Emerging Technologies in Food Processing

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Annu. Rev. Food Sci. Technol. 2011. 2:203–35

First published online as a Review in Advance on November 15, 2010

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

This article's doi:
10.1146/annurev.food.102308.124129

1941-1413/11/0410-0203$20.00

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Keywords

nonthermal food processing, high pressure, pulsed electric fields, ultrasound, cold plasma

Abstract

High hydrostatic pressure (HHP), pulsed electric fields (PEFs), ultrasound (US), and cold plasma (CP) are emerging technologies that have already found application in the food industry or related sectors. This review aims to describe the basic principles of these nonthermal technologies as well as the state of the art concerning their impact on biological cells, enzymes, and food constituents. Current and potential applications will be discussed, focusing on process-structure-function relationships, as well as recent advances in the process development.

INTRODUCTION

The development of emerging technologies in food processing addresses specific consumer needs toward safe, healthy, and minimally processed foods. These innovative processes also lead to environmentally friendly and sustainable food manufacturing techniques with low energy requirements and reduced water use that overcome some limitations given by current food processing practices (Toepfl et al. 2006). Taking advantage of specific potentials and opportunities of these new processes, including the understanding and control of the complex process-structure-function relationships, offers the possibility for a science-based development of tailor-made foods. High hydrostatic pressure (HHP), pulsed electric fields (PEFs), ultrasound (US), and cold plasma (CP) are used to exemplify scalable and flexible food manufacturing techniques. In this review, we discuss the state of the art regarding the research and application of these emerging technologies and demonstrate the potential of establishing new routes of process and product development by interfacing food science and food manufacturing.

Significant, science-based achievements have been made to better understand the basic principles underlying HHP and PEF processing (Hendrickx & Knorr 2002, Raso & Heinz 2006). In addition, the collection of kinetic data, especially on microbial and enzyme inactivation by HHP treatment and on metabolite recovery and microbial inactivation treatment by PEFs, as well as the generation of data related to mechanisms including the use of plant cell cultures as model systems (Doona & Feeherry 2007, Dörnenburg & Knorr 1993, Wouters et al. 2001), has laid the groundwork for targeted processing.

The food and beverage industry offers many possibilities for the use of US. The basic phenomena, such as microstreaming and cavitation, and the resulting hydrodynamic shear-forces, make US an alternative technology for the realization of homogenization, dispersion, and emulsification, as well as for the disintegration of tissue to enhance mass transfer processes (Mason et al. 1996, Povey & Mason 1998).

CP-based surface treatments, such as ultrafine cleaning, etching, surface functionalization, and thin film deposition, and environmental applications, such as exhaust air cleaning, are proven techniques and already applied using industrial scale plasma systems (Foest et al. 2005, Roth 1995). A strong increase of plasma applications in medical device technology and therapeutic medicine is currently taking place, including applications such as plasma decontamination, and research is focusing on the interaction between plasma and biological cells and tissue as well as on the plasma diagnostics with regard to the understanding and control of the complex behavior of CP (Daeschlein et al. 2010, Weltmann et al. 2009). Similar research is undertaken in the field of food science to explore the potential for CP application in the food industry (Mastwijk & Nierop Groot 2010), which will be discussed below.

Understanding the impact and potential of such technologies on food systems at the cellular level will enable the design of tailor-made foods and to establish process-structure-function relationships. Based on this knowledge, completely new process design and the incorporation of HHP, PEFs, US, and CP in traditional processes, as well as the generation of improved equipment design, will be possible. Consequently, the use of such nonthermal processes for maintenance or even improvement of product quality via processing to fulfill the PAN (consumer preference, acceptance, and needs) concept of the European Technology Platform Food for Life (**http://etp.ciaa.eu**), and thus the reverse food engineering approach will be one major innovative concept within the food industry.

HIGH PRESSURE PROCESSING

The demand for high-quality food opens high pressure (HP) as the most developed emerging processing technology for gentle preservation of food, the possibility to move from the research

and development environment into widespread industrial-scale applications. The pressure levels range from several tens of MPa in common homogenizers or supercritical fluid extractors to several hundreds of MPa in ultra-HP homogenizers or HP pasteurization units. In addition to the inactivation of microorganisms to enhance the shelf life of the treated food, there are numerous other interesting applications, such as food structure engineering (Diels & Michiels 2006, Knorr et al. 2006, Sharma & Yadav 2008).

Process Description

Without doubt, the inactivation of vegetative microorganisms to extend the shelf life of food is currently the largest commercial application of isostatic HP at an industrial scale by far. Typical industrial HP units consist of a horizontal HP vessel and an external pressure generating device. The simplest practical system of an intensifier is a single-acting, hydraulically driven pump. (Rovere 2002).

For the HP treatment, the packaged food is deposed in a carrier and automatically loaded into the HP vessel, and the vessel plugs are closed. The pressure-transmitting media, usually water, is pumped into the vessel from one or both sides. After reaching the desired maximum pressure the pumping is stopped, and in ideal cases no further energy input is needed to hold the pressure during dwell time. In contrast to thermal processing in which temperature gradients occur, all molecules in the HP vessel are subjected to the same amount of pressure at exactly the same time because of the isostatic principle of pressure transmission (Heinz et al. 2009, Rastogi et al. 2007).

Given that pressure and temperature are closely connected parameters, the thermodynamic effect of the adiabatic heat of compression, which occurs during the compression and decompression of the treated food and the pressure-transmitting media, has to be taken into account. Following the first and the second law of thermodynamics and by a rearrangement of the Maxwell equations, heating during compression and cooling during decompression can be described as a function of thermophysical properties of the compressible product (Perry 1984, Reineke et al. 2008). This quasi-adiabatic heating or cooling occurs instantly. Hence, pressure-induced temperature changes are predictable and homogeneous throughout the product, assuming it is homogeneous in composition. This ideal adiabatic process does not occur in practical applications, but the extent of temperature increase could be estimated with 3°C–9°C per 100 MPa dependence of the treated food or food composition, respectively (Ting et al. 2002).

Another possibility for the treatment of liquid unpacked products, such as fruit juices, is to fill the vessel with the liquid product, which also acts as the pressure transmitting media and realize the compression directly via a moving piston (Patterson et al. 2007, Rovere 2002).

A further HP process for the treatment of liquid food is the HP homogenization (150 MPa $< p <$ 400 MPa), which became more important during the last decade. It is used not only for the preparation or stabilization of emulsions and suspensions, or for creating physical changes such as viscosity changes in products (Pandolf 1998, Paquin 1999), but also for cell disruption of yeasts or bacteria to release intracellular products. It can be anticipated that HP homogenization for the production of emulsions or suspensions will also cause a partial inactivation of the microbial population (Diels & Michiels 2006).

History

The first HP treatment of food was reported in the late 1890s dealing with the inactivation of microorganisms in milk (Hite 1899), showing extended shelf life of bovine milk after HP treatment. Bridgman (1914) reported the coagulation of egg albumin by HP and determined

different product properties as compared to gels obtained by heat coagulation. Furthermore, he presented an extensive data set for the phase diagram of pure water (Bridgman 1912). Since the early 1980s, HP treatment has been evaluated as a food processing alternative to classical heat treatment technologies (Knorr et al. 1998). Decisive for the emerging research effort in this field was the growing consumer demand for minimally processed, fresh-like, safe, high quality food products (Hendrickx & Knorr 2002).

The first industrial HP application for the commercial preservation of food was installed in 1991 in Japan (Yaldagard et al. 2008). The food industry and related research institutes have extensively explored this field and introduced the HP technology to a broad range of products. As a result, during the past 10 years the number of industrial HP systems increased steadily, and at present more than 150 industrial scale installations with a maximum volume of 687 liters and total annual production of more than 300,000 tons are in use worldwide (Tonello Samson C., personal communication).

Research State of the Art

In general, small molecules are only slightly affected by HPP because no covalent bonds are split at pressure below 2 GPa. However, the impact of HPP on macromolecules, microorganisms, and complex systems such as food are manifold and hence discussed separately.

Impact on biological cells. In order to find an alternative to conventional thermal processing while maintaining a maximum level of food safety, intensive research on the HP inactivation of vegetative microorganisms and bacterial spores has been carried out in the past. However, in HP applications, thermal effects cannot be fully ruled out because of the adiabatic heat of compression, and HP inactivation of vegetative microorganisms is almost always connected to a thermal treatment (Smelt et al. 2001). The specific effects and damages of pressure on vegetative microorganisms are complex and cannot be evaluated detached from these effects. Primarily, the lethal effects of HP on vegetative microorganisms are attributed to enzyme inactivation and cell membrane rupture (Ananta 2005, Ardia 2004). In the course of identifying inactivation mechanisms, flow cytometry was used as a potent tool to gain insights to the states and mechanisms of cell damage of pressure-treated microorganisms (Black et al. 2007, Mathys et al. 2007).

Pressure treatments do not necessarily weaken biological cells. At low pressure levels, increased resistance of microbial cells was observed, and pressure-induced thermo-tolerance of lactic acid bacteria occured after HP treatment between 100 and 200 MPa (Ananta & Knorr 2003). As a result of this phenomenon, pressure-induced stress response was found to offer promising processing options such as pretreatment of lactic acid bacteria before drying or freezing for the purpose of starter culture production (Ananta 2005).

Microbial inactivation of more than five-log cycles in food products is reported by several authors to occur at pressures between 300 and 800 MPa (Ananta et al. 2005, Hendrickx & Knorr 2002). The special shape of isokinetic lines at higher pressure (**Figure 1**) for the inactivation of microorganisms shows the synergisms between pressure and temperature. This behavior is typical for vegetative cells but was also observed for bacterial spores (Ardia 2004, Margosch et al. 2006), viruses (Isbarn et al. 2007), and proteins (Heinz & Kortschack 2002, Smeller 2002). By increasing the process temperature, it is possible to decrease the applied pressure, but unwanted reactions (e.g., based on residual enzyme activity) that would lead to quality losses also have to be taken into account.

According to Smelt et al. (2001) the HP-induced effects resulting in vegetative cell death can be summarized as follows:

1. Proteins and enzymes: HP induces unfolding of globular proteins. It is assumed that the combined, complete, or partial inactivation of numerous enzymes and metabolic pathways leads to the inability to proliferate and cell death (Bunthof 2002).
2. Membranes: Other than the inactivation of enzymes, membrane damage is considered as one of the key events related to microbial cell death. Membranes undergo phase transitions and solidify under pressure, and perturbations are promoted (Schlueter 2004, Winter 1996). In addition, pressure leads to the detachment and inactivation of membrane proteins (Ulmer et al. 2002).
3. Ribosomes: The disintegration of ribosomes in their subunits is promoted by pressure and may be related to cell death (Niven et al. 1999).
4. pH: The maintenance of intracellular pH is crucial for the survival of cells. Some authors related cell death predominantly to intracellular pH changes, which are related to inactivation of enzymes controlling the acidity and membrane damage (Molina-Gutierrez et al. 2002).

Bacterial spores have a higher barotolerance than vegetative bacteria and survive pressures above 1,200 MPa at ambient temperatures (Ananta et al. 2001, Margosch et al. 2006). Early approaches toward spore inactivation aimed at a pressure-induced germination at moderate pressure. However, combination processes with spore germination at pressures below 200 MPa and an additional moderate heat treatment could not guarantee sufficient inactivation because a small population of spores could not be germinated and remain in the dormant state (Heinz & Knorr 1996). The HP inactivation of bacterial spores is not yet fully understood and is still highly relevant in modern HP sterilization research activity. A detailed discussion of HP-related spore inactivation mechanisms is given by Mathys (2008).

Impact on Enzymes

HP is regarded as a mild process by which the primary structure of proteins is not affected. However, it could have an impact on hydrophobic interactions, which stabilize the quaternary and tertiary structure through reversible unfolding, and the secondary structure through irreversible unfolding. Generally, pressure-induced changes in proteins and enzymes between 100 and 300 MPa at ambient temperature are reversible, whereas an increase of pressure above 400 MPa could cause an irreversible unfolding, leading to inactivation of the enzyme. Pressure also favors an unfolding of protein chains as well as a dissociation of oligomeric proteins (Buckow 2006, Tauscher 1995).

Owing to conformational changes, unfolding of an enzyme can alter its functionality and result in a decreased or increased biological activity and could even change its substrate specificity (Buckow & Heinz 2008, Ludikhuyze et al. 2002). The pressure stability of enzymes can vary significantly ranging from pressure-sensitive enzymes such as phosphohexose isomerase from bovine milk ($p < 400$ MPa) (Rademacher & Hinrichs 2006) to extreme pressure resistant enzymes such as horseradish peroxidase ($p > 700$ MPa) (Smeller & Fidy 2002). However, a categorization of enzymes as a result of their pressure stability is not appropriate, because there is structural variability among enzymes catalyzing the same reaction (Buckow & Heinz 2008).

Figure 2 depicts the inactivation of 90% of different polyphenol oxidases (PPO) after 10 min in the pressure and temperature landscape. Furthermore, the pressure-temperature resistance of enzymes shows a significant dependence on matrix conditions such as the pH value (Riahi & Ramaswamy 2004, Zipp & Kauzmann 1973), whereas even isoforms of an enzyme from the same origin can vary in physical stability between several hundred MPa (Buckow et al. 2005, Rodrigoa et al. 2006).

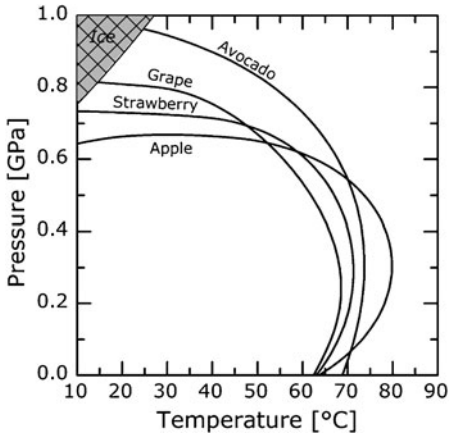

Figure 2

Pressure-temperature isorate diagram for 90% inactivation of polyphenol oxidase after 10 min under isobaric and isothermal conditions, from apple (Buckow et al. 2009), advocado (Weemaes et al. 1998), white grapes (Rapeanu et al. 2005), and strawberry (Dalmadi et al. 2006) (Figure taken from Buckow & Heinz 2008).

Moreover, owing to the fact that pressure and temperature often act antagonistically, this could further result in an enhanced thermostability of enzymes at specific pressures (Heremans & Smeller 1998). Such stabilization of enzymes could occur when the volume difference between the folded and unfolded state of the protein is positive, which might be caused by the promoted formation of noncovalent bonds under the applied pressure.

Impact on food constituents. HP could well be used for food texture engineering, owing to its influence on the properties of food ingredients. The primary structure of low molecular weight molecules such as peptides, lipids, vitamins, and saccharides is rarely affected by isostatic HP because of the very low compressibility of covalent bonds at pressures below 2 GPa (Cheftel & Culioli 1997, Oey et al. 2006, Van den Broeck et al. 1998). Conversely, HP can change the native structure of macromolecules such as starch similar to thermal-induced structure changes.

Several studies have investigated the gelatinization of different starches under HP. Potato starch was found to be less affected by pressure treatment than the other starches investigated, such as tapioca or wheat starches. Several studies revealed that the swelling of starch granules as measured by loss of birefringence depends on pressure treatment conditions as well as on the type of starch granules. Furthermore, damaged starch granules are less pressure resistant than intact starch granules (Bauer & Knorr 2005, Douzals et al. 1996, Michel & Autio 2002).

In consideration of the impact of pressure and dwell time on the pressure-induced starch gelatinization, suspensions of starch can also possibly act as a potential indicator for pressure, time, and temperature treatment of foods (Rumpold 2005). Another possible HP application is the modification of proteins. Dumay et al. (1998) observed gelatinization by HP treatment of solutions of a ß-lactoglobulin isolate. The pressure-induced gel matrix offered small particles in highly packed ß-lactoglobulin, unlike the gels after heating, which exhibit fine-stranded aggregates (Dumay et al. 1998). Furthermore, Zeece et al. (2008) reported that an HP treatment from 400 MPa to 800 MPa at 20°C improved considerably the digestibility of ß-lactoglobulin. Effects of HP on protein and polysaccharide mixtures have been studied extensively by Michel & Autio (2002) with the aim to establish and facilitate the creation and control of new textures.

Current Applications/Developments

HP pasteurization is currently the main application in industrial HP processing. The success of HP-treated products is primarily because HP-treated foods, in addition to their microbiological safety, retain more of their original fresh taste, texture, and nutritional content. These products are consequently products with superior quality compared to their thermally treated counterparts (Patterson et al. 2007).

At present, there is a wide range of HP-processed products available, from meat products, fruit juices, and seafood to dairy products and ready-to-eat meals. More than 30% of the total vessel volume is used to process meat products like sliced ham, turkey or chicken cuts, and ready-to-eat-meals, primarily to inactivate *Listeria* and to increase shelf life of the treated product. Thirty-four percent of the total vessel volume is utilized to inactivate enzymes (e.g., PPO in avocado-based products) and to modify the texture of vegetables and fruits and related products such as fruit desserts, smoothies, or ready-to-eat vegetable dishes. In 13% of the total vessel volume, fruit juices are processed to increase the shelf life by maintaining their sensory quality.

Another 14% of the total vessel volume is used for the pressure treatment of seafood, mainly oysters and shellfish. A further advantage, other than an increased shelf life (destruction of *Vibrio vulnificus*) during the treatment of oysters, is an easier opening of the oyster shell and improved meat extraction of lobsters and crabs (**www.nchyperbaric.com**).

Process-Structure-Function Interactions

The potential use of HP in food structure engineering implies diverse advantages when pressure is applied with moderate temperatures. Knorr et al. (2006) investigated HP applications for food biopolymers regarding protein stability, enzymatic activity, and starch gelatinization. The pressure-induced gelatinization differs from the thermally induced gelatinization of starches. For instance, HP-treated starches retain their granular structure and exhibit reduced swelling and can form weak gels. Therefore, they offer different functional properties than heat-treated starches do. Pressure, temperature, pressure dwell time, type of starches, and the content of free available water can influence the phase transition of various starches (Rumpold 2005).

Additionally, pressure-induced protein gels retain their original flavor and color. Furthermore, they are glossy, unlike heat-induced gels. Hence, HP treatment can be applied for the manufacturing of milk products to improve yogurt texture (Johnston et al. 1993) or increase cheese yield (López-Fandino et al. 1996).

Process Developments

HP offers several interesting applications for the food industry. For example, HP-treated starches have reduced digestibility and might be used to substitute fat in dietary foods (Sharma et al. 2008, Zhang et al. 2008). Furthermore, the inactivation behavior of enzymes under pressure could be used as an indicator for reaching HP pasteurization or sterilization conditions and to detect temperature differences in the pressure vessel during processing (Grauwet et al. 2010).

A further possible application of HP is its combination with temperatures below 0°C for subzero storage of food in the liquid state or a cold denaturation of proteins to form gels with unique properties. Moreover, HP could be used to reduce the thawing time for frozen products such as fish (LeBail et al. 2002, Schubring et al. 2003).

Research Needs and Challenges

The continuous increase of HP research over the past decades has already generated the basis for several commercially available HP-processed high quality products. Besides the cold pasteurization, isostatic HP can also be used to generate novel functional features, such as specific textures or health-promoting properties to develop tailor-made foods. A very promising field for further research is to use HP to modulate microbial fermentations or enzymatic conversations. Furthermore, HP might also influence the biosyntheses pathways and could lead to the formation of product variants with novel functional properties (Aertsen et al. 2009). A better understanding of the mechanisms underlying the pressure and temperature stability could also enable the possibility to construct HP-resistant enzymes. In spite of extensive research, there is still a lack of data about the behavior of nutrients, allergens, and food-spoiling viruses under the defined matrix (e.g., pressure stable buffer solutions) (Mathys & Knorr 2009) and treatment conditions (isothermal and isobaric conditions during dwell time), which could be used for modeling or the investigation of the underlying mechanisms.

Further research is needed on the process conditions that are necessary to inactivate pressure-resistant bacterial endospores. However, to successfully introduce the HP thermal sterilization in the food industry, a pressure-temperature resistant indicator microorganism has to be identified, and the microbial targets that lead to an inactivation have to be understood. It should also be noted that physicochemical properties of food constituents such as water vary under HP or high temperature conditions, making process development and understanding of mechanisms challenging (Mathys & Knorr 2009). To reduce the processing cost as well as to investigate the temperature distribution during the HP treatment, especially under sterilization conditions, modeling and simulation of the behavior of HP-treated biomaterials plus the temperatures distributions in the HP vessel will also present a challenge (Delgado et al. 2008).

PULSED ELECTRIC FIELDS

Process Description

When exposed to high electric field pulses, cell membranes develop pores that may be permanent or temporary, depending on the intensity and treatment conditions (Angersbach et al. 2000, Zimmermann et al. 1974). Pore formation increases the membrane permeability, resulting in the loss of cell content or intrusion of surrounding media (Vorobiev & Lebovka 2008). Low-intensity treatment has the potential to induce stress reactions in plant cells, resulting in the promotion of a defense mechanism by increased production of secondary metabolites (Dörnenburg & Knorr 1993, Galindo et al. 2009, Gomez Galindo et al. 2008). An irreversible perforation of the cell membrane reduces permanently its barrier effect, causing cell death, which can be applied for plant and animal raw material disintegration (Angersbach & Knorr 1998, Toepfl & Heinz 2007), as well as for the nonthermal inactivation of microorganisms (Lelieveld et al. 2007).

PEF processing consists of the application of very short electric pulses (1–100 μs) at electric field intensities in the range of 0.1–1 kV cm^{-1} (reversible permeabilization for stress induction in plant cells), 0.5–3 kV cm^{-1} (irreversible permeabilization of plant and animal tissue), and 15–40 kV cm^{-1} for the irreversible permeabilization of microbial cells. Depending on cell size and shape, the before-mentioned field intensities lead to the formation of a critical transmembrane potential, which is regarded to be the precondition for membrane breakdown (Tsong 1996).

Because the mechanism of electroporation is based mainly on a mechanical electrocompressive force affecting the cell membrane, the PEF technology is considered a nonthermal cell disintegration or preservation process. It provides an alternative to mechanical, thermal, or enzymatic

cell disintegration of plant and animal raw materials, providing a short-time (milliseconds), low-energy treatment, as well as to the traditional thermal pasteurization of liquid food products (Barbosa-Cánovas et al. 1999, Raso & Heinz 2006).

Generally, high-intensity electric pulses can be generated by the switched discharge of a suitable capacitor bank. The characteristics of the discharge circuit determine the shape of the time-dependent potential at the treatment chamber where the product is exposed to the electric field (Barsotti et al. 1999). Depending on the product and application, parallel plate electrode treatment chamber configuration or colinear type treatment chambers are most commonly used. A comprehensive review on treatment chamber configurations can be found in Huang & Wang (2009).

History

A first commercial application of electrical energy for pasteurization was the Electropure process established in the 1920s for the extension of shelf life of milk, based on ohmic heating. Unlike the Electropure process, based on Joule heating, which involves the passage of an electrical current resulting in the generation of heat by the resistivity of the food material, a technology using high voltage electricity up to 32 kV for a pulsed discharge application across two electrodes has been investigated since the 1950s and resulted in a process called electrohydraulic treatment (Gilliland & Speck 1967).

Pioneering experimental work of the PEFs application for food processing was undertaken by Heinz Doevenspeck (Doevenspeck 1961). The first systematic studies on the nonthermal lethal effect of PEFs on microorganisms were conducted at Unilever Research Center in the United Kingdom (Sale & Hamilton 1967). Krupp Maschinentechnik was recognizing the technique's potential and developed the processes Elcrack® and Elsteril® (Krupp 1988). The first commercial PEF application was installed in 2005 in the United States for fruit juice preservation (Clark 2006). Food and Drug Administration (FDA) clearance had been available since 1996, indicating the technique's potential for safe and gentle preservation.

Research State of the Art

Effective inactivation for most of the spoilage and pathogenic microorganisms has been shown, and colony count reductions depending on treatment intensity, product properties, and type of microorganism in the range of 4–6-log cycles are comparable to traditional thermal pasteurization. Bacterial spores and viruses are not affected by the PEF treatment (Lelieveld et al. 2007). Reports on the effects of PEFs on enzymes are limited, and different experimental setups and processing parameters make them difficult to compare (Van Loey et al. 2002). Thermal effects were also found to contribute to enzyme inactivation during PEF treatment (Jaeger et al. 2009a, Jaeger et al. 2010). Industrial large-scale applications have been realized for the disintegration of plant raw materials such as sugar beet and fruit mashes (Bluhm & Sack 2009). Industrial equipment is available up to capacities of a single system of 2,200 liters h^{-1} for PEF processing of liquids for nonthermal pasteurization with total treatment costs of 0.6 cents kg^{-1} and 22 ton h^{-1} for cell disintegration applications with related total treatment costs of 0.5 Euro ton^{-1} (DIL 2009).

Impact on biological cells. Until now there has been no clear evidence on underlying mechanisms at a cellular level, but two main effects have been described to be triggered by the electric field: the ionic punch-through effect (Coster 1965) and the dielectric breakdown of the membrane (Zimmermann et al. 1974). The factors that affect microbial inactivation during PEF treatment are process factors such as electric field intensity, pulse width and shape, treatment time and

temperature, microbial factors such as type, shape, size, concentration, and growth stage of microorganism, and media factors such as pH, antimicrobials, ionic compounds, electrical conductivity, and medium ionic strength.

Membrane damage and inactivation of microorganisms due to PEFs, first considered as an all-or-nothing event in some studies (Russel et al. 2000, Simpson et al. 1999), revealed a required differentiated approach, even if the critical parameters for the electrical breakdown of cell membranes are exceeded. Membrane damage and sublethal injury is repairable under certain conditions, and the extent to which cells repair their injuries was found to depend on treatment intensity and microorganism and treatment medium pH (Garcia et al. 2005, Somolinos et al. 2008a, Somolinos et al. 2008b).

Impact on enzymes. The evaluation of the effect of PEFs on enzymes is complex. Available reports on mechanisms are limited, and different experimental setups and processing parameters make them difficult to compare (Schuten et al. 2004, Van Loey et al. 2002, Yang et al. 2004). PEF side effects such as changes of pH at the electrode surface due to electrochemical reactions (Saulis et al. 2005), as well as the occurrence of temperature hot spots due to ohmic heating effects within a nonuniform electric field (Jaeger et al. 2009a), may contribute to the observed overall enzyme inactivation during PEF processing.

Although enzymes do not contain membrane structures, which are the target for an inactivation based on electroporation, the possible impact of PEF side effects indicates that process modifications toward the inactivation of microorganisms and enzyme structures are also possible (Aguiló-Aguayo et al. 2010, Martín-Belloso & Elez-Martínez 2005). A further discussion of PEF impact on proteins and enzymes is conducted in the following section.

Impact on food constituents. Only small amounts of data are available regarding PEFs effects on other food constituents, especially proteins (Barsotti et al. 2001). Perez & Pilosof (2004) reported a partial modification of the native structure of β-lactoglobulin when subjecting the concentrate to an electric field of 12.5 kV cm^{-1}, whereas bovine immunoglobulin G subjected to PEFs at 41 kV cm^{-1} for 54 μs did not show any detectable changes in the secondary structure or the thermal stability (Li et al. 2005). No effects of PEF treatment on the physicochemical properties of lactoferrin were found by Sui et al. (2010) for treatment intensities up to 35 kV cm^{-1} and a total specific energy input of 41 kJ kg^{-1} (treatment time 19 μs) with temperatures below 65°C.

Fernandez-Diaz et al. (2000) studied the effects of PEFs on ovalbumin solutions (2%; pH 7; 5 mS cm^{-1}) and dialyzed egg white (pH 9.2; 4–5 mS cm^{-1}), applying an electric field strength in the range of 27–33 kV cm^{-1}. Partial protein unfolding or enhanced sulfhydryl (SH) group ionization of ovalbumin was observed after PEF treatment and was found to increase with increasing the total specific energy input.

Recent investigations by Marco-Moles et al. (2009) focused on the PEF effect on protein and lipids in liquid whole egg and the microstructure of these components studied by low temperature scanning electron microscopy (Cryo-SEM). A partial denaturation and insolubilization of the protein was observed during conventional pasteurization and resulted in a thickening of the lipoprotein matrix as observed by Cryo-SEM. Microstructure of PEF-treated samples showed some discontinuities in the lipoprotein matrix.

Owing to the application of PEFs, changes in the conformational state of proteins might cause changes in enzyme structure and activity (Bendicho et al. 2003, Tsong 1990). In general, the mechanisms involved in the inactivation of enzymes by PEFs are not fully understood (Ohshima et al. 2006). Possible mechanisms are proposed (Castro et al. 2001, Perez & Pilosof 2004). If the duration of the pulse is long enough, the effects of PEFs on proteins could entail polarization of

the protein molecule, dissociation of noncovalently linked protein subunits involved in quaternary structures, changes in the protein conformation so that hydrophobic amino acid or sulfhydryl groups are exposed, attraction of polarized structures by electrostatic forces, and hydrophobic interactions or noncovalent bonds forming aggregates.

The effect of a PEF treatment of suspensions of potato and corn starch was under investigation by Han et al. (2009a) and Han et al. (2009b). Physicochemical properties of potato and corn starch granules were found to be significantly affected because of the electric field exposure, with electric field strength intensities in the range of 30–50 kV cm^{-1} (no other PEF treatment parameters were reported). Treatment effects included an intragranular molecular rearrangement and a partial loss of the crystalline structure.

The same research group reported the effect of a PEF processing (electric field strength 20–50 kV cm^{-1}, no other PEF parameters reported) of peanut oil on fatty acid composition, acid value, and peroxide value after treatment and storage (Zeng et al. 2010). Although the applicability and feasibility of a PEF treatment of an electrically insulating media such as oil may be questionable, the authors found a significant increase of the peroxide value of the treated oil after 100 days of storage at 40°C, whereas the acid value decreased. No differences were found between the control and the PEF-treated sample immediately after treatment. Further research is required concerning the PEF effect on lipids and carbohydrates in order to confirm first results obtained in the above-mentioned studies. However, the electric field application and its effect on physicochemical material properties may have a potential for a targeted modification of functional food characteristics.

Current Applications

PEF technology is on the verge of industrial application with various pilot scale units available worldwide (Lelieveld et al. 2007, Raso & Heinz 2006). Controlled reversible permeabilization offers the potential for a sublethal stress induction on biological cells triggering a metabolic response (Bonnafous et al. 1999, Gomez Galindo et al. 2008) and an increased production of secondary metabolites such as phenols or phytosterols, leading to increased antimicrobial and antioxidative effects (Dörnenburg & Knorr 1995). It also offers the potential for the infusion of precursors or other desired constituents into cells as well as the recovery of metabolites from cells while maintaining their viability and productivity (Tryfona & Bustard 2008).

The irreversible rupture of plant membranes offers various applications to replace or support conventional thermal, as well as enzymatic, processes for cell disintegration (Vorobiev & Lebovka 2008). Irreversible permeabilization allows significant improvement of mass transfer especially for drying, expression, concentration, and extraction, resulting in higher product yields, shorter processing times, and consequently reduced energy consumption (Toepfl et al. 2006).

Microbial inactivation of vegetative cells via PEFs offers pasteurization with low energy input, selective inactivation of microorganisms depending on cell size or shape (Toepfl et al. 2007), and retention of bioactive heat-sensitive food compounds while inactivating pathogenic microorganisms and increasing product shelf live and safety (for a comprehensive overview on the various fields of application of PEFs in the food industry, follow the **Supplementary Material link** from the Annual Reviews home page at **http://www.annualreviews.org**) (Guerrero-Beltrán et al. 2010, Jaeger et al. 2009b, Sui et al. 2010).

Supplemental Material

Process-Structure-Function Interactions

The effect of PEFs on food constituents such as proteins and carbohydrates and the resulting changes of functional properties were discussed above. The following section focuses on the PEF effect on structured foods.

PEFs affect the cell membranes and thus can be expected to influence the texture of products in which the structure is largely dependent on the integrity of cells. The possible use of PEFs in food processing has now been investigated for a number of years. These studies have mainly focused on the effect of electric pulses on inactivation of different types of microorganisms in different states and also electric permeabilization of plant cells to increase the yield of different material, such as juices. Other than the changes occurring on a cellular level, there has been very little research on the effect of PEF treatment on microstructures of the raw material or the food, which continues developing during further processing of the PEF-treated raw material.

Fundamental research on the modification of textural properties of plant and animal raw materials represents the basis for further possible applications. Lebovka et al. (2004) studied the impact of PEFs on apple, carrot, and potato tissue. Stress deformation and relaxation tests were performed to analyze the changes in tissue texture. PEF treatment, in combination with a mild heat pretreatment, leads to complete elimination of the textural strength of tissue. It was shown that by proper selection of PEF treatment conditions, it was possible to obtain a controlled degree of tissue softening.

Suitable methods such as impedance measurement (Angersbach et al. 1999) and acoustic impulse response (Grimi et al. 2010) were developed and are in use as efficient tools to quantify structural modifications. Improved water binding during cooking of meat was found to occur after PEF pretreatment because of enhanced microdiffusion of brine and water-binding agents. Hydrocolloids will influence protein swelling and water-binding activity, and their microdiffusion into the meat tissue can be enhanced by PEF pretreatment (Toepfl 2006).

The impact of a PEF treatment on microstructure and texture of salmon was investigated by Gudmundsson & Hafsteinsson (2001). Fish muscle was found to be more susceptible for gaping due to PEF treatment in comparison with chicken meat, most likely due to the lower content of connective tissue (0.6% in comparison to 2% for chicken meat). No direct-effect PEF treatment on protein denaturation was found by electrophoresis, so the changes in microstructure were related to permeabilization of the cell membrane and leakage of cell fluids into extracellular space. The understanding of the impact and the potential of the PEF technology on food systems at the cellular level will allow the design of tailor-made foods, establishing process-structure-function relationships.

Process Developments

Application of PEF technology as a short-time (milliseconds) continuous operation will improve sustainability of food processing and/or reduce energy requirements while maintaining or improving food quality and safety. Even if PEFs require an additional input of electrical energy, it has beneficial effects on total energy consumption of mass transfer processes such as extraction, pressing, or drying. Processing times are reduced, utilization of production capacities is improved, and water, as well as raw material, consumption is decreased (Toepfl et al. 2006).

Application of PEFs in combination with mild heat seems to be a promising technique for a gentle, multihurdle preservation process. PEF treatment in combination with mild heat provides a potential to reduce the thermal load and retain native enzyme activity. A reduction of thermal load could be used to increase operating time of heat exchangers by reducing the amount of biofouling.

Another key aspect for the successful application of PEF pasteurization is its selective inactivation capability, given that the pore formation process and the required *trans*-membrane potential depend on the size of the treated cell. Larger cells such as yeasts require a smaller intensity of the external electric field, and thus they are more sensitive to electropermeabilization. Hence,

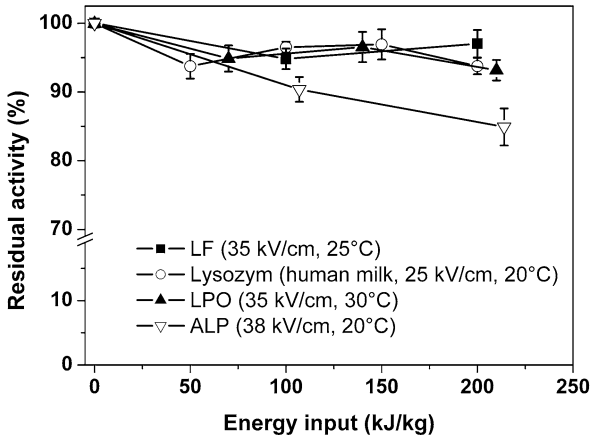

Figure 3

Impact of pulsed electric field treatment on enzymes and bioactive, antimicrobial components in milk (LF, lactoferrin; LPO, lactoperoxidase; ALP, alkaline phosphatase) according to Jaeger et al. (2009b).

inactivation of yeast in a product containing desirable probiotic bacteria can be realized without a loss of bacterial cell vitality.

Selectivity of PEF inactivation based on cell size and other factors that affect the electroporation mechanism are different from the susceptibility of microorganisms to thermal treatment. Thermotolerant microorganisms can be affected by PEFs, and inactivation of the more PEF-resistant species can be conducted by thermal effects; consequently, a combination of thermal and PEF treatment can improve the inactivation effectiveness in addition to the synergistic temperature effect on PEF inactivation below the thermal inactivation level.

As the PEF effect on proteins and enzymes, as well as on other food constituents, remains small, possible applications could include the pasteurization of bioactive antimicrobial milk fractions such as lactoperoxidase, lactoferrin, or immunoglobulins, as well as heat-sensitive vitamin solutions, which are destroyed during thermal pasteurization. **Figure 3** shows the retention of milk bioactives after pasteurization with PEFs. On the other hand, process improvements like treatment chamber design have offered the ability to selectively retain enzyme activity or to inactivate them via temperature effects caused by the electrodes and flow conditions in the treatment chamber.

Research Needs and Challenges

The application of PEFs to induce stress reactions in biological systems and the basic understanding of underlying mechanisms on a cellular and metabolic level will be the main focus of the research undertaken in the field of reversible permeabilization of plant cells. First attempts that have been described above are already showing the potential for the modification and the improvement of the production of valuable secondary metabolites.

The application of PEFs for the irreversible cell disintegration of plant and animal raw material was limited by the availability of large-scale pulse modulators, but a forward-looking technical development was already undertaken in the last years to overcome production scale limitations. In order to implement the cell disintegration processing step into existing processes, an integrative approach will be required that considers pre- and post-PEF processing unit operations, such as mechanical disintegration of solid-liquid separation in the case of extraction of juice recovery to

successfully transfer the cell disintegration provided by PEFs into improved process results, such as higher juice yields.

For PEF-assisted pasteurization, the design and optimization of the PEF treatment chamber is the most challenging point with regard to different product properties, such as viscosity and electrical conductivity, as well as with regard to uniform treatment conditions in terms of electric field and temperature distribution. Many authors (Fiala et al. 2001, Gerlach et al. 2008, Jaeger et al. 2009a, Lindgren et al. 2002, van den Bosch et al. 2002) have described the temperature distribution in a PEF treatment chamber and reported the occurrence of high local temperatures due to the inhomogeneous distribution of the electrical field, limited flow velocity, and recirculation of the liquid. Numerical simulations using computational fluid dynamics gain interest for this purpose because experimental measurement of the related parameters is not possible in most cases, owing to small dimensions of the treatment chamber as well as the interference of the measuring device with the product flow and electric field. Treatment homogeneity and the avoidance of overprocessing of the product, including the occurrence of local high temperatures, are key aspects to guarantee predictable cell disintegration and microbial inactivation while maintaining heat-sensitive food constituents.

In PEF systems working at higher electric field intensities, electrochemical reactions can occur at the electrode surface (Morren et al. 2003). Related unwanted effects such as a partial electrolysis of the solution, the corrosion of the electrode material, and an introduction of small particles of electrode material in the liquid can be limited or avoided by suitable selection of electrode materials and by adaptation of the electrical pulse shape and duration (Roodenburg et al. 2005, Saulis et al. 2005). Its consideration is a crucial prerequisite during the study of inactivation kinetics to exclude other simultaneously occurring side effects.

Protective effects existing in real food systems may limit the process effectiveness of PEFs compared with inactivation studies conducted in model solutions, and the occurrence of sublethally injured cells has to be taken into account with regard to food safety aspects (Jaeger et al. 2009c). A comprehensive statement concerning food safety aspects of PEF treatment can be found in Knorr et al. (2008). Furthermore, in addition to the complexity of treatment media, the consideration of microbial growth state, adaptation to the treatment media, and the existence of inhomogeneous microbial populations with less sensitive subpopulations seem to be the most challenging aspects when transferring inactivation results to real products and industrial implementation.

ULTRASOUND

Process Description

During the past years US-assisted processing has attracted growing interest in the field of food science and technology. Positive effects have been reported for various applications such as the assistance of thermal treatments, the improvement of mass transfer processes, and food preservation, as well as for texture manipulation and analysis.

When sound energy is transferred into a medium, longitudinal waves are formed causing continuous compression and rarefaction of elastic materials (Povey & Mason 1998). The resulting physical, chemical, and biochemical effects strongly depend on the energy applied (Knorr et al. 2004). The application of US in food technology can be divided into two different approaches: low energy diagnostic US in the MHz range used for nondestructive testing and high power US in the kHz range applied for material alteration. At low treatment intensities and amplitudes, the pressure waves induce acoustic streaming, whereas high intensities and amplitudes result in local pressures below the vapor pressure of the liquid. This leads to a constant growth of gas bubbles in

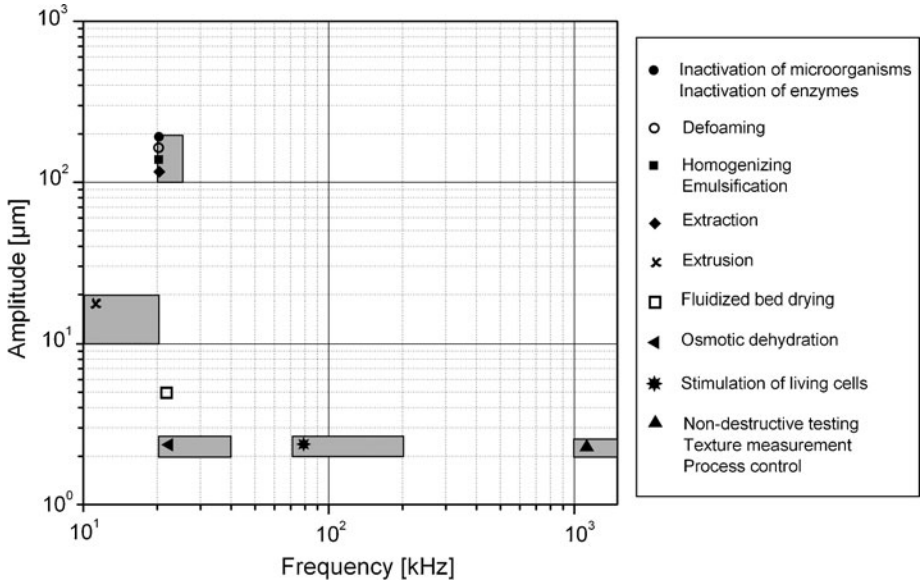

Figure 4

Comparison of treatment parameters (amplitude and frequency) of selected sonication processes in food science and technology (for references for the parameters used in the above-mentioned applications, see Supplemental Materials).

Supplemental Material

the medium, resulting in their violent collapse (cavitation) (Patist & Bates 2008). Cavitation and associated phenomena are responsible for the majority of the effects of high power US in food processing.

History

The first generation of ultrasonic waves goes back to the 1830s. Research for the potential of US in food processing started in the 1920s. Since the 1950s, US has been used on industrial equipment for cleaning and homogenization (Mawson & Knoerzer 2007). The initial ultrasonic tester at the industrial level in 1950 was followed by the commercial adoption of ultrasonic cutting in food processing. In the 1990s, the sealing of packaging materials and welding of plastic emerged. The development of piezoelectric transducers led to an increase in US research within the past decades and with the technical feasibility of higher treatment intensities, US was applied in disintegration of food materials, homogenization, and extraction (Mawson & Knoerzer 2007).

At present, US research is highly versatile, and US-assisted processes have been proposed for nearly every aspect of food production (**Figure 4**).

Research State of the Art

US research focuses mainly on three different aspects of food processing: the stimulation of fermentations and enzyme reactions at low treatment intensities; the application of high power US for preservation using synergistic effects of US, heat, and pressure; and the alteration of food consitutuents or structure leading to new product characteristics or improved mass and heat transfer during extraction or drying processes, where US can furthermore affect boundary layers.

Impact on biological cells. Biological cells are important raw materials and processing units for the food industry. Low-intensity US was reported to improve fermentation processes because of increased mass transfer through the cell wall and membrane and its influence on boundary layers (Sinisterra 1992). Increased fermentation rates could be observed because of the sonication-assisted removal of CO_2, which otherwise can inhibit fermentations (Matsuura et al. 1994).

A bactericidal effect of US was first reported in the 1920s (Harvey & Loomis 1929). The upcoming developments in ultrasonic equipment lead to higher acoustic densities and an increase in inactivation rates. US-induced cell damage is primarily explained by cavitation phenomena such as shear disruption (microstreaming), localized heating, and free radical formation (Hughes & Nyborg 1962). Cell wall and membrane of biological cells can be damaged by surface rubbing, leading to fracture and leakage (Kinsloe et al. 1954). Equally, separation of the cytoplasmic membrane due to ultrasonic treatments has been reported (Alliger 1975), and free radicals are assumed to cause DNA damage (Hughes & Nyborg 1962).

Several studies showed an additive or even synergistic effect when US was combined with other lethal effects such as elevated temperatures [thermosonication (TS)], pressure [manosonication (MS)], or both [manothermosonication (MTS)] (Knorr et al. 2004), which has been explained by the weakening of the cell wall, making it more susceptible to the effects of cavitation (Patist & Bates 2008). Ultrasonic pasteurization carried out at 50°C could present a high potential in preserving physicochemical properties, color, and flavor compared with conventional thermal pasteurization techniques (Patist & Bates 2008).

A comparative study on the inactivation of *Escherichia coli* by all four treatments (sonication, TS, MS, and MTS) showed significantly shortened treatment times to achieve a five-log reduction with combined treatments. In the case of sonicated samples, extensive cell damage and breakage could be shown by SEM analysis (Lee et al. 2009).

Although the overall results seem promising, the specific energy requirement of sonication-assisted processes was reported to surmount that of a comparable thermal process (Zenker et al. 2003) and has to be considered as the most important limitation for the application of US in food preservation processes today.

Impact on enzymes. Enzymes are valuable processing agents for the food industry. Positive effects on enzyme activity can be achieved by the application of low energy US. Substrates can be made available in large amounts, and the transport of substrate toward immobilized enzymes is increased by microstreaming (Mason et al. 1996).

The research in inactivation of enzymes for food preservation revealed increased effects for TS, MS, or MTS compared with sonication alone. Nevertheless, the sensitivity can be very different from one enzyme to another. Positive results have been reported for tomato pectic enzymes as well as for α-chymotrypsin and porcine lipase, whereas phospholipase A_2 was nearly insensitive to MTS, and the sensitivity of trypsin was found to be temperature dependent (Vercet et al. 2001, Vercet et al. 2002).

Impact on food constituents. The effects of sonication on food are various and range from compression and rarefaction to temperature peaks up to 5,000°C and local pressures up to 100 MPa (Suslick 2003). This leads to wide-ranging impact on food constituents with positive as well as critical results.

Sonication can modify proteins and product structure, improve flow behavior and increase heat transfer as it was exemplified with continuous formation of an edible coating of raw sausages (Knorr et al. 2004). A heated tubular sonotrode was used to form a skin of denatured proteins around the raw emulsion inside.

Critical results were obtained in the case of rabbiteye blueberries with reduction of product quality after an ultrasonic treatment (Stojanovic & Silva 2006). The application of US for the preservation of fruit juices was considered promising, as sonication resulted in only minor color changes and retention of 94% of the anthocyanins in blackberry juice (Tiwari et al. 2009). However, the application of US for food preservation could be limited because of a destruction of the physicochemical properties of the food, if parameters have to be designed with the objective to inactivate extremely heat-resistant microbial forms (Knorr et al. 2002).

Current Applications

Although US research in the field of food technology is versatile and shows a lot of promising effects, so far only a few treatments have reached industrial level. This discrepancy can be explained by the fact that ultrasonic equipment has to be custom designed for every single application and by a lack of appreciation of the food industry (Mawson & Knoerzer 2007), which has to be overcome by closer collaborations between research and industry in future.

One of the most common pieces of sonication equipment at the industrial level are ultrasonic filtration systems as add-ons to existing vibratory screens, while the combination of US and membrane filtration is still in the early phase of development (Patist & Bates 2008). Airborne US is used for defoaming of carbonated beverages and fermentation systems (Gallego-Juárez 1998).

Ultrasonic pulverization techniques are applied for the destruction of residual cell wall material and vegetable purees achieving significant modifications of textural and rheological properties, as by releasing pectin from cell walls, which contributes to the formation of continuous matrices (Bates et al. 2006, Mawson & Knoerzer 2007). In the case of the preparation of biomaterials for further processing by fermentation or enzyme digestion, US-assisted pulverization of cell matrices is used to facilitate the release of substrates or nutrients (Matsuura et al. 1994, Mawson & Knoerzer 2007, Wu et al. 2000).

Process-Structure-Function Interactions

Process-structure-function interactions are the basis for several processes in food technology. Consequently, the potential of US in assisting and influencing these processes has been widely studied.

Proteins are used for texturizing and thickening of sauces, dressings, dairy products, and gels. A study about the physical properties of US-treated soy proteins showed significant texture changes in model food systems (Jambrak et al. 2009). Soy protein isolate creamed during the treatment with an ultrasonic probe (20 kHz, 15 min) and soy protein concentrate showed changes in conductivity, increased solubility, and increased specific surface area, which is of importance for food texture and functionality. Contrarily, sonication did not improve emulsifying or foaming properties.

Extrusion processes can be improved by the ultrasonic excitation of a metal tube or extrusion dye and ultrasonic vibrations can lead to a reduction in drag resistance and improved flow characteristics (Knorr et al. 2004, Mousavi et al. 2007).

In meat processing, sonication has been successfully applied to improve binding strength, water holding capacity, product color, and yield of processed meat (Vimini et al. 1983), and was reported to be beneficial in the meat tenderization process (Roberts 1992).

Ultrasonic pretreatments of vegetables and fruits led to cavitation damage, an increased distortion of cells and the creation of microscopic channels (Fernandes et al. 2009, Jambrak et al. 2007). These changes in tissue structure were shown to have potential for increasing mass transfer processes.

Process Developments

Within the past few years, US research has covered nearly every aspect of food technology. Cavitation and associated phenomena such as microstreaming, pressure fluctuation, and local heating effects led to numerous improvements at laboratory scale.

Thermal processes can be improved by enhanced heat transfer, microstreaming at boundary layers, reduced fouling due to cavitation phenomena, and a faster formation of gas bubbles in evaporation processes. Airborne US was reported to have a positive impact on hot-air drying of carrots (García-Pérez et al. 2006). An influence on mass transfer rates has likewise been observed during the osmotic dehydration of apple cubes (Simal et al. 1998).

During extraction processes, cavitation can improve the penetration with the solvent and disrupt cell walls when high intensities are applied (Li et al. 2004). The mass transfer resistances during the extraction of vanillin from vanilla pods and of phospholipids from palm-pressed fiber could be reduced by the application of US (Chua et al. 2009, Jadhav et al. 2009). Positive results for sonication-assisted supercritical extraction of ginger indicate that cavitation, which will not occur in supercritical fluids due to the absence of liquid/gas boundaries, is not the only US effect having an important influence on the mass transfer rate (Balachandran et al. 2006). Acoustic streaming and the presence of gas pockets in the solid causing cavitational collapse have been discussed (Patist & Bates 2008).

Cavitation, shear forces, and an influence on boundary layers provide the opportunity to improve emulsification and homogenizing (Jafari et al. 2007, Villamiel & de Jong 2000). In the case of milk homogenization, subsequent yogurt fermentation led to a product with decreased syneresis, improved water-holding capacity, and increased viscosity (Wu et al. 2000).

A change in the sonication parameters can lead to the opposite effect and emulsions can be split into their components (Pangu & Feke 2004). Acoustic radiation force was reported to hold particles in position in a stationary field leading to coalescence, which has been presented as a novel principle for particle separation (Masudo & Okada 2006).

Sonication during freezing processes can promote ice nucleation and enhance heat and mass transfer processes (Zheng & Sun 2006). Crystal size distribution can be controlled, leading to reduced cell destruction and cavitation effects can minimize fouling in surface freezers (Acton & Morris 1992).

At lower treatment intensities US can be applied for the detection of foreign bodies in packaged and nonpackaged food (Knorr et al. 2004, Leemans & Destain 2009) and for nondestructive testing. Conventional methods such as microscopy, textural analysis, and rheology require laboratory practice and are unsuitable for real-time applications (Ting et al. 2009). Low-intensity US has been successfully tested for the monitoring of the gelation process in milk and tofu (Dwyer et al. 2005, Ting et al. 2009) and the measurement of the mechanical properties of cheese products (Benedito et al. 2000).

Research Needs and Challenges

The presented overview of ultrasonic research in the food industry underlines the versatility of sonication processes. One of the most important challenges for the industrial application of US is the definition of optimized parameters for every single process and product, which demands many research capacities and a close collaboration between researchers, equipment suppliers, and the food industry. In addition to the promising effects of sonication in food technology, detailed knowledge about quality aspects together with a thorough analysis of energy requirements are the basis for the successful scale-up from laboratory tests to industrial scale. Furthermore, technology transfer from other fields such as medical applications may prove beneficial.

PLASMA TREATMENT

Process Description

Plasmas can be described as quasineutral particle systems in the form of gaseous or fluid-like mixtures of free electrons and ions, frequently containing neutral particles (atoms, molecules), with a large mean kinetic energy of the electrons and/or all of the plasma components and a substantial influence on the charge carriers and their electromagnetic interaction on the system properties (Rutscher 2008).

In the field of plasma research, a complex nomenclature considering the temperature of the electrons and the bulk gas and/or the surrounding pressure can be found (Goldston & Rutherford 1997, Roth 1995). According to Fridman et al. (2005), all varieties of plasma-chemical systems are traditionally divided into two major categories: thermal and nonthermal plasmas, with specific advantages and disadvantages. Thermal plasmas [usually arcs or radiofrequency (RF) inductively coupled plasma discharges] are associated with Joule heating and thermal ionization, and enable the delivery of high power (to over 50 MW per unit) at high operating pressures. Besides other limitations, very high gas temperatures limit their applicability to food systems.

In nonthermal plasmas, the electron temperature is much higher than the bulk gas temperature. Whereas the electron temperature can reach several ten thousands K, the gas temperature remains at temperature levels below 40°C (Mastwijk & Nierop Groot 2010). Nonthermal plasmas may be produced by a variety of electrical discharges at different pressure levels. Working pressure below atmospheric conditions are mainly suitable for dried food materials or packaging materials, since a vacuum will support liquid to gaseous phase changes in high-moisture food products. The most suitable system for food processing is an atmospheric-pressure plasma device in which no extreme conditions are required and low temperatures can be realized. Atmospheric-pressure plasma is commonly generated by corona discharge, dielectric barrier discharge (DBD), or plasma jet (Keener 2008). For the treatment of nonuniformly shaped products, the application of plasma jets offers advantages due to various options regarding design and construction (Foest et al. 2005).

History

In the year 1808, Sir Humphry Davy developed the steady-state DC arc discharge, and in the 1830's, Michael Faraday and others developed the high-voltage DC electrical discharge tube. Rapid progress was made in electrical discharge physics during the nineteenth century, nearly all of it in a few laboratories in England and Germany. In 1857, Siemens designed an ozone generator that has evolved into the cylindrical dielectric type that covers most of the commercially available ozone generators in use. In 1840, Schönbein named the substance, which gave off this odor, ozone from the Greek word ozein, to smell (Rubin 2001), after van Marum already noticed in 1785 that air near his electrostatic machine acquired a characteristic odor when electric sparks were passed. In 1801, Cruickshank confirmed that a certain odor occurs at the anode during the electrolysis of water. In 1898, Sir William Crookes introduced the term ionization to describe the breakup of the neutral atom into an electron and a positive ion. Irving Langmuir introduced the term plasma in 1928, by which he meant an approximately electrically neutral collection of ions and electrons that may or may not contain a background neutral gas and that is capable of responding to electric and magnetic fields. More detailed information on the history of plasma research is given by Roth (Roth 1995).

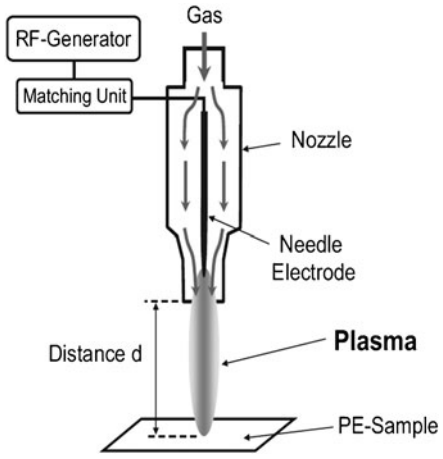

Figure 5

Experimental setup of a plasma jet according to Brandenburg et al. (2007).

Research State of the Art

Recent research activities in food-related application of plasma focus mainly on inactivation of microbes, but little is known about the effect of plasma on food matrices. Because emitted reactive species react with bacteria, they may also affect food components such as water, lipids, proteins, and carbohydrates (Deng et al. 2007b, Keener 2008). Owing to recent technical developments, plasma sources can operate at ambient conditions, keeping the processing temperature low. For example, RF-driven plasma jets can be used for studies on treatment of food-related materials. Such a plasma source consists of a needle electrode in the center of a ceramic nozzle and a grounded outer electrode. The RF voltage is coupled via a matching network to the needle electrode. The gas flowing between the electrodes is ionized and then ejected from the source (Brandenburg et al. 2007). Design principles of a plasma jet system are shown in **Figure 5**.

Impact on microorganisms. Inactivation of food-related microorganisms by plasma treatment is commonly conducted using model systems. An overview of microbial inactivation in model systems using nonthermal plasma published during the last three years is given by Wan et al. (2009).

The antimicrobial activity of nonthermal plasma against Gram-negative and Gram-positive bacteria, yeast and fungi, biofilm formers, and endospores was shown in various studies (Brandenburg et al. 2007, Kelly-Wintenberg et al. 1999, Laroussi 2005, Montie et al. 2000, Vleugels et al. 2005). Although several reviews focus on the inactivation mechanisms of plasma (Boudam et al. 2006, Gaunt et al. 2006, Moisan et al. 2001, Moreau et al. 2008), it is not yet fully understood. Moisan et al. (2001) stated that three basic mechanisms are involved in plasma inactivation: (*a*) ultraviolet (UV) irradiation of genetic material, (*b*) intrinsic photodesorption, and (*c*) etching. Most researchers claim that UV plays a minor role in the inactivation of microorganisms at atmospheric pressure, and the inactivation process is controlled by chemically reactive species. However, it was shown that in some cases UV photons can play a role in the inactivation process of microorganisms at atmospheric pressure (Boudam et al. 2006). Moreau et al. (2008) compared plasma inactivation effects to the effects of micropulses. Similar to the effects of micropulses, the cell membranes of microorganisms are perforated after plasma treatment. Besides

the perforation of the cell membrane, the inactivation effect of plasma is induced by the bombardment of the cell membrane by radicals (OH or NO). These radicals are absorbed onto the bacteria surface, and volatile components are formed and eliminated from the cells (etching). Two mechanisms of plasma inactivation described by Gaunt et al. (2006) are the electrostatic disruption of cell membranes and lethal oxidation of cellular components.

Impact on enzymes. Information regarding the impact of plasma on enzymes is only given in a very few papers. Dudak et al. (2007) found a decrease of enzyme activity after treatment with plasma generated in RF-driven glow discharge with the highest decrease in activity within the first 10 minutes of treatment. They showed plasma-chemical oxidation as well as fragmentation of the proteins. Additionally, changes in the secondary protein structure due to the plasma treatment were detected. A fragmentation of proteins was also found by Deng et al. (2007b) after DBD treatment. In this process, atomic oxygen was shown to play a dominant role in destruction and degradation reactions. Oxygen plasma generated by RF discharge led to a reduction of C-H and N-H bonds in casein protein and to a modification of the secondary protein structure (Hayashi et al. 2009).

Impact on food constituents. Although much work has already been performed on the effects of nonthermal plasma on microorganisms, information of plasma interaction with food components is rare. This is mainly due to the fact that the application of plasma was long limited to heat- and vacuum-resistant materials. As mentioned before, today plasma can operate at ambient pressure and low processing temperature.

Applying CP to improve the shelf life of fresh or freshly prepared food is new, and little is currently known about the effect of plasma treatment on bioactive plant substances. The degradation of mycotoxins in microwave-induced atmospheric pressure argon plasma has recently been shown by Park et al. (2007). Plasma treatment resulted in a significant time-dependent decrease in aflatoxin B1, deoxynivalenol, and nivalenol, coming along with a dose-dependent reduced cytotoxity. A time- and dose-dependent degradation has also been observed for flavonoids, known for their high antioxidant activity, which protects cells against the damaging effects of reactive oxygen species. The degradation rate strongly depended on the polyphenolics substitution pattern. Although glycosidic flavonoids showed a rather inert behavior throughout plasma treatment, aglycosid derivates were quickly degraded (Grzegorzewski et al. 2010b).

The potential of nonthermal plasma surface treatment to decontaminate food surfaces is investigated in various studies, often neglecting the effects on quality parameters. However, organoleptic analysis of plasma-treated nut samples likewise showed no relation between treatment and perceptual sensory character (Basaran et al. 2008). On a molecular level, SEM analysis of different cabbage and lettuce species yet revealed that under certain conditions, plasma treatment may lead to changes of plant surface hydrophobic wax layers (Grzegorzewski et al. 2010a). On the other hand, Ragni et al. (2010) observed no negative effects of plasma treatment on egg quality.

Current Applications

Plasma technologies in food processing are not yet established, but investigations using complex food raw materials have been performed. Some studies focus on the plasma-related decontamination of bacteria at the surface of several fruit and vegetable samples like apples, cantaloupe, and lettuce without evaluating the obtained product quality (Niemira & Sites 2008). A five-log reduction of *E. coli* inoculated on almonds was found after 30 s nonthermal plasma treatment at 30 kV and 2000 Hz (Deng et al. 2007a). Basaran et al. (2008) treated various nut samples inoculated with

Aspergillus parasiticus and tested the antifungal efficiency of low pressure CP (LPCP). They used air gases and sulfur hexafluoride (SF_6) and found that SF_6 plasma application (five-log reduction) was more effective than air gas plasma treatment (one-log reduction). In contrast, the efficiency of air gas plasma against aflatoxin was greater than the efficiency of SF_6 plasma. Equally, seeds inoculated with *Aspergillus* spp. and *Penicillium* spp. were treated with LPCP using air gases and SF_6 (Selcuk et al. 2008). The fungal attachment was reduced below one percent by the treatment while the germination quality of the seeds was preserved (for additional examples on the inactivation of microorganisms on food surfaces, see **Supplemental Table 1**).

Supplemental Material

Process Developments

The industrial use of applied plasma technology in the nonfood sector is diverse (e.g., plasma switches for power networks, cost-effective light sources, high-definition large area flat panel displays, plasma-improved printability of foils, etc.). Recent research increasingly concentrates on plasma treatment of living vegetative or mammalian cells and tissues (Shashurin et al. 2008, Stoffels et al. 2008). Much work has already been done in the field of plasma medicine and related topics. Using CP jets, eradication of yeast grown on agar (Kolb et al. 2008), blood coagulation, and tissue sterilization (Fridman et al. 2006), as well as ablation of cultured liver cancer cells (Cho et al. 2008), have been shown.

In the past decade, concerted research efforts were expended to understand and to apply atmospheric pressure plasmas as a sterilization method. Modular and selective plasma sources were developed. These combine the technological advantages of atmospheric pressure plasmas (avoidance of vacuum devices and batch processing) with the flexibility and handling properties of modular devices (Ehlbeck et al. 2008). Along with the applications in food processing, progress in germ-reduction technologies is important for medical and biomedical application, biotechnology, the pharmaceutical industry, and the packaging industry. The treatment of components with complex geometry requires the development of plasma sources for surface treatment and of cavity-penetrating plasmas. Because energy transfer of a low-temperature plasma to a surface is small, the treatment of heat-sensitive materials is feasible. In environmental applications, plasma technologies can be applied to flue gas cleaning and can substitute wet-chemical processes that generate waste water by environmentally desirable dry processes (Weltmann et al. 2008).

Another possible application of nonthermal plasma is the treatment of packaged products. Schwabedissen et al. (2007) described the different application fields of the *PlasmaLabel*™, e.g., fresh food conservation or packaged goods. However, further investigations are required to characterize the plasma applied and to better understand the interactions of reactive species with organic surfaces as well as vital biosystems (Mastwijk & Nierop Groot 2010). This will allow for control of the effects of plasma and the ability to design highly specified and efficient plasma processes.

Research Needs and Challenges

Approaches to the study of the effects of plasmas in industrial plasma engineering often regard plasma as a black box with inputs and outputs. Also, studies focusing on plasma application to foods as the desired output of a plasma-related process are mainly achieved by adjusting inputs until the desired result is obtained. Within such approaches, no serious attempt is made to understand the plasma-physical, plasma-chemical, or plasma-biological processes occurring in this black box. Future research needs to involve more interdisciplinary studies to allow a better understanding of the complex interactions during plasma processing, thus resulting in the design of beneficial and controlled plasma applications for food processing, which may encompass microbial inactivation

as well as the modification of functional food properties. Further, plasma processing has to be considered as a surface treatment, and the impact of potential toxicological effects resulting from chemical reactions based on plasma-air-food surface interactions have to be viewed in relation to the surface to volume ratio of the particular product.

CONCLUSION

HHP treatment has a high potential to produce microbiologically safe, high quality, tailor-made foods under gentle processes conditions. Process improvement can only be achieved by understanding and applying the different temperature, time, or pressure dependencies of wanted and unwanted reactions. Detailed studies regarding inactivation kinetics and mechanisms of pathogens should be performed in the respective food matrix and have to result in constant process parameters, and if not possible, in optimally controlled process parameters. Controlled and reproducible studies on pressure effect of HP on nutrients biopolymers, toxins, and allergens are also needed.

The interactions between products and PEF process and possible undesired changes during PEF treatment still remain uncertain and require further investigation. For example, high-value products such as enzyme or vitamin solutions or protein fractions isolated from milk, which are all heat sensitive, are potential products for a nonthermal pasteurization by PEFs. The combination of techniques that deliver effective preservation without the excessive use of any single conventional process parameter such as time or temperature allows the selective retention or inactivation of food constituents. The combination of PEFs with other stress factors like mild heat, antimicrobial compounds, pH, or organic acids, as well as the combination with other thermal or nonthermal decontamination techniques, will determine further development. The impact of PEFs on the structure of food matrices and on mass transfer within food matrices and the subsequent understanding of PEF-related process-structure-relationships will allow the development of unique tailor-made foods.

US-assisted processing offers advantages for a large variety of food production processes. The unique characteristics of sound waves provide opportunities to treat products with specifically adapted parameters. For instance, US with low maximum pressure amplitude will cause microstreaming in fluids, gently manipulating mass transfer, whereas high sound pressure amplitudes cause cavitation associated with high sound pressure and temperature peaks permitting changes in cell structure as well as homogenization of disperse systems. However, the large range of attainable effects is one of the most important obstacles for the transfer of laboratory scale results to industrial level. Equipment and parameters have to be directly adapted to every single product and objective. Furthermore, undesired changes in product structure and quality have to be known and minimized. A better understanding of basic mechanisms of ultrasonic treatments in dependency of product and process parameters is necessary to allow drawing general conclusions and to simplify the design of new processes and applications.

CP treatment at atmospheric pressures offers various opportunities in food processing, e.g., surface decontamination, modification of surface properties, and enhancement of mass transfer with respect for foods and food-related materials. Attempts to limit the heat transfer to sensitive materials such as food products resulted in the development of new atmospheric plasma jets and will allow efficient in-line integration in production lines; however, further research is required in light of the lack of data regarding the plasma-matrix-interactions and to ensure the development of safe and tailor-made processes for food application. Further investigations focusing on the spatial composition of plasma, physicochemical reaction kinetics, penetration depths, etc. should be supported by validated mathematical models and simulation approaches.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by the Federal Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn), Project AiF 15610 N; by Federal Institute of Food, Agriculture and Consumer Protection (BMELV) and supported by the Federal Institute for Agriculture and Food (BLE), project FriPlas (FKZ 28-1-63.003-07) and Verbundprojekt Hochdrucktechnologie; by the Federal Ministry of Education and Research, project BioMed; by the Commission of the European Communities, Framework 6, Priority 5 Food Quality and Safety, Integrated Project NovelQ FP6-CT-2006-015710 and Framework 7 (FP7/2007-2013) under grant agreement n°222233.

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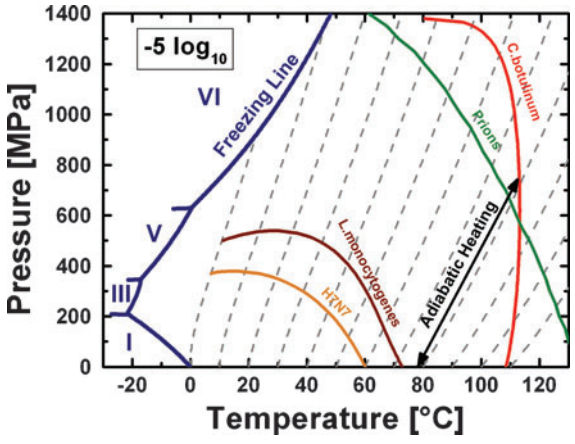

Figure 1

Isokinetic lines for a five-log10 reduction of H7N7, surrogate for bird flu virus H5N1 (chicken-meat suspension 15 s^{-1}) (Isbarn et al. 2007), with adiabatic lines due to compression (–) of water in comparison with significant sensory-rheological changes in raw turkey or chicken meat (Tintchev 2007).

Annual Review
of Food Science
and Technology

Volume 2, 2011

Contents

Errata

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