19), the bactericidal effect observed in electrically treated milk supplied to the city of Liverpool in England was not only due to heat generated during the process, but was also due to the electric field itself.

During the 1960s, procedures were developed to create pores in cell membranes by subjecting cells to high voltages. A natural pressure gradient exists across cellular membranes, so that when a cell is placed in an electric field, the transmembrane potential increases. If the applied electrical field exceeds a certain critical value, then cell wall rupture occurs. While the complete mechanism of cell wall breakdown in an electric field is not clearly understood, this observed phenomenon is regularly applied in order to create pores within cell membranes. The technique involved is known as electroporation and is applied in the field of biotechnology for the introduction of foreign DNA into cells.
In another application, cells are fused together when placed in an electric field. This process termed electrofusion has been used in the food industry to convert nonflocculant brewer's yeast to flocculant yeast (20).

3.1 Technical Considerations

A simplified schematic design of a pulsed electric field (PEF) system is shown in Figure 9. The main components are the high voltage generator, switch, capacitor and electrodes.

![Figure 9. A simplified general design of a pulsed electric field apparatus.](image)

The microbial inactivation in foods due to an imposed electrical field is dependent on the strength of the electric field, treatment time (which is in turn determined by the number of pulses applied) and the pulse type. Several technical issues that are important in the industrial application of PEF have been noted (21). These include:

- Determining the optimum electric field strength for inactivating bacteria
- Provision to cool the food material that heats up due to Joule heating effects
- Dielectric breakdown in foods
- Proper selection of power and flow rates
- Operational safety issues.

3.2 Pulse Generation
Food materials contain ions that make them good conductors of electricity. When a large flux of electrical current flows through a food material, a high voltage pulsed electric field is generated within the food. The electric current is allowed to flow through the food material for an extremely short period of time (of the order of microseconds). A capacitor is therefore needed to generate pulses. The capacitor slowly charges and then quickly discharges its stored electrical energy.

Two types of pulses, namely exponential decay and square pulses, have been considered for PEF applications. An electrical circuit shown in Figure 10 may be used to generate an exponential decay pulse (21), while that shown in Figure 11 may be used to generate a square pulse. In square pulses, the voltage increases instantaneously to a peak value, where it is held for some time before decreasing to zero almost instantly. With exponential pulses, the long tail section of the pulse is not effective in killing bacteria. On the other hand, it generates excess heat. Square pulses can maintain their peak voltage for a longer time than can exponential pulses. Square pulses in addition generate less heat than do exponential pulses. Although more complex circuits are required for the generation of square pulses, they are preferred for their advantages in food applications.
While PEF is desirable for microbial inactivation, it causes undesirable arcing or dielectric breakdown in a material. Arcing occurs when the applied field strength becomes equal to the dielectric strength of a material. When a liquid food is subjected to PEF, vapor bubbles present cause arcing. Gases or vapors have a much lower dielectric strength than do pure liquids. Roughness of the electrode surface also causes dielectric breakdown of food materials. Zhang et al. (21) recommend consideration of the following points in order to avoid the occurrence of arcing:

- Use of smooth electrodes
- Use of carefully designed treatment chambers to provide uniform electric field strength
Degassing
- Pressurizing the liquid in the treatment chamber to prevent bubble formation.

3.3 Design of PEF Treatment Cells

Several different designs of PEF treatment chambers have been investigated (22). A static chamber used at Washington State University contains disk-shaped electrodes (area 27 cm²) made of stainless steel polished to a mirror-like surface, with a gap that could be set at either 9.5 or 5.1 mm. Electric field strengths of up to 70 kV/cm can be used in this model. Electrodes contain built-in jackets that allow the circulation of water in order to maintain low temperatures. A modified version of this static cell has been used for continuous application. Baffled flow cells within the treatment chamber facilitate continuous pumping of liquid food through the cell. A pulse width of 2 to 15 microseconds with a repetition rate of 1 Hz has been tested and the flow rate of the test liquid food through this cell was reported to be either 1 200 or 600 cm³/min.

In studies conducted with PEF systems, it is recommended that high electric fields and short time pulses be used to minimize heat generation due to the Joule heating effect and thus thermal degradation of the treated food. There are operational problems with the use of monopolar pulses (21). These occur due to the fact that many constituents of food material, such as electrolytes, proteins and living cells, have a net electric charge and thus a tendency to accumulate on charged electrode surfaces. This therefore results in the creation of a shielding layer at the electrode surface, making the electric field nonuniform. Undesirable shielding layers are prevented with the use of bipolar pulses (21).

3.4 Applications of PEF Treatment in Food Processing

A PEF unit for treatment of fresh orange juice at a pilot scale was described by Qiu et al. (23). This system consisted of a continuous pilot-scale PEF unit integrated with an aseptic packaging machine, as shown schematically in Figure 12. A 40 000 V/17 MWp high voltage pulse generator with a multiple state cofield PEF treatment chamber was used by these investigators. An aseptic packaging machine was used to package PEF-treated food under either nitrogen or sterile air headspace. The pumping system (Moyno pump) was used to transport juice at a uniform rate ranging between 75 and 200 litres per hour. The pulse generator, shown in Figure 12, had a 40 kV command charging power supply. For switching, a 0 kV/5 kA hollow anode thyatron was used. The maximum repetition rate of the pulse generator was set at 1 000 Hz. Different pulse shapes; namely, square wave, exponential decay wave and an underdamped RLC waveform could be produced through alteration of the network. A set of cofield tubular treatment chambers with cooling capabilities was also used. The diameter of the treatment zone was 0.48 cm and the separation between the electrodes was set at 0.48 cm. The system was operated at 30°C and the feed was allowed to flow through 12 PEF treatment chambers. A system flow rate of 75 litres per hour was obtained, with an average of 3.3 pulses delivered to the feed stream in each cell. These investigators
concluded that the PEF treatment inactivated 99.9 percent of microbial flora, with the square waves being most effective. Compared with heat pasteurization, the PEF-treated orange juice retained more vitamin C and flavor.

As seen in the preceding example, the goal of many studies using PEF treatments is to extend the shelf-life of foods by minimizing spoilage caused by microbial growth. In these studies, the level of microbial reduction is a key parameter for evaluating foods treated with PEF against those treated using traditional technologies.

Some illustrative examples of foods treated with PEF are shown in Table 3. These applications are still under development stage. Considerable more research is necessary to obtain data on the effects of PEF treatments on the sensory properties as well as nutritional content of foods.

Figure 12. A simplified setup for an integrated pulsed electric field and aseptic system for juice processing.

CHAPTER 4. PULSED-LIGHT TREATMENT
Pulsed-light treatment involves the use of a flash of high-intensity light for the purpose of killing microorganisms on the surface of either food or packaging materials. This procedure, developed under the trade name PureBright (PurePulse Technologies, Inc., San Diego, California, USA), uses a light spectrum containing wavelengths ranging from ultraviolet to near-infrared. The light spectrum generated with the use of this equipment is similar to that of sunlight reaching the earth's surface. As shown in Figure 13, the peak of intensity is in the blue-violet region and the PureBright spectrum contains wavelengths in the 200 to 300 nm region, which are not present in sunlight reaching the earth's surface. Sunlight, on the other hand, has more radiation in the infrared region than does PureBright. The intensity of PureBright is 20,000 times that of sunlight measured at the earth's surface. The intense flashes of light produced by the PureBright system are used in the destruction of microorganisms.

![Figure 13. A comparison of Pure-Bright spectrum with sunlight spectrum at sea level.](image)

4.1 Equipment

The pulsed-light system essentially consists of two components: the power unit and the lamp unit.

The power unit is used to generate high voltage, following which resulting high-current pulses are employed in the lamp. As shown in Figure 14, AC power is first converted to high voltage DC power, which is then used to charge a capacitor. After the capacitor is
charged to certain voltage, a high voltage switch discharges the capacitor into a lamp. The system is properly contained in order to protect personnel from high voltage (Figure 15). Cooling water is used to minimize heating of the treated product.

Figure 14. The pulsed light generation system (Courtesy: PurePulse, San Diego).
The treatment unit contains one or more inert gas lamps (Figure 16). When a high current pulse is applied to the lamp, gas contained within the lamp emits an intense pulse of light. The frequency of flashing, number of lamps and flashing configuration depend on the treatment application.
4.2 Monitoring Controls

Monitoring of the lighting system is extremely important to ensure that the treatment area is properly treated. The PureBright system uses two types of diagnostic monitors, the lamp output (fluence) and the lamp current. Fluence is the measure of incident light energy per unit of surface area (J/cm²). Lamp fluence is measured in order to ensure that ultraviolet fluence generated by the lamp is sufficient for the inactivation of microorganisms. This is accomplished with the use of a silicon photodiode that is capable of detecting whether the lamp has the required output of ultraviolet light. A decreasing output would signal the need for replacement of the lamp. This control is also required in order to shut down the operation in cases where objects do not receive treatment above some predetermined threshold level (Figure 17).

A second monitoring control used in the PureBright system measures the lamp current for every flash. The current level is an indication of the intensity and spectrum of radiation. If the current level falls below a preselected threshold, the operation is shut down.
4.3 Operating Procedures

The PureBright system involves illuminating the desired treatment area with 0.1 to 3 J/cm² per flash, with total accumulated fluences of 0.1 to 12 J/cm². Flashes are, in general, applied at a rate of 0.5 to 10 Hertz, for duration of several hundred microseconds. Figure 18 shows a system set up for the treatment of packaging materials.
4.4 Application of Pulsed Light Systems in Food Processing

The application of pulsed light can reduce up to 9 logs of vegetative microorganisms and more than 7 logs of bacterial spores on smooth, nonporous surfaces such as those of packaging materials. When the surfaces are more complex and porous, such as in case of food materials, microbial reduction is of the order of only 2 to 3 log cycles.

The pulsed light systems affect only the surface of the product being treated. Photoproducts resulting from this treatment are much fewer than those produced by thermal treatments, thus minimizing product degradation. Pulsed light treatment has been reported to be effective in extending the shelf life of foods such as bread, shrimp and meats (26). Pulsed light is effective in treating water, owing to the transparency of water and the fact that it permits the penetration of light. The reported costs of equipment amortization, lamp replacement, electricity and maintenance indicate expenditures of only a few tenths of a cent (U.S.) per square foot of treated area (24).

CHAPTER 5. OSCILLATING MAGNETIC FIELDS

Published studies in the literature show contradicting results on the inhibition of microorganisms when placed in oscillating magnetic fields (OMF). Some studies indicate that magnetic fields have an inhibitory effect on microbial populations, while others note no effect or in some cases even a stimulating effect. Mechanisms describing these observations are under scientific inquiry. In one study, foods with high electrical resistivity were placed within a magnetic coil in an apparatus and subjected to one or more pulses of OMF with an intensity of 2 to about 100 Tesla and a frequency of 5 to 500 kHz (27). It was observed that a single pulse of magnetic field generally decreased the microbial population by at least two orders of magnitude. OMF involves little thermal energy input, thus avoiding thermal denaturation of food constituents during treatment. However, more research is needed to understand the changes in microbial population and other constituents of foods when treated with OMF.

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**Figure 13.** A comparison of Pure-Bright spectrum with sunlight spectrum at sea level (Courtesy: PurePulse, San Diego).

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</tr>
</thead>
<tbody>
<tr>
<td>atmosphere standard</td>
<td></td>
<td>1</td>
<td>0.101325</td>
<td>1.01325</td>
<td>1.0332</td>
</tr>
<tr>
<td>MPa</td>
<td>9.8692</td>
<td>1</td>
<td>10</td>
<td>10.197</td>
<td>145.0377</td>
</tr>
<tr>
<td>bar</td>
<td>0.98692</td>
<td>0.1</td>
<td>1</td>
<td>1.0197</td>
<td>14.50377</td>
</tr>
<tr>
<td>kg/cm²</td>
<td>0.9679</td>
<td>0.0981</td>
<td>0.9807</td>
<td>1</td>
<td>14.2236</td>
</tr>
</tbody>
</table>
Table 2. Some examples of products processed using high-pressure technology and changes in quality attributes other than microbial changes.

<table>
<thead>
<tr>
<th>Product</th>
<th>Process and Quality Attributes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avocado Puree</td>
<td>Prevent discolouration. Inhibition of undesirable browning reactions in presence of low pH.</td>
<td>(8)</td>
</tr>
<tr>
<td>Banana Puree</td>
<td>Prevent discolouration. Reduction in Polyphenoloxidase activity when combined with blanching.</td>
<td>(8)</td>
</tr>
<tr>
<td>Black Beans</td>
<td>Cooking. Increased water absorption and reduced cooking time.</td>
<td>(8)</td>
</tr>
<tr>
<td>Cheese</td>
<td>Rennet coagulation. Reduction in rennet coagulation of milk.</td>
<td>(9)</td>
</tr>
<tr>
<td>Jam</td>
<td>Commercial Production (Meiji-ya, Japan). Improved retention of colour and flavor of fresh fruit.</td>
<td>(10)</td>
</tr>
<tr>
<td>Meats</td>
<td>Thawing. Reduction in drip loss and minimal colour change</td>
<td>(11)</td>
</tr>
<tr>
<td>Meats, tenderized</td>
<td>Commercial production (Fuji Chiku and Mutterham, Japan). Improved retention of sensory characteristics.</td>
<td>(10)</td>
</tr>
<tr>
<td>Orange juice, fresh-squeezed</td>
<td>Preservation. Retention of colour and cloud stability during storage.</td>
<td>(8)</td>
</tr>
<tr>
<td>Pink grapefruit juice, fresh-squeezed</td>
<td>Preservation. Retention of colour and cloud stability during storage.</td>
<td>(8)</td>
</tr>
<tr>
<td>Pork sausage</td>
<td>Manufacturing. Moister, denser, and more tender sausages with more retention of colour than if heat treated.</td>
<td>(8)</td>
</tr>
<tr>
<td>Potato</td>
<td>Freezing. Reduction in freezing time in potato cylinders</td>
<td>(8)</td>
</tr>
<tr>
<td>Product</td>
<td>Process and Quality Attributes</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Rice paste with herbs</td>
<td>Commercial Production (Japan). More desirable sensory properties than if heat treated.</td>
<td>(7)</td>
</tr>
<tr>
<td>(Yomogimochi)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soya proteins</td>
<td>Manufacturing. Less firm but more elastic and extensible gels. Improved preservation of colour and initial aroma.</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surimi</td>
<td>Control of Enzyme activity. Enhanced activity of transglutaminase in surimi with increased gel strength</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surimi, Pacific Whiting</td>
<td>Gelation. Increased gel strength in surimi.</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tofu</td>
<td>Freezing. Production of small-size ice crystals.</td>
<td>(13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato juice</td>
<td>Juice production. Modification of physical and sensory characteristics deemed desirable.</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yogurt</td>
<td>Storage. Reduced syneresis.</td>
<td>(14)</td>
</tr>
</tbody>
</table>

Table 3. Examples of foods processed using pulsed-electric fields and change in their quality attributes other than microbial.
<table>
<thead>
<tr>
<th>Food Product</th>
<th>Processing Method</th>
<th>Notes</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice, fresh-squeezed</td>
<td>Pasteurization. Minimal loss of flavor compounds, colour and vitamin C.</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Salsa</td>
<td>Preservation. Better flavor and appearance than comparable products</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Spaghetti Sauce</td>
<td>Aseptic processing. Acceptable after 2 years and 80° F storage.</td>
<td>(8)</td>
<td></td>
</tr>
</tbody>
</table>

**PART II: SAFETY ELEMENTS**
CHAPTER 7. INTRODUCTION

Interest in the development of new food processing technologies has increased dramatically over the past decade primarily due to consumer demand for food with fresh-like taste, crisp texture and natural colour. Consumers are also increasingly becoming aware of food-borne disease hazards and are concerned about the safety of their food supply. Developments in non-thermal technologies have been advanced by both industry and academia in an attempt to meet the challenge of producing safe processed food of a high quality. There is no doubt that high quality food can be produced through the use of non-thermal processing technologies. The safety and microbiological quality of food processed using these technologies, however, needs to be affirmed. The safety of foods processed using selected non-thermal technologies will be addressed in this Chapter. Emphasis is given to high pressure processing (HPP), and processing with the use of pulsed electric fields (PEF) and pulsed light.

CHAPTER 8. NEW TECHNOLOGIES AND NEW SAFETY STRATEGIES

Food is deemed unsafe if it constitutes either a physical, chemical or biological hazard to the consumer. Physical hazards may result, for example, from the presence of sharp pieces of metal or glass in foods. Physical hazards are unlikely to increase when traditional technologies are substituted by novel technologies. Chemical hazards occur when deleterious substances occur naturally within the food, or are either intentionally or accidentally added to it. Hazardous chemicals (e.g. nitrosamine produced during the curing of meat) may also be produced during food processing. Information pertinent to the potential development of hazardous chemicals during food processing by non-thermal technologies is currently lacking. Chemical hazards associated with these new technologies will not therefore be addressed in this Chapter. Biological hazards are associated with the presence of pathogens, i.e. viruses, bacteria, fungi and parasites (Table 1) that cause food-transmitted diseases. Food safety is currently compromised more often by biological than by physical or chemical agents. Non-thermal food processing technologies therefore target maximum impact against such biological hazards.

Food processors currently rely on a variety of methods for food preservation. These conventional methods include heating, dehydration, freezing, and the addition of preservative ingredients. Heat is the most commonly used preservation factor and heat-treated food generally has a good safety record. When properly applied, heat can eliminate bacteria, fungi, viruses, parasites, and enzymes, which are the biological agents that cause spoilage or compromise food safety. The dosage of conventional preservation factors can be varied to accomplish microbial inactivation over a broad spectrum. For example, when heat is applied to milk at 71.6°C for 15 seconds, a 5-6 log kill of non-spore forming bacterial pathogens occurs, and the resulting product is considered pasteurized. However, heating milk at 145°C for a few seconds produces a commercially sterile Ultra High Temperature (UHT)-treated product. This UHT treatment is presumed to be a 12-D process when targeting *Clostridium botulinum* spores.
Non-thermal technologies have been advanced to replace conventional heat treatments. These new technologies cannot however achieve the broad microbial lethality that are currently attainable by heat treatment. Current PEF and HPP technologies can only accomplish the equivalent of pasteurization when applied at their maximum lethal doses. The achievement of commercial sterility by these non-thermal technologies is currently not feasible.

The application of non-thermal technologies to foods is more likely to result in stress or injury than to cause the death of microorganisms. An abundance of injured microbial cells in non-thermally-processed food may create new challenges to food processors and regulatory agencies. The detection of low levels of pathogens in food is a difficult task particularly when cells are injured. The safety of the non-thermally processed product is compromised if food and storage conditions favour the recovery of injured cells. Stress of pathogens by non-thermal technologies is a concern and the adaptation of cells to such stress may constitute a microbial hazard. Non-thermal technologies therefore introduce new challenges, and thus warrant the implementation of new safety strategies.

CHAPTER 9. KINETICS OF MICROBIAL INACTIVATION BY NON-THERMAL TECHNOLOGIES

The inactivation of microorganisms during food processing either by conventional or novel technologies, is dependent on (i) processing variables, (ii) properties of the treated food and (iii) characteristics of the treated microorganism. This Section will review the dependence of microbial inactivation on processing variables and will emphasize the contribution of these factors to inactivation kinetics.

Both thermal and non-thermal preservation technologies cause microbial inactivation in a dosage-dependent fashion. Where heat treatment is applied for example, temperature and time define the thermal process. The higher the temperature and the longer the heating period, the greater the degree of microbial kill. Dependence of microbial inactivation, at a given temperature, on treatment time follows a pattern similar to that of a chemical first-order kinetic model. Linearity of survivor plots makes it possible to measure inactivation rate parameters and allow for reasonable predictability of the treatment process. Data from survivor plots are commonly used to measure the decimal reduction time (D-value) using the following formula:

\[
D\text{-value} = -\frac{\log N_t}{t} = \log \frac{N_0}{N_t}
\]

where \(N_t\) is the count of survivors at time \(t\), and \(N_0\) is the initial count at time 0.

The D-value is an important parameter which describes thermal inactivation kinetics at a given temperature. In practical terms it is equivalent to the treatment time required to decrease the number of the treated microorganisms by one log cycle. A semi-
logarithmic plot of D-values vs. temperature (T) allows for measurement of a thermal resistance parameter or z-value, which is calculated follows:

\[ z\text{-Value} = \frac{T_1 - T_2}{B} \]

\[ \log D_1 - \log D_2 \]

where z-value represents the change in temperature required to cause a ten-fold change in the D-value. A concise review of kinetic models for fitting microbial inactivation data is provided by Xiong et al. (1).

The relationship between microbial inactivation and non-thermal treatment dosage is however more complex. Several interrelated processing variables (critical process factors) in non-thermal technologies require closer monitoring than in case heating.

### 9.1 High pressure processing

Critical process factors (parameters) in HPP include the process pressure (the constant holding pressure during the treatment), treatment time and process temperature. Process pressure varies between 100 and 800 MPa and is limited by equipment design. Total treatment time (in seconds or minutes) includes compression, hold-at pressure and decompression times. Process temperature is dependent on the initial temperature of the product, the amount of adiabatic heating generated during compression and eventually the regulation of temperature during processing.

Compression time (i.e., time required to attain a given high pressure, or "come-up time") varies with the design of the HPP equipment and the magnitude of the targeted pressure (process pressure). Treatment of a medium containing microorganisms (e.g., food) with high pressure, results in some inactivation during the pressure come-up time. However, measurements of inactivation kinetics are valid only during the pressure hold time.

Linear survivor plots (count of survivors at a given process pressure vs. pressure hold time) were reported when HPP was applied in the inactivation of vegetative microorganisms (2). Inactivation kinetic models, similar to those applied in thermal processing, have been fitted to these linear inactivation patterns (2,3). Linearity of these survivor plots allows for measurement of the D-value at the tested pressure. A series of D-values at various process pressures (P) may follow this model:

\[ z_P\text{-Value} = \frac{P_2 - P_1}{\log D_2 - \log D_1} \]

where \( z_P\)-value is the change in pressure required to cause a change in the D-value of one log cycle. Although linear survivor plots have been reported in scientific literature, a shoulder (an initial delay in inactivation) or a tailing effect is occasionally encountered (4,5). The foregoing discussion clearly shows the deficiencies of current thermal kinetic models in describing microbial inactivation by non-thermal technologies.
9.2 Pulsed Electric Field

As in case of HPP, inactivation of microorganisms by PEF is also dependent on several processing variables, food properties and characteristics of the treated microorganism. Processing variables of greatest significance (i.e., critical process factors) include electric field intensity (E), treatment time, treatment temperature, and the shape of the pulse wave (6). Treatment time is the product of the number of pulses received by the food and duration of the pulse. Exponentially decaying, square-wave and bipolar pulse wave shapes are commonly used in experimental PEF treatments. In general, the efficacy of PEF against microorganisms increases proportionally to the electric field intensity, total treatment time, and treatment temperature and with a square pulse wave.

It is generally agreed that the PEF process can be reasonably defined by the electric field strength and total treatment time. Monitoring these two critical process parameters allows a reasonably good prediction of microbial inactivation (7). Inactivation kinetics for PEF may therefore be simplified by plotting counts of survivors at a given electric field strength and the corresponding treatment times. For a linear survivor plot, the D-value at the tested electric field strength can be calculated as indicated earlier for thermal treatments. Measured D-values can then be described as a function of electric field strength using a dose-response model similar to that applied for heat treatment (B). Nonlinear data led investigators to search for alternative models, which better describe the kinetics of microbial inactivation during PEF processing. A kinetic model that correlates the fraction of survivors (N/N₀) with electric field strength (E), and treatment time (t) was applied by Hülsheger et al. (7) as follows:

\[ \frac{N}{N₀} = \left( \frac{t}{t_c} \right) k \]

Where \( t_c \) is a critical treatment time, \( E_c \) is a critical field strength and \( k \) is a constant.

More recently, Peleg (8,9) applied another kinetic model to sigmoid microbial inactivation curves resulting from PEF treatment. The model describes the ratio of survivors (N/N₀) as a function of the electric field strength (E) as follows:

\[ \frac{N}{N₀} = \frac{1}{1 + e^{\frac{E-E_d}{k'}}} \]

where \( k' \) is related to the slope of the steep segment of the dose-response plot, and \( E_d \) is a critical electric field value.

9.3 Pulsed light

Pulsed light technology uses intense pulses of white light to sterilize or decrease the microbial load on surfaces of packaging materials and potentially on food (10,11). The degree of microbial inactivation is dependent on the light intensity (measured in J/cm²) and the number of pulses (flashes) delivered (12). Pulsed light equipment is commonly
run at 1 to 20 pulses/s for a pulse duration of 200 to 300 μs (11). Three flashes of 1.5 J/cm² each inactivated more than 10⁶ spores of Bacillus stearothermophilus, B. subtilis, B. pumilus and Aspergillus niger (12).

Scarcity of research on pulsed light technology makes it difficult to describe the kinetics of microbial inactivation by this method. It is reasonable, however, to adapt the rules of thermal kinetics (as described earlier) to microbial inactivation data caused by pulsed light. Experiments to inactivate L. monocytogenes by non-pulsed UV energy produced sigmoid curves (13). Linear portions of these curves were however used to estimate D-values in order to compare treatments. It is likely that pulsed light also causes nonlinear inactivation patterns and thus models other than first-order inactivation kinetics need to be used.

CHAPTER 10. MECHANISMS OF MICROBIAL INACTIVATION BY NON-THERMAL TECHNOLOGIES

Microorganisms are affected to various degrees by processes that cause structural or functional damage to the cell. Results of this functional or structural damage include (a) inhibition of growth, (b) loss of the ability of cells to multiply (loss of viability), or (c) cell death (inactivation). The most common types of structural damage affect both the cell wall and the cytoplasmic membrane, leading to cell injury in mild treatments or cell lysis in case of severe processing. Microorganisms are also inactivated when subjected to processes that impair the functions of enzymes, DNA, ribosomes, or other essential constituents of the cell. This Section describes a number of potential mechanisms for the inactivation of microorganisms by the novel non-thermal technologies.

10.1 High pressure processing

High pressure is believed to disrupt the secondary and tertiary structures of macromolecules such as enzymes and to alter their function. It also disrupts ionic bonds in macromolecules but does not affect covalent bonds. HPP therefore causes the irreversible denaturation of proteins. According to LeChatelier's principle, pressure enhances reactions which lead to a decrease in volume and inhibits reactions which result in an increase in volume. Hydrophobic interactions among protein molecules under high-pressure causes a decrease in volume and thus these reactions are favoured during HPP (14). Accordingly, inactivation of enzymes by HPP is dependent on their relative hydrophobicity (15).

It therefore follows from the foregoing discussion that inactivation of microorganisms by HPP is associated with enzyme inactivation. Membrane damage is also believed to be the direct cause of microbial inactivation by HPP. Benito et al. (16) found that the uptake of fluorescent stains (ethidium bromide and propidium iodide) was greater in pressure-sensitive than in pressure-resistant strains of Escherichia coli O157. Since these stains enter bacterial cells having damaged membranes, it follows that membrane damage occurs during high-pressure treatment. Sohn and Lee (17) investigated spore
inactivation by HPP and obtained results which support the hypothesis that HPP causes the inactivation of microorganisms by causing membrane damage.

10.2 Pulsed electric fields

A widely accepted mechanism for cell inactivation by PEF is based on the concept of the electrical breakdown of the cell membrane (18,19). The cell membrane can be likened to a capacitor filled with a dielectric substance having a dielectric constant of 2, when compared to water, which has a dielectric constant of 80. Free charges therefore accumulate on both sides of the membrane, the normal resting potential difference across the membrane being 10 mV. The application of electric field pulses across the membrane however causes an increase in the trans-membrane potential, causing attractive forces between the positive and negative charges on the opposite sides of the membrane to compress it, thereby reducing its thickness. Local breakdown of the membrane occurs when the applied electric field reaches a value sufficient to build a 1-V potential across the cell membrane. This breakdown is reversible if the size and number of the resulting pores are relatively small compared to the total membrane surface. Irreversible breakdown occurs at higher field strengths thus causing inactivation of the cell. A critical electric field strength ($E_c$) should be applied before a transmembrane potential of 1 V is attained and cell inactivation occurs. Systematic studies into the effect of pulsed electric fields on the inactivation of microorganisms were conducted by Sale and Hamilton (20, 21) and Hamilton and Sale (22). According to these workers, intense electric pulses cause either permanent or temporary loss of the integrity of microbial cell membranes. Their calculations showed that a minimum potential difference of 1 V across the membrane of the microorganism was required for loss of function as a semipermeable barrier between the cell and its environment.

The value of $E_c$ varies with cell diameter (23). According to these authors, cells having a diameter of 1 μm require an $E_c$ value of 10 kV/cm.

10.3 Pulsed light

Pulsed light includes wavelengths that range from the ultraviolet (UV) to the infrared regions (11). Short UV wavelengths (200 to 280 nm) are known to be lethal to food borne pathogens (13). It is therefore plausible to assume that the UV component of pulsed light contributes significantly to microbial lethality. UV wavelengths inactivate microorganisms through alteration of their DNA structures. Cell mortality occurs when excessive damage is caused by the UV dose. Contrary to this hypothesis, some researchers believe that the thermal effect of pulsed light is the cause of microbial lethality (24).

CHAPTER 11. RELATIVE SUSCEPTIBILITY OF MICROORGANISMS, COMPARISON WITH CONVENTIONAL TECHNOLOGIES

The inactivation of microorganisms during processing by both conventional and novel technologies is influenced by treatment parameters, properties of the food and characteristics of the targeted microorganisms. The susceptibility of food microflora to a given set of processing parameters is dictated by the physical and compositional
properties of the food, the genetic makeup of contaminating microorganisms and their physiological status.

The susceptibility of microorganisms to conventional processing has been extensively investigated. Some of the generalizations applicable to the relative susceptibility of microorganisms to these conventional technologies also apply to novel non-thermal processing methods. It is well established for example, that bacterial spores are resistant to all types of preservation processes, while vegetative bacterial cells are highly susceptible to most. Resistance to processing increases when microbial cells are at the stationary, rather than at the exponential phase of growth. In addition, microorganisms are generally more resistant to processing under conditions of low water activity, than under high water activity conditions. Food provides greater protection to microorganisms against inactivation by processing, than do simple microbiological media or buffers. Although these generalizations may illustrate the fundamental challenges that face the development of new food processing technologies, a more detailed picture and more comprehensive studies are required by the food industry in order to ensure the success of emerging non-thermal technologies. This Section describes various factors related to food or targeted microorganisms, which may account for differences in the susceptibility of microorganisms to the novel non-thermal technologies.

11.1 High pressure processing

The physical properties of a food have a minimal impact on HPP, when compared to conventional processing technologies. High pressure processing has been successfully applied to liquid, particulate and solid foods (e.g., juices, rice pudding, and cheese, respectively). Compositional properties of the food are, however, likely to have a considerable impact on the efficacy of HPP (25). Low pH and high aw increase the susceptibility of microorganisms to HPP. Oxen and Knorr (26) treated Rhodotorula rubra at 400 MPa in media with aw of 0.91 and 0.94 respectively, and observed the microorganism to have a higher barotolerance at the lower aw. Stewart et al. (27) pressure treated Listeria monocytogenes CA in media of different pH values, for 10 min at 353 MPa and 45°C. The initial population of 7x10⁸ Listeriae/mL decreased by approximately 3 log and 6 log cycles, at pHs of 6.0 and 4.0, respectively.

Bacteria, in the vegetative state, vary considerably in their sensitivity to HPP. Patterson and Kilpatrick (28) reported a 5 log reduction in counts of Yersinia enterocolitica, Salmonella Typhimurium, L. monocytogenes, S. Enteritidis, E. coli O157:H7 and Staphylococcus aureus when these cultures were pressure treated at 275, 350, 375, 450, 700 and 700 MPa, respectively for 15 min. These researchers discovered wide variability in sensitivity to pressure among strains of the same species. Benito et al. (16) also demonstrated variability in the sensitivity of strains of E. coli O157 to pressure. Some pressure-resistant strains of this organism showed resistance to heat, acid and oxidative and osmotic stresses. Hauben et al. (29) isolated pressure-resistant E. coli by repeated pressurization of pressure-sensitive strains.

The barotolerance of microorganisms is generally greater at the stationary phase, rather than at the exponential phase of growth (30). Bacterial spores exhibit high resistance to HPP (31,32). Nakayama et al. (33) compared barotolerance and thermotolerance of different Bacillus spp. spores and reported that there was no correlation between the
barotolerance and thermotolerance of these spores. *C. botulinum* produces one of the most pressure resistant spores (34).

### 11.2 Pulsed electric fields

Pulsed electric fields are more suited to the processing of homogeneous liquid than particulate or solid foods. Conductivity (i.e., ability to conduct electric current), pH and water activity of food have profound effects on microbial inactivation by PEF. Food having a low conductivity, high water activity and an acidic pH is an ideal medium for effective PEF treatment.

Microorganisms show variation in their susceptibility to PEF on the basis of their size, cellular structure, and physiological status. Yeasts are generally more susceptible to PEF than are bacteria, while among the bacteria, rods appear to be more susceptible than cocci (35, 20). On this basis, PEF therefore appears to be more effective against large than small microbial cells (Fig. 1). This hypothesis however raises a question about the potential lack of effectiveness of PEF against viral particles. Research is currently underway to investigate the efficacy of PEF against viral particles. Gram-positive bacteria have a peptidoglycan rich rigid cell wall while gram-negative bacteria have a less rigid but multilayered cell envelope. This difference in cellular structure may account for the greater susceptibility of Gram-negative bacteria to PEF. Susceptibility to PEF also increases when microbial cultures are at the exponential rather than at the stationary phase of growth (6).

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**CHAPTER 12. INCREASING THE EFFECTIVENESS OF NON-THERMAL TECHNOLOGIES THROUGH TREATMENT COMBINATIONS**

Heat is widely applied in food preservation because of its effectiveness against microorganisms, viruses and enzymes. The dose of heat applied in the preservation process can be adjusted to achieve almost any desired level of microbial safety provided that food quality remains acceptable. Non-thermal technologies, result in limited microbial lethality. At the highest doses currently feasible, non-thermal preservation methods cannot accomplish commercial sterility in most foods, owing to the resistance of bacterial spores. These technologies may however reduce the microbial risk associated with some foods to an acceptable level. Processes that eliminate 5 log of *E. coli* O157:H7 (e.g. pasteurization) may be considered adequate for the production of safe fruit juices (36). High pressure processing and pulsed electric fields can be used to accomplish this goal (37).

Current non-thermal technologies may not be adequate to deliver treatments equivalent to pasteurization, in some low acid foods. Treating milk with PEF for 600 μs at 30 kV/cm and 25°C, for example, eliminated only 3 log *L. monocytogenes* (38). Pasteurization of milk commonly eliminates at least 5-6 log of this pathogen (39). Limitations of non-thermal technologies can be overcome through the combination of...
treatments. Inactivation of *L. monocytogenes*, *S. Typhimurium* and *E. coli* O157:H7 by HPP or PEF was greatly enhanced in the presence of bacteriocins (40). Kalchayanand *et al.* (41) suspended Gram-positive and Gram-negative foodborne pathogens in 0.1% peptone water (~10⁸ CFU/mL) and treated them with 345 MPa at 25°C for 5 min. The high-pressure treatment decreased the count by 1.3 to 8 log. Further inactivation was achieved when Pediocin AcH (3000 AU/mL) was included during the treatment. The same authors found that the efficacy of HPP increased proportionally when treatment temperature increased from 35°C to 50°C.

Relatively few studies have focused on the combined effect of PEF and other preservation methods. Liu *et al.* (42) observed a synergistic effect between PEF and organic acids (sorbic and benzoic) against *E. coli* O157:H7 (Fig. 2). Calderón-Miranda *et al.* (43) observed a greater inactivation rate when *L. innocua* was treated with PEF in the presence of nisin, than when it was treated with PEF alone (Fig. 3). Inactivation of *L. monocytogenes* in milk was enhanced to a greater extent at 50°C than at 25°C (38).

Bacterial spores are generally, not affected by HPP. However, when ~10⁶ spores of *B. coagulans* /mL were treated with high pressure (400 MPa for 30 min) at 70°C in the presence of 0.8 IU nisin/mL, no viable spores were detected (44). Similarly, Sohn and Lee (17) inactivated 6.0 x 10⁶ *B. subtilis* spores/mL in phosphate buffer medium (pH 7.0), using a combination of HPP at 800 MPa and heat (80°C) over a 20 min period. A combination of HPP and heat can be used for the production of high quality, commercially sterile food (45).

Bacterial spores and fungal ascospores are resistant to pulsed electric fields. Grahl and Márkl (35) reported no inactivation subsequent to treatment of endospores of *B. cereus* and *C. tyrobutiricum* and ascospores of *Byssochlamys nivea* with 22.4 kV/cm for up to 0.2 ms at temperatures of less than 45 to 50°C. Pagán *et al.* (46) used 75, 2-μs pulses at 60 kV/cm and 60°C against *B. subtilis* spores. No spore inactivation was observed, even when this treatment was combined with 5000 IU lysozyme/mL. Yin *et al.* (47) treated *B. subtilis* spores with 30 kV/cm for 1800 μs and obtained less than one log decrease in spore viability. A similar treatment, but in the presence of a germinant (L-alanine), resulted in 2 log spore inactivation. Marquez *et al.* (48) suspended *B. cereus* spores in 0.1M NaCl solution and applied PEF at 25°C using an electric field of 50 kV/cm and 50 pulses. Contrary to all other findings reported, this treatment inactivated 5 log spores/mL.

**CHAPTER 13. MEASURING EFFICACY AND USE OF NON-THERMALLY PROCESSED FOODS**

Non-thermal technologies were introduced for the production of safe food having fresh-like qualities. The efficacy of these technologies is ideally tested in food that has been inoculated with pathogens (challenge studies). The test pathogen to be used in these challenge studies varies in accordance with the food concerned. *Listeria*-inoculated milk and *Salmonella*-contaminated liquid egg for example are commonly used to test processes designed for the production of safe pasteurized milk and liquid egg,
respectively (e.g., 49; 28). Pasteurized milk and liquid egg may be characterised as refrigerated low-acid foods. Shelf-stable low acid foods (e.g., canned peas) are ideally tested using spores of *C. botulinum*. Until recently, high acid foods (e.g., fruit juices) were rarely tested with the use of pathogenic microorganisms since the main goal of processing is the elimination of aciduric spoilage microorganisms. Outbreaks of diseases due to the consumption of *E. coli* O157:H7-contaminated apple cider and juice (50;51) and *Salmonella*-contaminated orange juice (52) have however prompted the food industry to test pathogen-inoculated high acid foods.

Challenge studies cannot be run in a commercial food processing facility since pathogen-contaminated foods require careful handling in specialized laboratories (e.g., Biosafety Level-II). However, in order to run these studies in such specialized laboratories, laboratory-scale or bench-top processing equipment that closely mimics the commercial processing line is required. Scarcity of such equipment is another hurdle to be overcome in order to conduct challenge studies. The food industry has therefore been searching for "surrogate" microorganisms to allow safe testing of new technologies under real processing conditions. Surrogate microorganisms or "surrogates" are non-pathogenic microorganisms which show similarity with the targeted pathogen in its susceptibility to the processing technology. Ideal surrogates are (a) easy to culture in the laboratory, (b) easy to isolate on selective media and to enumerate on both selective and non-selective media, and (c) stable in morphological and biochemical properties. *C. sporogenes* PA 3679 has been effectively used as a surrogate to *C. botulinum* in heat inactivation studies. *L. innocua* has been used to study treatments that target *L. monocytogenes* (53). Fratamico *et al.* (54) constructed non-pathogenic strains of *E. coli* O157:H7 for use in challenge studies. The new strains carry the luciferase (*luc*) and the green fluorescent protein (*gfp*) genes. The recombinant *E. coli* strains were similar to their parent strains in biochemical and immunological assays and growth kinetics, yet easily detectable using fluorescence techniques. Industry may still be reluctant to use such surrogates in food processing facilities. The presence of these surrogates in the processing environment may result in false positives when environmental samples from these facilities are tested for the presence of pathogens.

**CHAPTER 14. ENSURING THE SAFETY OF NON-THERMALLY PROCESSED FOODS**

The application of non-thermal technologies in food processing should be preceded by extensive studies in order to ensure the safety of the treated food. These studies should determine both the critical treatment parameters and the magnitudes of these parameters that are sufficient to eliminate predefined levels of targeted pathogens. The following are examples of the critical processing parameters in selected non-thermal technologies:

a. Pulsed electric field: 
   - Electric field intensity (kV/cm),
   - Treatment time (μs)
b. High pressure processing:

- Process pressure (MPa),
- Pressure hold time (minutes)
- Product temperature (°C).

c. Pulsed light:

- Light intensity (J/cm²),
- Number of pulses
- Pulse duration.

The targeted pathogen is dependent on the food in question. Raw milk for example, occasionally causes Listeriosis in humans. This disease is caused by *L. monocytogenes*, which is naturally present in raw milk at levels that normally do not exceed $10^2$ CFU/mL. A non-thermal process designed to produce safe milk (i.e., cold pasteurization) should be applied at an intensity that is adequate to eliminate at least 5-6 log of *L. monocytogenes* (39). Such a treatment ensures that the processing of raw contaminated milk results in a product containing less than one *Listeria* organism per kg. It is important to caution that the previous example over simplifies a complicated safety question. The process just described minimizes appreciably, but does not totally eliminate the risk of Listeriosis in processed milk. In addition to *L. monocytogenes*, other pathogens (e.g., *Mycobacterium* spp.) are of concern in raw milk. If *L. monocytogenes* is more resistant to the non-thermal process than are other pathogens, then treatments designed to minimize the risk of Listeriosis should be adequate to significantly reduce other microbial risks. Studies should therefore be conducted to determine the relative susceptibility of pathogens of concern in a given food, to a non-thermal process. The most resistant pathogen should be considered as the target of the process.

Once the critical process parameters and the targeted pathogen are established, a validation process should follow. Validation entails inoculation of the food with the targeted pathogen and treatment under conditions similar to an actual processing run. If the non-thermal processing equipment is located in a commercial processing facility, pathogens may be substituted with suitable surrogate microorganisms. The non-thermal treatment is applied to inoculated food at pre-calculated levels of critical process parameters. Validation is accomplished if the non-thermal processing treatment decreases the population of the targeted pathogen (or its surrogate) below the pre-defined level.

Validation of a non-thermal process substantiates the feasibility of using the technology in commercial applications. An increasingly popular approach to ensure the safe commercial production of food is the hazard analysis critical control point (HACCP) system (55). Use of HACCP is recommended in non-
thermal food processing. Essential steps in developing a HACCP plan with emphasis on the non-thermal process include:

1. Assessing potential hazards (microbial, chemical or physical) associated with the non-thermally processed food (see earlier discussion).

2. Determining critical control points (CCPs) required for control of the recognized hazards. The non-thermal processing steps (e.g., HPP, and PEF) along the production line are typical CCPs.

3. Establishing critical limits at each CCP. An upper and lower limit should be defined for each critical process parameter, and food must be kept within these limits during non-thermal processing.

4. Developing and setting up procedures to monitor the CCP. In HPP processing, the HPP unit operation is a CCP that is monitored by gauges which measure vessel pressure, product temperature and treatment time (come-up, hold, and depressurization times). For PEF processing the PEF treatment step is a CCP that can be monitored by an oscilloscope (to measure field intensity, pulse width and pulse rate), a flowmeter (to measure product flow rate) and a temperature sensor (to measure product temperature before and after the treatment).

5. Corrective actions should follow if the critical limits are breached during food processing. Where process deviations occur, the flow of any under-processed product can be diverted by a valve for reprocessing.

6. Establishing procedures to verify the control of hazards. The absence of the targeted pathogen in non-thermally processed food verifies safe processing.

7. Establishing effective record keeping. Values of critical process parameters for each production run must be recorded and retained for future reference.

CHAPTER 15. ECONOMIC CONSIDERATIONS

Foods produced by HPP are already available on both the Japanese and US markets. These foods generally cannot be processed by conventional methods. Guacamole (prepared from avocado) is traditionally prepared immediately prior to consumption owing to its sensitivity to heat and other preservation methods. High pressure-processed guacamole, having a refrigerated shelf-life of several
weeks, is now produced by Avomex (Avomex Inc., Keller, Texas) for the US market. Pressure-processed fruit juices with sensory qualities similar to those of the freshly prepared products are scheduled to appear in the US market in the near future. The recent application of HPP to fresh seafood allows the sale of minimally processed oysters with a level of contamination that otherwise could not be reached without affecting the sensory properties of the product (56). Pressure-processed fresh oysters will soon be available on the US market. The novelty of pressure-processed products justifies their relatively high price and the high processing start up costs.

Currently no commercial food processing operations are reliant on PEF technology. The fruit juice market is likely to be the first to benefit from this technology. Unlike HPP, PEF is inherently suitable for continuous operation and large product flow rate. Although high initial costs constitute a significant obstacle in applying PEF in commercial operations, operating costs are low (6).

CHAPTER 16. CONCLUSION

Interest in non-thermal food processing technologies has increased appreciably in the past decade. These technologies promise to maintain the critical balance between safety and marketability of a new generation of foods. Some of these technologies have been optimized for the cold-pasteurization of foods and a limited number of such products are now commercially available. Non-thermal technologies can be used to produce safe fresh-like acid foods (e.g., fruit juices), but extensive research is needed to adapt these technologies for the production of shelf-stable low acid foods. Current limitations of emerging non-thermal technologies can be overcome when they are combined with conventional preservation methods.

CHAPTER 17. REFERENCES


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