7
Pulsed Electric Field Processing, Power Ultrasound and Other Emerging Technologies

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7.1 Introduction

The extended preservation of food has been a challenge for mankind throughout the ages. Preservation processes such as drying, curing, pickling and fermenting have been carried out for generations and examples of these processes can be found throughout the world. The products resulting from these processes, almost without exception, are radically changed in comparison to the fresh counterpart. Nevertheless these products have become established in the diet throughout the world and are important in their own right.

The advent of rapid freezing technologies fundamentally shifted the consumer's expectations upward in terms of the 'quality' of extended shelf life products. In the western world, freezing remains an excellent method for preserving foods in a cost-effective manner and in some cases the frozen product is the closest to fresh that the consumer is ever likely to get. Peas for example rapidly deteriorate after picking and the frozen product is better, in terms of sensory and nutritional value, than 'fresh' as purchased over the counter.

The application of heat for food preservation is a long established production process for which we must thank pioneers such as Nicolas Appert and Louis Pasteur. Thermal preservation has an excellent safety record and near universal consumer acceptance. However, even relatively mild thermal processes can result in a food product that is substantially different in terms of colour, flavour and texture to the unprocessed food.

A wide range of novel preservation processes have been studied over the last 100 years. A general mistrust of 'artificial' preservation and consumer demand for convenient 'fresh like' products has given fresh impetus to research into so called 'nonthermal' preservation methods. A selection of the most prominent emerging preservation technologies of interest to the food industry is summarised in Table 7.1. Many of these technologies remain very much in the research arena, some are on the brink of commercialisation. This chapter provides an overview of some of the main nonthermal emerging technologies showing promise for commercial food processing. It is not intended to be ex-

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Table 7.1 Summary of emerging preservation technologies attracting interest worldwide.

High pressure processing
Pulsed electric field processing
Power ultrasound
High intensity pulsed light
Oscillating magnetic fields
Irradiation (X-ray, electron beam, γ ray)
High voltage arc discharge
Plasma processing
Microwave and radio frequency heating
Ohmic and inductive heating
Ultraviolet

haustive, merely illustrative of the range of techniques that could be available to food manufacturers in the coming years.

High pressure processing (HPP) has been the subject of intense research effort over the last 15–20 years. Food products pasteurised by high pressure are now commercially available in a number of countries including Japan, France, Spain, North America and the UK. Since HPP is covered in Chapter 6, it will not be discussed here.

Irradiation, which is covered in detail in Chapter 5, has perhaps been more widely investigated than any other novel preservation method. Its torturous route to commercialisation and the arguments for and against the technique have been widely debated. Today, despite the availability of commercial systems and the proven efficacy of the process, industrial use is on a limited scale. In the UK, this is largely due to strong consumer resistance. Since 1999, no food has been irradiated in the UK for commercial purposes [1].

There are around 15 facilities in the EU that are approved for food irradiation [2]. The exact amount of food irradiated per year in the EU is not certain, but an estimate for 2001 is around 22 000 t [1]. Herbs, spices and poultry products account for the major proportion of this total.

In the USA, there appears to have been a softening of consumer attitudes towards irradiation, in part due to concerns over *Escherichia coli* in ground meat products and the need for an effective intervention method. Electron beam irradiated beef burgers and ground meat 'chubs' have been successfully introduced in the US market. Over 5000 US retail stores in 48 states now carry products that have been pasteurised using electron beam irradiation [3]. The US supermarket chain Wegmans is reported to offer irradiated ground beef products at stores throughout the chain and Dairy Queen is similarly reported to offer irradiated ground beef products in all of its Minneapolis stores.

Irradiation has been so comprehensively studied that it can almost be considered as a 'conventional' nonthermal preservation method. Consumer confidence is the main barrier to irradiation being considered as a credible preservation

method in the UK. Any review of nonthermal preservation would be incomplete without a discussion of irradiation and so it is briefly mentioned here for completeness (see also Chapter 5).

The main focus of this chapter will be on Pulsed Electric Field (PEF) and power ultrasound. Intensive research into PEF has brought the technology to the brink of commercial uptake. Power ultrasound has interesting potential as a novel preservation method but is some way from being utilised commercially for this purpose. It does, however, have numerous nonpreservation applications, some of which are already being used commercially. The remainder of the chapter will provide an overview of a range of technologies that are attracting research interest, but that are some years away from being viable commercial processes.

7.2 Pulsed Electric Field Processing

7.2.1 Definition of Pulsed Electric Fields

Pulsed electric field processing is a technique in which a food is placed between two electrodes and exposed to a pulsed high voltage field (typically 20–80 kV cm^{-1}) [4]. For preservation applications, treatment times are of the order of less than 1 s, achieved by multiple short duration pulses typically less than 5 μs. This process reduces levels of microorganisms whilst minimising undesirable changes in the sensory properties of the food. It is important to stress that although heat may be generated in the food product (and may need to be controlled by cooling), microbiological inactivation is achieved by nonthermal means, that is, due to the electrical field not just due to any induced thermal effects. However, there is a clear synergy between a moderate degree of heating (for example 40–45 °C) and the applied PEF [5].

7.2.2 Pulsed Electric Field Processing – A Brief History

The inactivation of microorganisms and enzymes using electric discharges started as early as the 1920s with the 'ElectroPure' process for milk production. This process consisted of heating the milk to 70 °C by passing it through carbon electrodes in an electric heating chamber to inactivate *Mycobacterium tuberculosis* and *E. coli* [6, 7]. The electric field was small, only 220 V AC, and was not pulsed; and the inactivation mechanism was purely thermal [7]. There were around 50 plants using the ElectroPure system in the USA up until the 1950s [8].

An 'electrohydraulic' process was developed in the 1950s as a method for inactivating microorganisms in liquid food products. A shock wave generated by an electric arc and the formation of highly reactive free radicals was thought to be the main mechanism for microbiological inactivation [6]. The process did not

find widespread use in the food industry because particulates within the food were damaged by the shock waves and there were issues surrounding electrode erosion and the potential for contaminating the food [8].

The roots of pulsed electric field processing can be traced back to Germany and a patent by Doevenspeck [5, 9]. This inventor pioneered the design of pulsed electric field equipment and until the time of his death remained involved in collaborative projects in the PEF field, a career spanning over 50 years [5]. Unilever scientists Sale and Hamilton made valuable contributions to the field in the 1960s, studying the mechanisms of PEF inactivation of microorganisms [5, 10, 11]. PEF has been used for a number of years, in nonpreservation applications, at relatively low field strengths (5–15 kV cm^{-1}) as a means of inducing pores in cell membranes. This application finds widespread use in biotechnology for the insertion of foreign DNA into living cells to modify their characteristics [5, 12]. PEF as a preservation method differs from these reversible electroporation techniques in the equipment design, field strengths used (20–80 kV cm^{-1} typically) and the pulse duration (typically <5 μs versus tenths or 100ths of microseconds) [5].

7.2.3 Effects of PEF on Microorganisms

Two mechanisms have been proposed to explain the inactivation of microorganisms using pulsed electric fields, 'electrical breakdown' and 'electroporation' [4].

7.2.3.1 Electrical Breakdown

According to Zimmermann [4, 8], the bacterial cell membrane can be considered to be a capacitor that is filled with a dielectric material. The normal resisting potential difference across the membrane (the transmembrane potential) is around 10 mV [4]. If an external electric field is applied, this increases the potential difference across the cell membrane. This increase in potential difference causes a reduction in the membrane thickness. When the potential difference across the cell reaches a critical level (normally considered to be around 1 V), pores are formed in the membrane. This leads to an immediate discharge at the membrane pore and, consequently, membrane damage [4]. Breakdown of the membrane is reversible if the pores are small in relation to the total membrane surface, but when pores are formed across large areas of the membrane then destruction of the cell membrane results. Figure 7.1 shows a *Bacillus cereus* cell before and after PEF treatment.

The transmembrane potential developed in the direction of an applied electric field is given by [8]:

$$U(t) = 1.5\,rE \tag{7.1}$$

where $U(t)$ is the transmembrane potential in the direction of the applied field (V), r is the radius of the cell (μm) and E is the applied electric field strength (kV mm^{-1})

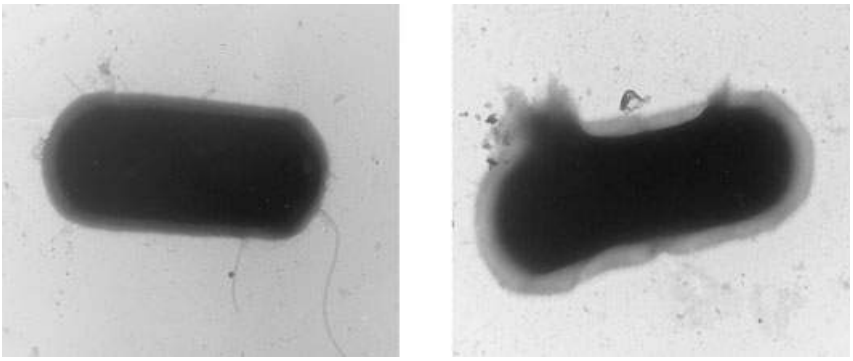

Fig. 7.1 PEF treatment of *Bacillus cereus* cell.

For a typical cell radius of 0.5 μm, the electrical field strength (E) required to induce poration would be 13.33 kV cm^{-1}.

7.2.3.2 **Electroporation**

A second proposed explanation for microbiological inactivation using PEF is that of electroporation. When a microorganism is subjected to a high voltage electric field, the lipid bilayer and proteins of the cell membrane are temporarily destabilised [4]. Changes in the conformation of lipid molecules are induced, existing pores are expanded and structurally stable hydrophobic pores are formed which can conduct current. This leads to localised heating that changes the lipid bilayer from a rigid gel to a liquid crystalline form [8]. Once the semipermeable nature of the membrane is impaired, swelling and eventual rupture of the cell is induced [4, 8].

7.2.4
Critical Factors in the Inactivation of Microorganisms Using PEF

Three key areas – process factors, product factors and microbial factors – determine the effectiveness of PEF for microbiological inactivation using PEF [4].

7.2.4.1 **Process Factors**

The intensity of the electric field will affect the transmembrane potential of the microbial cell (as described earlier) and therefore an increase in inactivation can be expected with an increase in electric field intensity. The pulse width used affects the level of electric field intensity that is required to achieve inactivation. Larger pulse widths reduce the field intensity that is required to produce a transmembrane potential large enough to initiate pore formation. Unfortunately, longer pulses also increase the degree of heating observed in the food so a careful balance must be established to maximise inactivation whilst minimising product heating. In general, an increase in treatment time (number of

pulses multiplied by pulse duration) also increases the level of inactivation. The pulse waveshape also influences the degree of inactivation achievable with PEF. Square wave pulses are more energy efficient and more lethal than exponentially decaying waveforms. Bipolar pulses cause additional stress to the cell membrane, enhance microbial inactivation and are energy efficient. Finally the process temperature has an impact on the lethality of PEF. Moderately elevated temperatures have a synergistic effect when combined with PEF. This may be due to changes in membrane fluidity and permeability, or an increase in the conductivity of the liquid being treated.

7.2.4.2 **Product Factors**

The electrical conductivity of the product to be treated is a very important parameter for PEF processing. Foods with a large electrical conductivity are not suitable for processing with PEF because the peak electric field across the chamber is reduced. The ionic strength of a food material directly influences its conductivity and as the conductivity rises, the lethality of a process decreases. Reducing the pH of the product is thought to increase the inactivation achievable for a given field strength. However, work by Berlin University of Technology suggested that pH modifications down to pH 5.5 had minimal effect on the lethality of PEF processing of *B. subtilis* [13].

Particulates in the liquid also pose processing problems because high energy inputs may be needed to inactivate microorganisms in the particulates and there is a risk of dielectric breakdown of the food.

7.2.4.3 **Microbial Factors**

In general, the order of resistance of microorganisms to PEF (lowest to highest) is considered to be yeasts, Gram negative bacteria and Gram positive bacteria. The lifecycle stage of the microorganisms affects the lethality of the process: organisms in the log phase of growth are generally more sensitive to PEF than those in the lag or stationary phases of growth. There is also some evidence to suggest that higher initial concentrations of microorganisms can impact on the lethality of the process [4].

7.2.5 **Effects of PEF on Food Enzymes**

Work on PEF effects on food enzymes has been relatively limited to date and variable results have been obtained [14]. Work using simulated milk ultrafiltrate in a continuous flow unit (45 ml min^{-1}) resulted in a 20–90% reduction of plasmin (bovine milk). The process conditions used varied between 15–45 kV cm^{-1}, with a 2-μs pulse, a frequency of 0.1 Hz and between 10–50 pulses [14].

Studies on a protease (*Pseudomonas flourescens* M3/6) highlighted the effect of substrate on the levels of achieved inactivation. A 60% reduction in activity was

found in skimmed milk (15 kV cm^{-1}, 2-μs pulse, frequency of 2 Hz, 98 pulses, 50 °C), whereas no effect was found using the same processing parameters in casein tris buffer [14].

Results on the inactivation of alkaline phosphatase in raw milk have been variable. Washington State University demonstrated a 96% reduction in activity using 13.2 kV cm^{-1} and 70 pulses, whereas unpublished data by Verachtert and others showed no inactivation using 13.3 kV cm^{-1}, a frequency of 1 Hz and 200 pulses of 2 μs [14].

Ho et al. examined the effects of PEF on a number of enzymes in model systems [14, 15]. They found that the activity of a wheat germ derived lipase in deionised water (pH 7) could be reduced by up to 85%, using a treatment of 87 kV cm^{-1} at a frequency of 0.5 Hz using 30 pulses of 2 μs duration. The level of inactivation increased with increasing electric field strength. For example, at 20 kV cm^{-1} only a 20% reduction in activity was achievable. Glucose oxidase activity in pH 5.1 buffer could be reduced by 20–75%, using 17–63 kV cm^{-1}, a frequency of 0.5 Hz and 30 pulses of 2 μs at 20 °C. Inactivation of α-amylase (from *B. licheniformis*) in deionised water (pH 7) varied between less than 5% to around 85% using 20–80 kV cm^{-1}, a frequency of 0.5 Hz and 30 pulses of 2 μs at 20 °C.

Work at the Katholieke Universiteit Leuven (KUL) in Belgium [14] has increased the complexity of the materials used in enzyme studies, moving from initial trials in simple model systems towards real food products. In distilled water, KUL studies found no better than a 10% reduction in activity of a range of commercial enzymes: lipoxgenase (soyabean), pectinmethylesterase (tomato), α-amylase (*B. subtilis*), polyphenoloxidase (mushroom) and peroxidase (horseradish). Processing conditions evaluated were 10, 20 and 30 kV cm^{-1}, frequencies of 1–100 Hz, pulse widths of 5–40 μs and 1–1000 pulses [14]. In raw milk, no inactivation of alkaline phosphatase was observed using field strengths of up to 20 kV cm^{-1} at a frequency of 1 Hz with 200 pulses of 2 μs duration. The only treatment that brought about a reduction in activity (74%) was one using an extended pulse duration (40 μs). This resulted in a temperature rise within the milk of up to 70 °C and this is likely to have been responsible for the inactivation. Similarly, lactoperoxidase in milk proved resistant to PEF processing. A maximum of 13% inactivation was achieved using 13 kV cm^{-1}, a frequency of 1 Hz and 200 pulses of 10 μs duration. In this experiment, the milk reached a temperature of 52 °C. PEF had very little effect on lipoxygenase in pea juice, the maximum reduction in activity that could be achieved being 9%. In apple juice, polyphenoloxidase was similarly resistant with only a 10% reduction in activity being achievable using 31 kV cm^{-1}, a frequency of 1 Hz and 1000 pulses of 1 μs duration.

In a joint piece of work by the University of Lleida, Spain, and Washington State University, the activity of endopolygalacturonase (endoPG) was reduced by 98% after a treatment for 32 ms at 10 kV cm^{-1} [16].

Debate continues regarding the effects of pulsed electric field processing on enzymes. Much of the research conducted in Europe would suggest that PEF

has minimal effects, whereas research in the USA more frequently suggests that a significant degree of inactivation is achievable. Varying experimental approaches and equipment design features cloud the issue. Historically, it has proved difficult to compare results between laboratories because of these differences in equipment design and the processing parameters selected. Separating temperature effects from PEF effects with respect to enzyme inactivation is critical. Some researchers firmly believe that it is temperature increases that give rise to any observed enzyme inactivation; others maintain that inactivation is a nonthermal effect.

7.2.6 Basic Engineering Aspects of PEF

A number of excellent reviews have been published on the engineering aspects of pulsed electric field processing e.g. [4, 8, 12, 17]. To generate a high voltage pulsed electric field of several $kV\,cm^{-1}$ within a food, a large flux of electrical current must flow through the food within a treatment chamber, for a very short period of time (μs) [12]. This process involves the slow charging of a capacitor followed by a rapid discharge. The typical components of PEF processing equipment include [4, 12, 17]:

- A power supply: this may be an ordinary direct current power supply or a capacitor charging power supply (this latter option can provide higher repetition rates).
- An energy storage element: either electric (capacitive) or magnetic (inductive).
- A switch which may be either closing or opening. Devices suitable for use as the discharge switch include a mercury ignitron spark gap, a gas spark gap, a thyratron, a series of SCRs, a magnetic switch or a mechanical rotary switch [12].
- A pulse shaping and triggering circuit in some cases.
- A treatment chamber (a wide variety of designs have been developed by individual laboratories).
- A pump to supply a feed of product to the chamber.
- A cooling system to control the temperature of the feed and/or output material.

7.2.6.1 Pulse Shapes

PEF can be applied in a variety of forms, including exponential, square wave, instant charge reversal, bipolar or oscillatory pulses [4]. Figure 7.2 shows a simple circuit for the generation of an exponentially decaying pulse. Exponential waveforms are characterised by a rapid rise to the target voltage followed by a slow decay towards zero volts [4]. This waveform has been widely used by researchers in the field, one reason for this being that they are relatively simple to generate and modify.

Square waveforms are also quite widely used in PEF studies. The generation of square pulses is more complex than the generation of exponential decaying

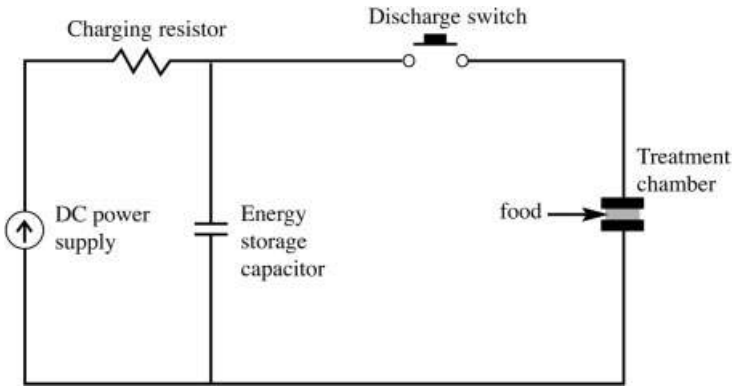

Fig. 7.2 Basic circuit for exponential waveform.

pulses and requires a pulse forming network (an array of capacitors, inductors and solid state switching devices [4]). To generate the square waveform, the pulse forming network and treatment chamber must have matching impedance and, practically, this is difficult to achieve [12].

Although both exponential and square waveforms are effective for inactivating microorganisms, the square waveform is more lethal [4] and is generally considered to be the better option of the two because it maintains peak voltage for longer than the exponential form and is more energy efficient. The prolonged tailing associated with the exponential waveform can lead to excessive heat generation in the food and additional cooling is required in comparison with the square wave [12].

Oscillatory pulses have been found to be the least efficient for microbial inactivation because although the microbial cell is subjected to multiple exposures to the high intensity field, each exposure is only for a short duration of time and irreversible breakdown of the membrane over a large area is prevented [4, 18].

Bipolar pulses, in which the polarity of the applied electric field reverses after a relaxation time, are more effective than monopolar pulses because additional stress is thought to be induced in the cell membrane. Rapidly reversing the electric field orientation changes the movement direction of charged groups in the cell membrane, causing structural fatigue and enhanced electrical breakdown [4, 8]. Biopolar pulses also minimise the deposition of solids at the electrode surfaces and the consequent detrimental effect on field uniformity within the chamber [4, 12].

Instant charge reversal pulses are characterised by having a positive and negative component with various pulse widths and peak field strengths [18]. This type of pulse can significantly reduce the energy requirements for the PEF process to as low as 1.3 J ml^{-1} [4]. Instant charge reversal differs from standard bipolar pulsing because there is no relaxation time between the changes in polarity. There is evidence to suggest that instant charge reversal pulses reduce the

critical electric field strength that needs to be applied in order to induce poration of the microbial membrane [18].

7.2.6.2 **Chamber Designs**

A wide range of experimental PEF treatment chambers have been designed and built by researchers active in this field. Chambers can be broadly categorised as batch or continuous in design. Early chambers were designed for batch processing of static volumes and used parallel plate electrodes separated by an insulating spacer [5, 19]. An alternative batch design is a U-shaped unit, which comprises two electrodes supported on brass blocks in a U-shaped polystyrene spacer [4, 19].

Perhaps of most interest from the point of view of a future commercial process are the continuous chamber designs. Coaxial chambers consist of an inner and outer electrode with the product flowing between them [5]. The electrical current flow is perpendicular to the fluid flow [19]. In co-field designs, electrical current flow is parallel to the fluid flow [19]. The co-field chamber consists of two hollow cylindrical electrodes, separated by an insulator, forming a tube through which the product flows [5].

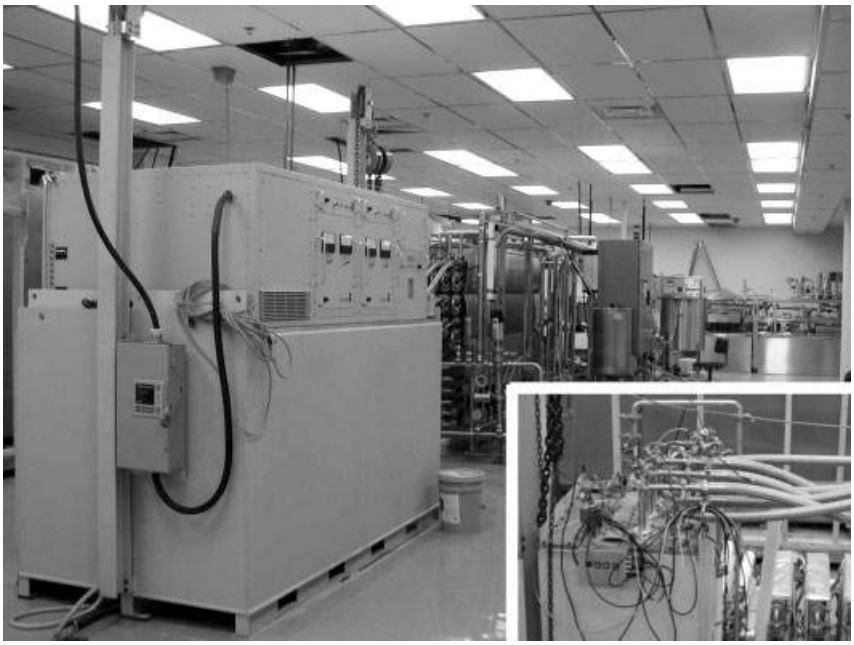

Fig. 7.3 Continuous-flow PEF processing: 'OSU-6' at the Ohio State University (picture courtesy of Prof. Howard Zhang, The Ohio State University).

The main hurdle restricting commercialisation of PEF has, until recently, been problems associated with scale-up of the equipment. The development of solid state switching systems in recent years has opened up the possibility of full scale-up to almost any throughput desired by the manufacturer. The work of a PEF consortium funded by the US 'DUST' programme (Dual Use of Science and Technology) has led to the manufacture of a large-scale processing unit that has been installed and commissioned at Ohio State University (Fig. 7.3). The 'OSU-6' consists of four treatment chambers with a cooling system before and after each one to control the temperature of the product. A throughput of up to 2000 l h^{-1} is achievable. The university has successfully conducted trials on the pasteurisation of products such as orange juice, tomato juice, salsas and yoghurt.

The capital cost for a commercial PEF unit capable of processing 5000 l h^{-1} has been estimated to be around £460 000 (€663 000). Depreciating the equipment over 5 years, the cost per litre of juice pasteurised using PEF has been estimated at around £0.02 l^{-1} (€0.03 l^{-1}). This includes all personnel, maintenance and utility costs. This is broadly in line with the costs associated with conventional thermal pasteurisation [20].

7.2.7 Potential Applications for PEF

7.2.7.1 Preservation Applications

A substantial body of research has demonstrated the microbiological effects of PEF. Many studies have been conducted in model food systems, but relatively few publications have related to real food products. From those that have, a range of pumpable food products have been identified that potentially could be preserved using PEF.

Juice The shelf life of apple juice (from concentrate) has been successfully extended from 21 days to 28 days using 50 kV cm^{-1}, ten pulses, a pulse width of 2 μs and a maximum process temperature of 45 °C [4]. Sensory panellists could determine no significant differences between treated and untreated juice. Work at Washington State University demonstrated that PEF could extend the shelf life of fresh apple juice and apple juice from concentrate to over 56 days and 32 days respectively when stored at 22–25 °C [4].

Rodrigo and others [21] demonstrated that, with process conditions of 28.6, 32.0 and 35.8 kV cm^{-1} for 10.3–46.3 μs, a 2.5 log reduction of *Lactobacillus plantarum* was achieved in orange/carrot juice (70% orange, 30% carrot).

Sitzmann [7], summarising work by Grahl, indicated that a five-fold reduction of *Saccharomyces cerevisiae* was achievable in orange juice using five pulses at a field strength of around 6.5 kV cm^{-1}. Work conducted by Zang and others [4] showed a 3–4 log reduction of total aerobic plate counts in orange juice processed under 32 kV cm^{-1}. The shelf life of this product at 4 °C was 5 months, vitamin losses were lower than heat-processed controls and colour was better preserved.

Milk A number of studies have demonstrated PEF inactivation of microorganisms in milk. Examples [22] include a 3 log reduction of *E. coli* using 21 kV cm^{-1}, a 4 log reduction of *Salmonella dublin* using 18 kV cm^{-1}, a 2.5 log reduction of *Streptomyces thermophilus* using 25 kV cm^{-1} and a 4.5 log reduction of *L. brevis* using 23 kV cm^{-1}. More recently, the shelf life of skimmed milk treated with PEF was reported to be 2 weeks at 4 °C using a process of 40 kV cm^{-1}, 30 pulses and a 2-μs treatment time [4].

Speaking at a CCFRA conference [23] Professor Barbosa-Cánovas of Washington State University discussed the potential of PEF processing for skimmed milk. In his experience of PEF skimmed milk processing, coliforms were inactivated with minimal treatment but naturally occurring flora survived. He also found that Gram positive species were not always more resistant to PEF than Gram negative, as is usually proposed. A relatively short shelf life was achieved in his studies on skimmed milk, primarily due to the surviving population of natural flora. From an engineering viewpoint, significant high voltage electrode damage was observed, along with solids deposition on the electrodes. A hurdle approach was recommended for effective PEF processing using a combination of mild heat and PEF.

Liquid Whole Egg Washington State University has conducted trials over a number of years, assessing the feasibility of PEF processing for liquid whole egg pasteurisation. Results have been promising. PEF has been shown to have minimal effect on the colour of liquid whole egg. Although undetectable levels of microbial populations were present in PEF-treated liquid whole egg, spoilage was found to occur within 25–28 days of storage at 4 °C according to Professor Barbosa-Cánovas [23]. Aseptic packaging of PEF-treated liquid whole egg was therefore recommended to optimise the product shelf life.

Other Liquid Products Research at Washington State University [24] showed that a 6.5 log reduction of *E. coli* (alone) and a 5.3 log reduction of *B. subtilis* (alone) were achievable in pea soup treated with 33 kV cm^{-1}, 0.5 l min^{-1}, 4.3 Hz and 30 pulses. Inactivation was reduced however when a combination of the two organisms were treated in pea soup: a 4.8 log reduction was observed using 30 kV cm^{-1}, 6.7 Hz and a flow rate of 0.75 l min^{-1}. The effects of PEF were limited if the bulk temperature of the liquid during processing was below 53 °C. The physical, chemical and sensory properties of the product did not appear to be changed following PEF treatment and 4 weeks of storage at chilled temperatures [4].

7.2.7.2 Nonpreservation Applications

Whilst the vast majority of research in the field of PEF processing has focused on microbiological inactivation and food preservation, a number of nonpreservation applications using PEF could also prove useful for the food industry.

Baking Applications PEF-treated wheat dough (50 kV, 20 min) is reported to have decreased water loss during baking and the shelf life of the bread subsequently baked from the dough is reported to be increased [25]. Another potential application is the treatment of brewer's yeast to convert nonflocculent yeast to a flocculent form [25].

Extraction/Cell Permeabilisation The irreversible permeabilisation of plant cell membranes and tissues using PEF has been demonstrated [26]. This offers interesting possibilities for improving expression, extraction and diffusion processes [26]. Potential applications include extraction processes such as those found in starch production, sugarbeet processing and juice extraction.

Pretreating vegetables with pulsed electric fields can dramatically reduce drying times. In trials on potato cubes which were dried in a fluidised bed with and without a PEF pretreatment, a one-third reduction in drying time was demonstrated [26].

Taiwo and others [27] studied the effects of a range of pretreatments (PEF, pressure treatment, freezing and blanching) prior to osmotic dehydration of apple slices. PEF pretreatments were conducted in a static, parallel plate chamber using an exponentially decaying wave shape and a field strength of 1.4 kV cm^{-1}. Twenty pulses (at 1 Hz) were administered, each with a duration of 800 μs. In the initial period of dehydration (up to 3 h) PEF processing was not the most effective treatment (in terms of enhancing water loss from the samples). However, beyond this point, the rate of change of moisture loss from PEF-treated samples stabilised at around 0.43 whereas in the other pretreatments investigated, the rate of change of moisture loss continued to fall and in some cases tended towards zero. As a result, after 6 h, the water loss from the PEF pretreated apples was around 79.7% versus 72.6, 40.5, 72.2 and 60.3% for pressure-treated, frozen, blanched and untreated samples, respectively. Uptake of sugar during dehydration was more pronounced in pressure-treated samples than PEF-treated samples. At the start of the dehydration process, PEF processing had a minimal effect on vitamin C levels (10.3±0.8 mg $(100\ g)^{-1}$ fruit versus 10.8±0.5 mg $(100\ g)^{-1}$ fruit in the control). As dehydration progressed, vitamin C levels dropped considerably regardless of pretreatment.

7.2.8 The Future for PEF

Significant steps forward have been made for pulsed electric field processing and it has reached a point where it is very close to commercial realisation. Large-scale equipment is now not only feasible but can be built to specification by companies such as Diversified Technologies Inc. (Mass., USA). It seems likely that a PEF-processed product will be launched in the not too distant future.

There are of course still some issues to be resolved, most notably that of establishing exactly how effective PEF really is with respect to microbial and enzyme inactivation. Numerous laboratory and pilot-scale trials have been con-

ducted using a range of custom-built PEF equipment. Unfortunately, this makes it extremely difficult to compare data obtained from different laboratories and assess exactly what levels of inactivation are truly achievable in a commercial system. Harmonisation of equipment and research protocols is beginning to take place and this will greatly help the situation. As research progress is made and knowledge increases regarding the most effective design parameters for maximising microbiological and enzyme inactivation whilst minimising product deterioration, then the full potential of PEF may be realised.

7.3 Power Ultrasound

7.3.1 Definition of Power Ultrasound

Ultrasonic techniques are finding increasing use in the food industry for both the analysis and processing of foods [28]. Normal human hearing will detect sound frequencies ranging from 0.016 kHz to 18.0 kHz and the power intensity of normal quiet conversation is of the order of 1 W cm^{-2}. Low intensity ultrasound uses very high frequencies, typically 2–20 MHz with low power levels from 100 mW cm^{-2} to less than 1 W cm^{-2}. This type of ultrasound is readily used for noninvasive imaging, sensing and analysis and is fairly well established in certain industrial and analytical sectors for measuring factors such as composition, ripeness, the efficiency of emulsification and the concentration or dispersion of particulate matter within a fluid [29].

Power ultrasound, in contrast, uses lower frequencies, normally in the range of 20–100 kHz (generally less than 1 MHz), and can produce much higher power levels, in the order of 10–1000 W cm^{-2}. Low frequency high power ultrasound has sufficient energy to break intermolecular bonds, and energy intensities greater than 10 W cm^{-2} will generate cavitation effects, which are known to alter some physical properties as well as enhance or modify many chemical reactions [28, 30, 31].

It has long been known that ultrasound is able to disrupt biological structures and produce permanent effects in the medium to which it is applied, but the proposed use of power ultrasound for microbial inactivation in foods is still in its infancy. Much work has been done to investigate the mechanism by which ultrasonic disruption of biological systems occurs, but cavitation effects are thought to be a major factor [32, 33].

Cavitation occurs when ultrasound passes through a liquid medium [34], causing alternate rarefactions and compressions. If the ultrasound waves are of sufficiently high amplitude, bubbles are produced. These bubbles collapse with differing intensities and it is this that is thought to be a major contribution to cellular disruption. The mechanisms involved in cellular disruption are multifactorial and may include shear forces generated during movement (subcellular

turbulance) of the bubbles or sudden localised temperature and pressure changes caused by bubble collapse.

A characteristic of ultrasonic waves is the ability to produce different effects in different media in such a way that sometimes these effects seem contradictory. For example, power ultrasound in liquid suspensions has the ability to break aggregated particles whereas using ultrasound in air or gas suspensions tends to produce particle agglomeration.

7.3.2 Generation of Power Ultrasound

Whatever type of system is used to apply power ultrasound to foods, it will consist of three basic parts [35]:

1. Generator: this is an electronic or mechanical oscillator that needs to be rugged, robust, reliable and able to operate with and without load.
2. Transducer: this is a device for converting mechanical or electrical energy into sound energy at ultrasonic frequencies.
3. Coupler: the working end of the system that helps transfer the ultrasonic vibrations to the substance being treated (usually liquid).

There are three main types of transducer: *liquid driven*, *magnetostrictive* and *piezoelectric*. Liquid driven transducers are effectively a liquid whistle where a liquid is forced across a thin metal blade causing it to vibrate at ultrasonic frequencies: rapidly alternating pressure and cavitation effects in the liquid generate a high degree of mixing. This is a simple and robust device but, because it involves pumping a liquid through an orifice and across a blade, processing applications are restricted to mixing and homogenisation (see Chapter 15).

Magnetostrictive transducers are electromechanical devices that use magnetostriction, an effect found in some ferromagnetic materials which change dimension in response to the application of a magnetic field. The dimensions of the transducer must be accurately designed so that the whole unit resonates at the correct frequency. The frequency range is normally restricted to below 100 kHz and the system is not the most efficient (60% transfer from electrical to acoustic energy, with losses mainly due to heat). The main advantages are that these transducers are rugged and able to withstand long exposure to high temperatures.

Piezoelectric transducers are electrostrictive devices that utilise ceramic materials such as lead zirconate titanate (PZT) or barium titanate and lead metaniobate. This piezoceramic element is the most common of the transducers and is more efficient (80–95% transfer to acoustic energy) but less rugged than magnetostrictive devices; piezoelectric transducers are not able to withstand long exposure to high temperatures (normally not $>85\ ^{\circ}C$).

7.3.3 System Types

The design, geometry and method by which the ultrasonic transducer is inserted or attached to the reaction vessel is essential to its effectiveness and efficiency – this is an important variable and any differences between laboratory and pilot plant design and application of ultrasound can often lead to very different results. For example, with ultrasonic baths, the transducer is bonded to the base or sides of the tank and the ultrasonic energy is delivered directly to the liquid in the tank. However, with probes, the high power acoustic vibration is amplified and conducted into the media by the use of a shaped metal horn; and the shape of the horn will determine the amount of signal amplification.

There are several ultrasonic systems available, which differ mainly in the design of the power generator, the type of transducer used and the reactor to which it is coupled. Typical ultrasonic systems are.

7.3.3.1 Ultrasonic Baths

Transducers are normally fixed to the underside of the vessel, operate at around 40 kHz and produce high intensities at fixed levels due to the development of standing waves created by reflection of the sound waves at the liquid/air interface. The depth of the liquid is important for maintaining these high intensities and should not be less than half the wavelength of the ultrasound in the liquid. Frequency sweeping is often used to produce a more uniform cavitation field and reduce standing wave zones.

7.3.3.2 Ultrasonic Probes

These systems use detachable 'horns' or shapes to amplify the signal; the horns or probes are usually a half wavelength (or multiples) in length. The amount of gain in amplitude depends upon the shape and difference in diameter of the horn be-

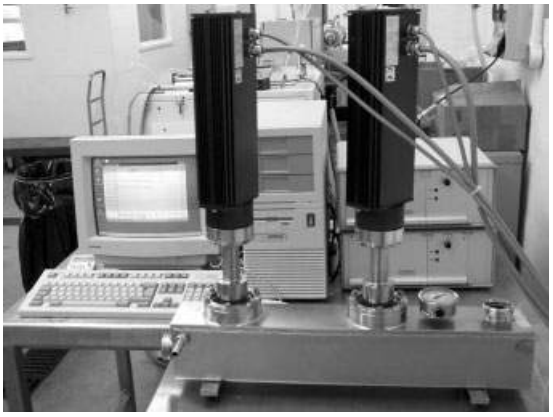

Fig. 7.4 Example of a probe type system from Dr Hielscher GmbH.

tween one face (the driven face) and the other (the emitting face). If the probe is the same diameter along its length then no gain in amplitude will occur but the acoustic energy will simply be transferred to the media (see Fig. 7.4).

7.3.3.3 **Parallel Vibrating Plates**

Opposing vibrating plates offer a better design for maximising the mechanical effect of ultrasound than a single vibrating surface. Often plates vibrate at different frequencies (for example 20 kHz and 16 kHz) to set up beat frequencies and create a larger number of different cavitation bubbles.

7.3.3.4 **Radial Vibrating Systems**

This is perhaps the ideal way of delivering ultrasound to fluids flowing in a pipe. The transducers are bonded to the outside surface of the pipe and use the pipe itself as a part of the delivery system (see Fig. 7.5). This system is very good for handling high flow rates and high viscosity fluids. A cylindrical resonating pipe will help focus ultrasound at the central region of the tube, resulting in high energy in the centre for low power emission at the surface; and this can reduce erosion problems at the surface of the emitter.

7.3.3.5 **Airborne Power Ultrasound Technology**

Air and gaseous media present problems for the efficient generation and transmission of ultrasonic energy. Due to the low density and high acoustic absorption

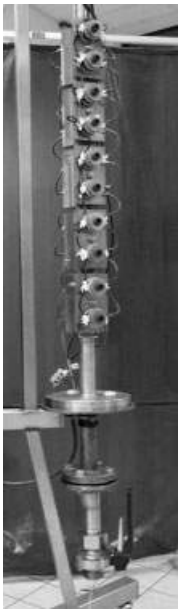

Fig. 7.5 Example of a radially vibrating system from Sonic Process Technologies.

of air and gases, ultrasonic generators require good impedance matching with the air or gas concerned, a large amplitude of vibration, highly directional or focused radiation and a high power capacity. Whistles and sirens were the most common type of ultrasonic device for use in air until the 'stepped-plate transducer' was developed in the late 1980s. In this case, a large diameter flexible radiating plate with a stepped profile is driven at its centre by a piezoelectric device. The extensive surface of the plate produces a high radiation resistance and power capacity and offers a good impedance matching with air. The special profile of the vibrating plate permits good control of the vibrating amplitude and focused radiation pattern that is very directional. Special power generators (1–2 kW macrosonic generators) are used to drive the transducer at resonance during operation and produce acoustic energy in the frequency range 10–50 kHz. These devices have found applications in food dehydration and defoaming of liquid in cans or batch tanks and could be applied to the agglomeration of particles in the air of a room or filling environment as well as gas sterilisation and mass transfer.

7.3.4 Applications for Power Ultrasound in the Food Industry

Power ultrasound is already used for the processing of food materials in a variety of ways such as mixing, emulsification, cutting, tenderising and ageing [30, 36]. Potential applications for high power ultrasound in the food industry are wide-ranging and include enzyme inhibition, hydrogenation of oils, crystallisation control, extraction of proteins and enzymes, the inactivation of microorganisms and improved heat and mass transfer [30, 31, 35]. A list summarising current and potential applications is shown in Table 7.2.

7.3.4.1 Ultrasonically Enhanced Oxidation

Ageing of fermented products and inducing rapid oxidation in alcoholic drinks for flavour development and early maturation has been developed, using higher frequency lower energy power ultrasound. In 1981, in Japan, the use of 1 MHz ultrasound was shown to alter the alcohol/ester balance [37] with possible applications for accelerating whisky maturation through the barrel wall being tested [38].

7.3.4.2 Ultrasonic Stimulation of Living Cells

Lower power sonication can be used to enhance the efficiency of whole cells without cell wall disruption. In this case, ultrasound appears to be increasing the transport of nutrients or affecting membrane/seed permeability by microstreaming. For example, work done in Belgium, at Undatim Ultrasonics, demonstrated that the use of ultrasound as a processing aid in yoghurt manufacture led to a reduction in production time of up to 40%. Sonication reduced the dependency of the process on the origin of milk and improved the consistency and texture of the product. Russian studies have shown that the application of

Table 7.2 List of current and potential applications for ultrasound in the food industry.

Application	Comments
Crystallisation of fats and sugars	Enhances the rate and uniformity of seeding
Degassing	Carbon dioxide removal from fermentation liquors
Foam breaking	Foam control in pumped liquids and during container filling
Extraction of solutes	Acceleration of extraction rate and efficacy; research on coffee, tea, brewing; scale-up issues
Ultrasonically aided drying	Increased drying efficiency when applied in warm air resulting in lower drying temperatures, lower air velocities or increased product throughput
Mixing and emulsification	Online commercial use often using 'liquid whistle'. Can also be used to break emulsions
Spirit maturation and oxidation processes	Inducing rapid oxidation in alcoholic drinks; 1 MHz ultrasound has possible applications for accelerating whisky maturation through the barrel wall
Meat tenderisation	Alternative to pounding or massaging; evidence for enhanced myofibrillar protein extraction and binding in reformed and cured meats
Humidifying and fogging	Ultrasonic nebulisers for humidifying air with precision and control; possible applications in disinfectant fogging
Cleaning and surface decontamination	Online commercial use for cleaning poultry processing equipment; possible pipe fouling and fresh produce cleaning applications; can reach crevices not easily reached by conventional cleaning methods
Cutting	Commercial units available capable of cutting difficult products – very soft/hard/fragile with less wastage, more hygienically and at high speeds
Effluent treatment	Potential to break down pesticide residues
Precipitation of airborne powders	Potential for wall transducers to help precipitate dust in the atmosphere; also removal of smoke from waste gases
Inhibiting enzyme activity	Can inhibit sucrose inversion, and pepsin activity; generally oxidases are inactivated by sonication, but catalases are only affected at low concentrations; reductases and amylases appear to be highly resistant to sonication
Stimulating living cells	Lower-power sonication can be used to enhance the efficiency of whole cells without cell wall disruption, for example in yoghurt, action of *Lactobacillus* was improved by 40%; also improved seed germination and hatching of fish eggs
Ultrasonically assisted freezing	Control of crystal size and reduced freezing time through zone of ice crystal formation
Ultrasonically aided filtration	Rate of flow through the filter medium can be increased substantially
Enhanced preservation (thermal and chemical)	Sonication in combination with heat and pressure has the potential to enhance microbial inactivation; this could result in reduced process times and/or temperatures to achieve the same lethality

20 kHz low amplitude/energy (40 μm/0.7 W cm^{-2}) ultrasound in an aqueous environment for 10 min improved the germination of lotus seeds by 30%.

7.3.4.3 **Ultrasonic Emulsification**

The most common use of power ultrasound in the food industry is the online commercial use of the 'liquid whistle' to emulsify a range of products. It can also be used to break emulsions. Trials have shown that fat globules undergo a substantial reduction in size (up to 80%) following ultrasonication and emulsions produced are often more stable than those produced conventionally, requiring little, if any surfactant. In trials, when temperatures of 70–75 °C were reached during ultrasonic homogenisation, a better particle distribution was achieved in comparison with lower temperature treatments. Ultrasound has been used industrially in the manufacture of salad cream, tomato ketchup, peanut butter and some cream soups and fruit juices (see Chapter 15) [31].

7.3.4.4 **Ultrasonic Extraction**

The mechanical effects of power ultrasound provide a greater penetration of solvent into cellular materials and also improve mass transfer [31]. Additionally, biological cells can be disrupted by power ultrasound, facilitating the release of cell contents. The benefits are acceleration of extraction rate and improved efficacy. Laboratory trials have taken place with coffee, tea, soya bean protein, sugar from sugar beet and rennin from calf stomachs and shown the benefits of improved yield. Scale-up issues could be a problem. However, a pilot-plant extraction process for soya protein was developed in the 1980s [39].

7.3.4.5 **Ultrasound and Meat Processing**

Power ultrasound has been used as an alternative to pounding, tumbling or massaging. There is evidence that enhanced myofibrillar protein extraction occurs and that binding in reformed and cured meats is improved following ultrasound application. The binding strength, water-holding capacity, product colour and yields of processed meats were evaluated after treating with either salt tumbling, sonication in an aqueous liquor or both. The samples that received both treatments were judged superior in all qualities [40]. Pilot studies in the early 1990s showed that sirloin steak connective tissue could be reduced when subjected to sonication at 40 kHz (2 W cm^{-2}) for 2 h [41].

7.3.4.6 **Crystallisation**

Controlled crystallisation of sugar solutions, hardening of fats, and the manufacture of chocolate and margarine are examples of food processes where crystallisation plays a vital part that can be improved by the application of power ultrasound (see Chapter 14). Power ultrasound acts in a number of ways during

crystallisation. It initiates seeding because the cavitation bubbles tend to act like crystal nuclei and so enhances the rate and uniformity of seeding. In addition, the ultrasound can break up any large crystalline agglomerates and can also effectively remove any encrustation from heat exchanger surfaces [35].

A successful, patented, full-scale ultrasonically assisted crystallisation operation has been used in the production of a crystalline drug for some years [42]. Examples of more recent applications involving ultrasonically assisted crystallisation include a patent by Kraft Jacobs Suchard issued in the late 1990s for the transformation of unstable to stable polymorphic crystals in edible fat manufacture [43] and a patent using sonication to retard fat bloom development in chocolate confectionery products [44]. Another area where ultrasound can be used to control crystal size is in the freezing operation where, under the influence of ultrasound, food materials such as soft fruits can be frozen with a reduced freezing time through the zone of ice crystal formation. In addition, it has been reported that more rapid and uniform seeding of ice crystals and reduced cellular damage can be achieved [45].

7.3.4.7 **Degassing**

Ultrasound has been used successfully for the control or removal of carbon dioxide from fermentation liquors. Japanese brewers have shown that nitrogen gas bubbling and ultrasonic vibrations can decrease dissolved carbon dioxide when brewing in cylindroconical tanks and can help control yeast metabolism, foam separation and froth height [46].

Another area of commercial application of power ultrasound is in foam control in pumped liquids and during container filling. Trials by NIZO Food Research in The Netherlands [47] showed that following the application of 1-s pulses of high-intensity ultrasound (20 kHz) for 3 min at 20 °C, up to 80% reduction in the foaming potential of supersaturated milk could be achieved. Low energy consumption was also reported. However, longer applications were required before any noticeable change in dissolved oxygen was observed.

7.3.4.8 **Filtration**

Ultrasound in filtration has two specific effects (see Chapter 14). Sonication will (a) cause the agglomeration of fine particles and (b) supply sufficient vibration energy to the filter to keep particles suspended above the medium and prevent clogging. This has been successfully applied to the reduction of water in coal slurry. Acoustic filtration can increase the rate of flow through the filter medium substantially and, when applied to fruit extracts and drinks, this technique has been used to increase the fruit juice extracted from pulp. Applying a potential difference across the pulp bed whilst at the same time applying ultrasound (electroacoustic filtration) can enhance the removal of juice from the pulp. In a pilot study, vacuum belt filtration reduced the moisture content of apple pulp from 85% to 50% but electroacoustic filtration reduced this further to 38% [22].

7.3.4.9 **Drying**

Applying power ultrasound to particles in a warm air convective drier can lead to increased drying efficiency, allowing lower drying temperatures, lower air velocities or increased product throughput (see Chapter 3). It is thought that initially sonication reduces the pressure above the particles and encourages water loss into the warm air passing over the bed of the drier. This approach has been evaluated for the hot air drying of carrots and results showed dramatic reductions in treatment times with a final moisture content of less than 1% attained easily. In addition, product quality was maintained, sample rehydration was greater than 70% and energy consumption was low. The technique may only be useful to specific food applications but scale-up and potential industrial applications seem promising [48].

7.3.4.10 **Effect of Ultrasound on Heat Transfer**

Increasing the rate of heat transfer from liquids to solid food particles by the use of high intensity 'power ultrasound' has been described [49]. Using ultrasonic power inputs of 0.14–0.05 $W\,g^{-1}$, Sastry and colleagues were able to demonstrate an increase in the convective heat transfer coefficient (h_{fp}) from about 500 $W\,m^{-2}\,K^{-1}$ to 1200 $W\,m^{-2}\,K^{-1}$: this was measured for aluminium particles in heated water. Later work [50] showed that, in a simple ultrasonic bath, this effect was dependent upon the position of the particle as well as the viscosity of the fluid medium. It was suggested that higher ultrasonication power levels would be required to ensure the enhanced heat transfer effects persisted in higher viscosity fluids. Studies at CCFRA have confirmed this effect and have also shown that, in simple batch heating trials, high intensity ultrasound has the potential both to significantly increase the rate of heat transfer and to reduce the thermal resistance of yeasts, moulds and bacteria. Heating trials have been conducted at CCFRA using water flowing over aluminium cylinders and 4% starch flowing through a packed bed of potato cubes. These trials demonstrated that, each time ultrasonic energy was applied to the carrier fluid during the heating in a mock holding tube, the centre of probed solids in the carrier fluid heated up more rapidly and more uniformly across the range of particles tested [51, 52].

7.3.5
Inactivation of Microorganisms Using Power Ultrasound

7.3.5.1 **Mechanism of Ultrasound Action**

Microbial cell inactivation is generally thought to occur due to three different mechanisms: cavitation, localised heating and free radical formation. There are two sorts of cavitation (transient and stable) which have been reported to have different effects.

Stable cavitation occurs due to oscillations of the ultrasound waves, which causes tiny bubbles to be produced in the liquid. It takes thousands of oscillatory cycles of the ultrasound waves to allow the bubbles to increase in size. As the ul-

trasonic wave passes through the medium, it causes the bubbles to vibrate, causing strong currents to be produced in the surrounding liquid. Other small bubbles are attracted into the sonic field and this adds to the creation of microcurrents. This effect, which is known as microstreaming, provides a substantial force, which rubs against the surface of cells, causing them to shear and break down without any collapse of the bubbles. This shear force is one of the modes of action, which leads to disruption of the microbial cells. The pressures produced on the cell membrane disrupt its structure and cause the cell wall to break down.

During transient cavitation, the bubbles rapidly increase in size within a few oscillatory cycles. The larger bubbles eventually collapse, causing localised high pressures and temperatures (up to 100 MPa and 5000 K) to be momentarily produced. It is widely believed that cellular stress is caused by the cavitation effect, which occurs when bubbles collapse. The pressures produced during bubble collapse are sufficient to disrupt cell wall structures, eventually causing them to break, leading to cell leakage and cell disruption.

Additionally, the localised high temperatures can lead to thermal damage, such as denaturation of proteins and enzymes. However, as these temperature changes occur only momentarily and in the immediate vicinity of the cells, it is likely that only a small number of cells are affected.

The intensity of bubble collapse can also be sufficient to dislodge particles, for example, bacteria from surfaces, and could displace weakly bound ATPase from the cell membrane, another possible mechanism for cell inactivation [53].

Free radical formation is the final proposed mode of action of microbial inactivation. Applying ultrasound to a liquid can lead to the formation of free radicals, which may or may not be beneficial. In the sonolysis of water, OH^- and H^+ ions and hydrogen peroxide can be produced, which have important bactericidal effects [54, 55]. The primary target site of these free radicals is the DNA in the bacterial cell. The action of the free radicals causes breakages along the length of the DNA and fragmentation occurs where small fragments of DNA are produced. These fragments are susceptible to attack by the free radicals produced during the ultrasound treatment and it is thought that the hydroxyl radicals attack the hydrogen bonding, leading to further fragmentation effects [32]. The chemical environment plays an important part in determining the effectiveness of the ultrasound treatment and it may be possible to manipulate or exploit these conditions in order to achieve a greater level of inactivation.

7.3.5.2 **Factors Affecting Cavitation**

The frequency of ultrasound is an important parameter and influences the bubble size [56]. At lower frequencies such as 20 kHz, the bubbles produced are larger in size and when they collapse higher energies are produced. At higher frequencies, bubble formation becomes more difficult and, at frequencies above 2.5 MHz, cavitation does not occur [33]. The amplitude of the ultrasound also influences the intensity of cavitation. If a high intensity is required then a high amplitude is necessary.

The intensity of bubble collapse also depends on factors such as temperature of the treatment medium, viscosity and frequency of ultrasound. As temperature increases, cavitation bubbles develop more rapidly, but the intensity of collapse is reduced. This is thought to be due to an increase in the vapour pressure, which is offset by a decrease in the tensile strength. This results in cavitation becoming less intense and therefore less effective as temperature increases. This effect can be overcome if required, by the application of an overpressure (200–600 kPa) to the treatment system. Combining pressure with ultrasound and heat increases the amplitude of the ultrasonic wave and it has been shown that this can increase the effectiveness of microbial inactivation. Pressures of 200 kPa (2 bar) combined with ultrasound of frequency 20 kHz and a temperature of 30 °C produced a decrease in the decimal reduction time (*D* value: the time taken to achieve a 1 log reduction in cell levels) by up to 90% for a range of microorganisms [57].

7.3.5.3 **Factors Affecting Microbiological Sensitivity to Ultrasound**

Bacterial cells differ in their sensitivity to ultrasound treatment [33]. In general, larger cells are more sensitive to ultrasound [58]. This may be due to the fact that larger cells have an increased surface area, making them more vulnerable to the high pressures produced during ultrasonication. The effects of ultrasound have been studied using a range of organisms such as *Staphylococcus aureus* and *Bacillus subtilis* and the Gram negative *Pseudomonas aeruginosa* and *Escherichia coli* [59]. Gram positive cells appear to be more resistant to ultrasound than Gram negative cells; and this may be due to the structure of the cell walls. Gram positive cells have thicker cell walls that provide the cells with some protection against sonication treatment. Other studies have indicated that there is no significant difference between the percentage of Gram positive and Gram negative cells killed by ultrasound [59]. Cell shape has been investigated and it has been found that spherical-shaped cells (cocci) are more resistant to ultrasound than rod-shaped cells [33]. *Bacillus* and *Clostridium* spores have been found to be more resistant to sonication than vegetative bacteria and many of the bacteria known to be resistant to heat are similarly resistant to ultrasound [60].

7.3.5.4 **Effect of Treatment Medium**

The characteristics of the food or substrate can influence the effectiveness of the ultrasound treatment applied. For instance, it has been found that the resistance of bacteria is different when treated in real food systems than when treated in microbiological broths [61]. In general, foods that contain a high fat content reduce the killing effect of the ultrasound treatment. Differences in effectiveness may be due to intrinsic effects of the environment on the ultrasound action (e.g. cavitation) or due to changes in ultrasound penetration and energy distribution. In a low viscosity liquid, ultrasound waves will pass through rela-

tively easily, causing cavitation to occur, but in a more viscous solution the ultrasound waves have to be of a higher intensity to enable the same level of penetration to be achieved. Low frequency, high power ultrasound is better at penetrating viscous products than higher frequency ultrasound. This is because ultrasound waves with higher frequency are more easily dispersed within the solution, causing a reduction in the overall intensity of the energy delivered.

7.3.5.5 **Combination Treatments**

With normal laboratory probes or cleaning baths, ultrasound applied on its own does not appear to significantly reduce bacterial levels. However, if it is combined with other preservation treatments such as heat or chemicals, the vital processes and structures of bacterial cells undergo a synergistic attack.

A varying response of microorganisms to ultrasound treatment depending on the pH of the surrounding medium has been observed [62, 63]. In particular, it has been found that, if the microorganisms are placed in acidic conditions, this leads to a reduction in the resistance of the organisms to the ultrasound treatment [63]. This may be due to the effects of the ultrasound on the bacterial membranes, making them more susceptible to the antimicrobial effects of the acid or unable to maintain essential internal pH conditions.

The most commonly used combination treatment is the use of heat with ultrasound, known as *thermosonication* (TS) or *manothermosonication* (MTS) if pressure is also included as a variable [34, 64, 65]. Several studies have shown that bacteria become more sensitive to heat treatment if they have undergone an ultrasound treatment either just before (presonication) or at the same time as the heat application. Increased cell death has been demonstrated in cells that have been subjected to a combined ultrasound and heat treatment compared with cells that were exposed to ultrasound treatment only or heat treatment only [64, 65].

Studies at CCFRA used a pilot-scale continuous-flow ultrasonic system manufactured by Sonic Process Technologies (SPT) Ltd, Shrewsbury. This system comprised a 1 kW unit operating at 30 kHz with radially mounted PZT ceramic transducers arranged in opposing pairs around the outside of a 32 mm diameter stainless steel pipe. Microbiological inactivation trials with this system in batch mode showed that in the case of *Zygosaccharomyces bailii* in orange juice there was a seven-fold increase in the inactivation of organisms using ultrasound in combination with heat, when compared with heating alone at 55 °C. With *Listeria monocytogenes* in milk there was a 20-fold increase in the inactivation achieved using ultrasound and heat, compared with heat alone at 60 °C. The amount of heat used in the ultrasound trials was about one-quarter of that used in the isothermal laboratory trials [51].

Spore formers have also been shown to have some degree of reduced resistance to subsequent heat treatments if they are exposed to power ultrasound at temperatures of 70–95 °C. The increased heat sensitivity caused by sonication can be quantified in terms of changes in the decimal reduction or D value; and

Table 7.3 shows the synergistic effect of heat and ultrasound for a range of bacterial species. Whilst these data show up to a 43% reduction in the heat resistance of the spore formers tested [66], other studies have shown no effect or a limited effect for other spore formers. Similar reductions have also been reported in the heat resistance data of spore formers such as *Bacillus cereus* and *B. licheniformis* after treatment with ultrasound (20 kHz) [67]. It is not fully understood why there is a limited effect of ultrasound on spores but it has been attributed to the fact that spores contain a highly protective outer coat, which prevents the ultrasound passing through, thus limiting the amount of perturbation that occurs within the spore.

During treatment with a combination of pressure and thermosonication (MTS), it has been shown that chemicals such as dipicolinic acid and low molecular weight peptides are released from spores of *B. stearothermophilus* [68]. In these combined treatments, spores are subjected to violent and intense vibrations due to increased cavitation effects [57]. The loss of substances from spores during this combination of pressure, heat and ultrasound suggests that spore cortex damage and protoplast rehydration may account for the subsequent reduction in heat resistance.

As previously discussed, the frequency of ultrasound used affects the type of cavitation response observed. Data from trials treating *L. monocytogenes* at 20, 38 and 800 kHz in whole milk indicate that 20 kHz was the most effective frequency, whilst 800 kHz had very little effect and resulted in a survivor tail. These data also suggested that the order in which heat and ultrasound are applied could have affected the inactivation observed.

Studies have also been conducted on the use of ultrasound in combination with chemical treatments. Ultrasound is able to disperse bacterial cells in suspensions, making them more susceptible to treatment with sanitising agents. One of the advantages of this type of combined treatment is that it could enable large reductions in the concentrations that are required when chemical treatments are used in isolation for sanitation and disinfection. This has additional

Table 7.3 Inactivation (*D* values) of a range of bacteria using heat and high-power ultrasound [34].

Organism	Heating temperature (°C)	*D* value (min)		
		Heat only	Heat + ultrasound	Ultrasound only
Bacillus subtilis	81.5	257.0	149.0	Not tested
Bacillus subtilis	89	39.2	22.9	Not tested
Bacillus licheniformis	99	5.0	2.2	No effect seen
Bacillus cereus	110	12.0	1.0	No effect seen
Enterococcus faecium	62	11.2	1.8	30
Salmonella typhimurium	50	50.0	30.0	No effect seen
Staphylococcus aureus	50.5	19.7	7.3	Not tested

advantages in that there is less likelihood of residual cleaning agents contaminating equipment after cleaning. Chemicals such as chlorine are often used to decontaminate food products or processing surfaces and it has been demonstrated that chlorine combined with ultrasound enhances the effectiveness of the treatment [69]. Trials were conducted using *Salmonella* attached to the surface of broiler carcasses. Treatment with ultrasound caused the cells to become detached from the surfaces, making it easier for the chlorine to penetrate the cells and exert an antimicrobial effect. For example, immersion in 0.5 ppm chlorine solution for 30 min reduced *Salmonella* by 0.89 log cycles, sonication for 30 min reduced the count by 1.4 log cycles, but a combination process reduced the count by 2.88 log cycles.

Similar results have been obtained in fresh produce cleaning and decontamination in trials at CCFRA using lettuce inoculated with *S. typhimurium*. A 2-l ultrasonic cleaning bath (30–40kHz) operating both with and without the addition of chlorine showed that the combination of ultrasound with chlorine resulted in just under 3 log reductions of *S. typhimurium* compared with about 1.5–2.0 log reductions for ultrasound or chlorine applications separately [70].

7.3.6
Effect of Power Ultrasound on Enzymes

Over 60 years ago, it was reported that pure pepsin was inactivated by sonication [71]. High-power ultrasound has been reported to inhibit various enzymes and work is still ongoing in this area. Recent examples have included the inactivation of enzymes involved in the inversion of sucrose [71] and inactivation of lipases and proteinases [72, 73, 74]. Wiltshire [75] reported that power ultrasound (20 kHz with a power intensity of 371 W cm^{-2}), when applied to peroxidase dissolved in a buffered (pH 7) solution of 0.1 M potassium phosphate at 20 °C, progressively reduced the original activity of the enzyme by 90% (1 log reduction) over a 3-h period. The main mechanisms by which enzyme inactivation is thought to occur are the same as those associated with the destruction of microorganisms (cavitation, localised heating and free radical formation).

7.3.7
Effects of Ultrasound on Food Quality

Power ultrasound has numerous nonpreservation applications, as previously discussed. There is a growing body of evidence to suggest that power ultrasound can be used in combination with heating to reduce bacterial populations and to bring about a substantial reduction in enzyme activity. However, what is uncertain, especially in relation to enzymes, is whether power ultrasound is effective when using processing conditions representative of typical commercial operations. For example, if power ultrasound were used for microbial inactivation in a continuous flow system the residence time would be of the order of seconds; it is uncertain as to whether this would be a sufficient exposure time to inacti-

vate enzymes. There is also a substantial knowledge gap regarding the extent to which the sensory qualities of foods are affected by power ultrasound. For example, there is surprisingly little published information regarding the effects of power ultrasound on food texture and nutritional composition. In order for power ultrasound to be commercially viable as a preservation technique, it must not only produce an acceptable level of food safety, it must also have a minimal effect on the sensory and nutritional qualities of the food being treated. This does not necessarily mean that there is no tolerance for minor effects. Thermal processing is known to result in vitamin C losses relative to a fresh counterpart. For example, Davidek [76] and others reported a 10–20% reduction in ascorbic acid resulting from pasteurisation. Sterilisation at 110–140 °C for 3.5 s was reported to result in a 17–30% loss. For ultrasonic processing to have any chance of commercial success, the process impact on nutritional composition should be comparable or reduced relative to conventional thermal processing. Trials have been carried out at CCFRA (unpublished) with the aim of investigating the effects of power ultrasound on the sensory qualities of foods to establish if possible undesirable effects of ultrasonication outweighed the potential benefits of the technology. In batch trials using a probe type system, no significant reduction in total vitamin C was observed in a sprout pureé after treatments of around 13–28 $W\,m^{-2}$ at 20 °C and 40 °C with holding times of between 1 min and 5 min. At 40 °C there did appear to be some reduction in total vitamin C, but a statistically significant correlation could not be established due to variation in the raw material vitamin C levels. Within the same project, a substantial reduction in viscosity was observed when a 5% solution of a modified waxy maize starch was sonicated for 1 min in a flow cell with a probe assembly. In this trial, the apparent viscosity of the solution at a shear rate of 10 s^{-1} was reduced from 2360 cP to 13 cP. However, it should be stressed that, in this type of system, the ultrasonic energy is transmitted to the food in a particularly intense and localised manner and alternative equipment designs could minimise this detrimental effect.

7.3.8 The Future for Power Ultrasound

Ultrasound currently finds numerous applications in the food industry, including emulsification of fats and oils, mixing, blending, cutting and accelerating the ageing processes in meats and wines. In the laboratory, it has the potential to be applied to the pasteurisation of a range of low viscosity liquid products as well as enhance the effectiveness of other processing methods. However, the process remains some way from being a viable preservation technology. This is in part due to a lack of knowledge regarding full-scale design and scale-up, but also in part due to a considerable knowledge gap relating to the optimisation of process conditions for food processing. Much more research is required to gain a greater understanding of issues such as:

- equipment design to optimise microbial and enzyme inactivation;

- ultrasonic enhancement of heat transfer to augment existing thermal processes;
- accurate mapping of field intensity variations within a treatment chamber to develop reliable scheduled processes using ultrasound;
- inactivation mechanisms for vegetative cells, spores and enzymes, which need to be clearly identified, especially when combination technologies are used;
- development of mathematical models for the inactivation of microorganisms and enzymes involving ultrasound;
- identifying the influence of food properties such as viscosity and particle size on process lethality as well as the implication of process deviations when using ultrasound.

7.4 Other Technologies with Potential

Several other technologies have been the subject of research interest for food preservation and processing, including oscillating magnetic fields, arc discharge, pulsed broad-spectrum light and UV light for preservation. Although not well advanced, they are considered here in outline as some of them, at least, are likely to develop further.

7.4.1 Pulsed Light

Pulsed light will not penetrate deeply into foods but has potential for the treatment of surfaces – on the product, on packaging and on surfaces used for food preparation. The light in question is broad spectrum white light – which can include light from the ultraviolet and infrared regions – with an energy density of 0.01–50.0 J cm^{-2} [77]. It is generally applied as a single pulse or a short series (up to 20) of short pulses (milliseconds duration). Although the light spectrum generated has a similar composition to sunlight, the main company involved in developing this technology suggest that the effects arise because the intensity involved is roughly 20 000–90 000 times that of sunlight at the earth's surface.

According to publicity materials from equipment manufacturers, pulsed light treatments have been reported to reduce spoilage of baked products by inactivation of moulds, *Salmonella* on egg shell and chicken surfaces, and *Pseudomonas* on cottage cheese. It has been suggested that up to 9 log reduction in viable vegetative bacteria can be achieved on nonporous smooth surfaces. The mechanisms of action that have been proposed include short-term, thin-layer temperature effects (the treated surface briefly reaching 300–700 °C), photochemical effects (formation of free radicals) and DNA damage.

Significant research and independent evaluation are still needed to determine the true potential of this approach, but the scale of the potential effects – at least for certain types of product and/or material – suggest that this is warranted.

In 2002, PurePulse Technologies (the main manufacturer of this technology) ceased trading, which has made the future of the use of this technology for food preservation a little uncertain.

7.4.2 High Voltage Arc Discharge

High voltage arc discharge processing involves rapidly discharging voltages through an electrode gap immersed in aqueous suspensions [78]. The discharge is believed to generate intense physical waves and chemical changes (through electrolytic effects) which can inactivate microorganisms and enzymes without any significant rise in temperature. However, the approach is not without its problems – for example, the shock waves can cause disintegration of food particles – and relatively little has been published to support or refute its use for food preservation. That having been said, the initial findings are likely to be explored further and it might emerge as having some specialist applications, if not a wider use.

7.4.3 Oscillating Magnetic Fields

Speculation surrounding the potential use of high intensity magnetic fields is largely based on a US patent [79] issued to Maxwell Laboratories (Pure Pulse). The patent suggested that a single pulse of an oscillating magnetic field with a strength of 5–100 Tesla and a frequency of 5–500 kHz, could bring about a 2 log reduction in the number of viable microbes in the food within the field. Multiple pulses could result in a commercially sterile product.

However, other studies have not universally corroborated these findings, with some suggesting that oscillating magnetic fields do not affect the microbial population or that the treatment can even stimulate microbial growth. As with some of the other technologies discussed here, such variation may be due either to differences in the treatment intensity or means of delivery, to differences in the media/food in which the microbes are treated, or to the target microbes. It does seem, however, that further research is needed to clarify the extent and mechanisms of any such effect before the approach can be fully assessed as a tool for food preservation.

7.4.4 Plasma Processing

The use of cold gas phase plasmas has been proposed for the inactivation of microorganisms [80]. The term 'cold plasma' refers to partially ionised or activated gases existing at temperatures in the region of 30–60 °C. Cold plasma irradiation could be used to inactivate microorganisms on the surface of a range of materials, including packaging and food surfaces such as fruit, vegetables and meat. Cold plasma irradiation has the advantage that it can be readily switched

on and off, making it much more controllable than something like irradiation using a radioactive source. To date, only highly exploratory studies have been carried out on plasma processing for food preservation [80]. In trials conducted by ATO in the Netherlands, structural changes were observed in microorganisms irradiated by a cold gas phase plasma. Gram negative organisms were completely fragmented after treatment. Gram positive organisms had some cell leakage. Using exposure times of 25 s to several minutes, a 5–6 log reduction was demonstrated. Factors which appeared to influence the efficacy of the process included the density of bacterial loading, exposure time and spatial location. Organisms located central to the plasma source were inactivated most readily. Interestingly, after treatment, not all of the nonviable microorganisms were structurally damaged and, conversely, some structurally damaged cells were still viable. This suggests that the mechanisms for inactivation may not simply be due to structural damage. The work at ATO has shown that cold gas phase plasmas can inactivate microorganisms, but a great deal more fundamental research is still required before the technique can be applied commercially.

7.4.5
Pasteurisation Using Carbon Dioxide

Praxair Technologies Inc. has developed a continuous nonthermal pasteurisation process for juice products [81]. The plant operates at around 34.5 MPa, which is required to solubilise the CO_2. The components of the system are relatively simple. The juice is supplied from a raw juice tank and is mixed with CO_2 under pressure. The conditions are maintained such that the CO_2 maintains a liquid state and does not freeze the product. After moving through a holding coil, the juice is de-aerated to strip off the CO_2. According to Praxair, at least a 5 log reduction for a range of pathogens is readily achievable using 5–20% w/w carbon dioxide. Inactivation of *E. coli* O157.H7, *S. muenchen*, *S. agona* and *L. monocytogenes* has been demonstrated using this technique. The process has been shown to be effective for processing orange juice concentrate and orange juice with and without pulp. In studies reported by Praxair, vitamin C and folic acid levels, brix, pH, titratable acidity and cloud stability were virtually unaffected when comparing freshly squeezed juice with a CO_2 pasteurised juice. Sensory trials by two independent laboratories found no significant differences between freshly squeezed and CO_2 pasteurised orange juice. Commercial systems are now available from Praxair operating at 40 US gallons min^{-1} (151.4 l min^{-1}).

7.5
Conclusions

This chapter has provided an overview of some of the main nonthermal preservation technologies attracting both academic and industrial interest. Some of these technologies may come to nothing while some, such as PEF processing,

are likely to succeed and find, at least, niche applications in the food industry. The road to commercialisation of a new technology can be rocky and history has shown that timing, industrial need and a little good fortune all play an important role in the successful adoption of a new technology. HPP, for example, was discovered over 100 years ago but it took 80 years before it could realistically be used for commercial food processing. The success of pressure-processed products, particularly in the USA, has relied heavily on the entrepreneurial spirit of relatively small companies and the careful selection of niche products with high added value. It is highly unlikely that any of the technologies discussed in this chapter will one day replace thermal preservation. They will, however, find niche applications for products where they can provide solutions that simply cannot be delivered by conventional technologies.

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