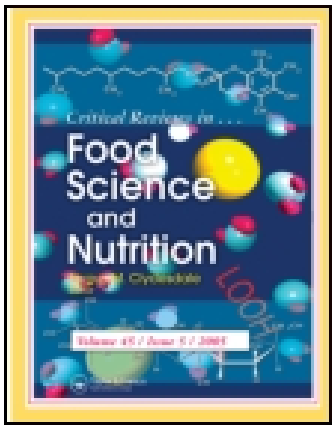

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Quality-Related Enzymes in Plant-Based Products: Effects of Novel Food Processing Technologies Part 2: Pulsed Electric Field Processing

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Quality-Related Enzymes in Plant-Based Products: Effects of Novel Food Processing Technologies Part 2: Pulsed Electric Field Processing

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Pulsed electric field (PEF) processing is an effective technique for the preservation of pumpable food products as it inactivates vegetative microbial cells at ambient to moderate temperature without significantly affecting the nutritional and sensorial quality of the product. However, conflicting views are expressed about the effect of PEF on enzymes. In this review, which is part 2 of a series of reviews dealing with the effectiveness of novel food preservation technologies for controlling enzymes, the scientific literature over the last decade on the effect of PEF on plant enzymes is critically reviewed to shed more light on the issue. The existing evidence indicates that PEF can result in substantial inactivation of most enzymes, although a much more intense process is required compared to microbial inactivation. Depending on the processing condition and the origin of the enzyme, up to 97% inactivation of pectin methylesterase, polyphenol oxidase, and peroxidase as well as no inactivation have been reported following PEF treatment. Both electrochemical effects and Ohmic heating appear to contribute to the observed inactivation, although the relative contribution depends on a number of factors including the origin of the enzyme, the design of the PEF treatment chamber, the processing condition, and the composition of the medium.

Keywords Pulsed electric field processing, enzyme inactivation, pectin methylesterase, polygalacturonase, polyphenol oxidase, peroxidase, β-glucosidase, lipoxygenase

INTRODUCTION

Pulsed electric field (PEF) processing is an effective method to inactivate vegetative cells of microorganisms in foods at low or moderate temperatures, which maintains the fresh-like characteristics of food better than conventional thermal processing. The application of PEF processing is often limited to liquid foods, such as fruit and vegetable juices, liquid egg, and dairy products with a relatively low electrical conductivity (<10 mS/cm) and do not contain or form gas bubbles (Butz and Tauscher, 2002). PEF has very limited effect on bacterial spores at sub-pasteurization temperature (<72°C) narrowing its potential applications to acid foods distributed in the cold chain (Butz and Tauscher, 2002; Mastwijk and Bartels, 2005; Clark, 2006). PEF processing involves the discharge of high-voltage electric pulses (15–80 kV/cm) of a few microseconds into the food product, which is placed between or flows through two electrodes (Butz and Tauscher, 2002). Application of PEF usually compromises the integrity of microbial, plant, and animal cell membranes by enlarging the pores on the cell membrane leading to the release of intracellular liquid and cell death. This is commonly referred to electroporation and is believed to be the main mechanism of PEF-induced microbial inactivation. In general, PEF treatment at room to moderate temperature has no or a very limited detrimental effects on the sensorial and nutritional quality of liquid foods (Min et al., 2003a, 2003b; Rivas et al., 2006; Sanchez-Moreno et al., 2009). The first reported investigation on the application of electric pulse for food preservation was performed by Fetteman (1928) in the 1920s.

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Further, interest in the application of this technique was renewed in the last decade of the 20th century.

It is generally assumed that PEF has limited effects on enzymes (Mastwijk and Bartels, 2005; Clark, 2006). This is perhaps due to the conflicting reports in the literature and the thermal effect associated with PEF, which is sometimes credited for the observed inactivation of enzymes (Van Loey et al., 2001). Nevertheless, several studies reported that PEF at very high intensity or in combination with mild heat causes substantial inactivation of most if not all food quality-related enzymes (Giner et al., 2000, 2001, 2002; Yeom et al., 2000, 2002; Espachs-Barroso et al., 2006; Zhong et al., 2007). Conformational changes in enzymes following PEF treatment have also been reported (Yeom et al., 1999; Zhong et al., 2005; Zhong et al., 2007; Zhao and Yang, 2010). However, compared to vegetative microorganisms much more intense PEF conditions are required to inactivate enzymes (Giner et al., 2000; Yeom et al., 2002; Byun et al., 2003). It has been estimated that four to five times the specific energy input is needed for enzyme inactivation by PEF compared to thermal processing. This includes the energy required to produce the electrical effect and the energy dissipated into the cooling medium (Giner et al., 2000; Espachs-Barroso et al., 2006). On the other hand, mild PEF treatment has been reported to enhance the activity of some enzymes such as fungal polygalacturonase (PG) (Giner et al., 2003), pepsin, and lysozyme (Ho et al., 1997), which may have beneficial applications in the food and pharmaceutical industries.

The mechanism of PEF inactivation of enzymes is not well understood (Zhong et al., 2005). The available scientific literature suggests that both electrochemical effects and thermal effects associated with PEF individually or in synergy may cause changes in the structure and conformation of enzymes (Tsong, 1990; English et al., 2009; Zhao and Yang, 2010), which can lead to inactivation. The catalytic activity of enzymes depends on their active sites and three-dimensional configuration and any changes in the structural conformation of an enzyme or its functional groups can alter its functionality resulting in increase or loss of biological activity (Hendrickx et al., 1998). The application of electric fields may cause association or dissociation of functional groups of proteins, movement of charged chains and changes in the alignment of helices (Tsong and Astumian, 1986). The structure of proteins is stabilized by a sensitive balance of various covalent and noncovalent interactions such as hydrogen bonds and hydrophobic, electrostatic, and Van der Waals interactions. The application of an external electric field may affect the local electrostatic fields in proteins and disrupt electrostatic interactions of peptide chains leading to conformational changes (Zhao and Yang, 2009). In addition, PEF-induced electrolysis and free radical formation may cause localized (*i.e.* near the electrodes) but strong pH shifts (Meneses et al., 2011) and oxidation of amino acid residues important for the activity and stability of enzymes (Rastogi, 2003). Meneses et al. (2011) observed pH shifts of up to 4.04 units during PEF treatment (10 kV/cm, 34 μs) of a salt solution (initial pH 7.1) with the pH values of 3.3 and 10.9 observed at the anode and the cathode, respectively. However, no such change in pH was observed in phosphate buffer solution. As a consequence, up to 30% inactivation of mushroom PPO was observed at the anode in the NaCl solution where pH drop was observed, whereas no inactivation was observed at the cathode, the center of the treatment chamber, and in phosphate buffer (pH 6.5) where no change in pH was observed under the same treatment conditions. This implies that the observed inactivation was solely due to the pH drop at the anode (Meneses et al., 2011). The instantaneous temperature increase during pulsing and localized hot spots in the PEF treatment chamber can also contribute to what is considered as PEF-induced denaturation of enzymes (Schilling et al., 2008; Jaeger et al., 2009; Buckow et al., 2010). For instance, in the study by Jaeger et al. (2009), numerical modeling of the temperature distribution in a co-linear PEF treatment chamber revealed temperature hot spots in the chamber with predicted temperature peaks exceeding 80°C compared to the outlet temperature of 61°C. The peak temperature was reduced to 68°C with a slight increase in the bulk temperature through the insertion of stainless steel and polypropylene grids while both the measured and predicted outlet temperatures remained the same. The insertion of the grids led to a more homogenous electric field, flow velocity, and temperature distribution and higher electric field strength in the case of the stainless steel grid for the same applied external voltage of 18 kV. In the case of the polypropylene grids, this resulted in a decrease in the level of inactivation of alkaline phosphatase in milk treated in the chamber at 28 kV/cm and 130 kJ/kg from 22% to 13%, although the PEF treatment condition remained the same indicating that PEF did not contribute to the observed inactivation. In the case of the stainless steel grid, the inactivation level was further reduced to 8% despite the fact that the electric field strength was increased to 37.6 kV/cm. The authors attributed the observed 8% inactivation to thermal effects during the holding of the PEF-treated milk at the outlet temperature of 61°C for seven seconds prior to cooling, estimating the expected inactivation based on their thermal inactivation kinetics data (Jaeger et al., 2009). A synergy between heat and PEF has been reported in a number of studies (Yang et al., 2004; Schilling et al., 2008; Riener et al., 2008a, 2008b, 2009), which is attributed to an increased mobility of charged groups at higher temperatures that affects electrostatic interactions and the stability of proteins (Yang et al., 2004).

PEF-induced changes on the secondary (Yeom et al., 1999; Zhong et al., 2005, 2007; Zhao and Yang, 2009) and tertiary (Zhao and Yang, 2008; Zhao and Yang, 2009; Luo et al., 2010) structures of enzymes have been reported in a number of studies. Yeom et al. (1999) observed PEF-induced loss of the α-helix structure of papain using far-UV circular dichroism (CD) spectroscopy after two milliseconds of PEF treatment at 50 kV/cm. The authors used a bench scale co-field PEF system consisting of four chambers with each pair of chambers connected to a cooling coil immersed in a water bath maintained at 10°C. According to the authors, the temperature did not exceed 35°C, which

excluded the possibility of thermal denaturation, since two-minute heat treatment at temperatures up to 80°C did not inactivate the enzyme. The authors noted that oxidation of the cysteine residue in the active site of the enzyme did not contribute to the inactivation of the enzyme (Yeom et al., 1999). However, application of 50 kV/cm for two milliseconds is roughly equivalent to 4–5 MJ/L (assuming a media conductivity of 1.0 mS/cm) and thus to an estimated temperature increase of over 1000°C. Although PEF application was partitioned over four treatment zones with cooling in between and probably several cycles through the system, it is questionable that the temperature could be kept at 35°C in all parts of the system. Thus, thermal denaturation may have contributed to the observed effect on the changes in the secondary structure of the enzyme. Zhong and co-workers (2007) also reported that the PEF inactivation of horseradish peroxidase (POD) at a maximum temperature of 40°C was accompanied by changes in the secondary structure of the enzyme as observed by far-UV circular dichroism and fluorescence spectroscopy (Zhong et al., 2005, 2007). Similar results were observed for mushroom polyphenol oxidase (PPO) (Zhong et al., 2007; Luo et al., 2010), lysozyme (Zhao and Yang, 2008), pepsin (Zhao and Yang, 2009), and soybean lipoxygenase (LOX) (Luo et al., 2010). Moreover, the cleavage of disulfide bonds has been observed following PEF treatment of some enzymes (Zhao et al., 2007). Aggregation following prolonged PEF treatment of enzymes has also been observed in a number of instances (Zhao et al., 2007; Zhao and Yang, 2009), although the inactivation of the enzymes preceded the aggregation. This has been attributed to increased intermolecular hydrophobic interaction due to PEF-induced exposure of buried hydrophobic side chains and disulfide linkage formation (Li et al., 2007; Zhao et al., 2009). Oxidation of amino acid residues susceptible to oxidation by free radicals generated during PEF processing may also contribute to the formation of disulfide-linked aggregates (Davies et al., 1987).

EFFECTS ON QUALITY-RELATED ENZYMES

Pectin Methylesterase (PME)

The majority of studies on the effect of PEF on enzyme activity are on PMEs from various plant sources including orange (Van Loey et al., 2001; Yeom et al., 2000, 2002; Giner et al., 2005b; Espachs-Barroso et al., 2006; Rivas et al., 2006; Sentandreu et al., 2006; Elez-Martinez et al., 2007; Hodgins et al., 2002), tomato (Giner et al., 2000; Van Loey et al., 2001; Espachs-Barroso et al., 2006), carrot (Espachs-Barroso et al., 2006; Rivas et al., 2006), banana (Espachs-Barroso et al., 2006), and commercial enzyme preparations of fungal origin (Giner et al., 2005a, 2005b). The results of most of the studies show that PEF treatment causes significant inactivation of PME's from different sources (Table 1). Most of these studies reported substantial inactivation of PMEs from orange and other sources (45–97.9%), following PEF processing at different conditions (see Table 1). Nevertheless, extremely high-specific energy inputs (up to 44 MJ/L) have been used in many of these studies to achieve such a level of PME inactivation (see Table 1), which is much more than typically required for the PEF inactivation of vegetative microorganisms. The treatment times used in many of these studies are also much longer than the practical limit (<200 microseconds) (Yang et al., 2004). In general, electric field strengths of 20–40 kV/cm and specific energy input of 50–1000 kJ/kg are required for the inactivation of vegetative microorganisms (Toepfl et al., 2006), although the exact condition depends on the physicochemical properties of the food product as well as the target microorganism (Toepfl et al., 2007) and the processing temperature (Heinz et al., 2003). Nevertheless, the goal should be to optimize the PEF process making use of the synergistic effects of PEF and mild heat on microbial and enzyme inactivation so that the specific energy input approaches that of conventional thermal pasteurization (20 kJ/kg assuming 95% heat recovery) (Toepfl et al., 2006). PEF treatments of 35 kV/cm for 59 microseconds for orange juice (Ayhan et al., 2001), 34 kV/cm for 166 microseconds ($T < 38$°C) for apple juice (Evrendilek et al., 2000), and 40 kV/cm for 57 microseconds (Tin = 45°C, Tout = 53°C) for tomato juice (Min et al., 2003b) have been reported to result in microbial inactivation comparable to thermal pasteurization. On the contrary, many of the studies on PME inactivation outlined in Table 1 used treatment times over 1000 microseconds. There are no studies on the effect of PEF on the thermostable fraction of orange PME. Nevertheless, it seems that the thermostable fraction is PEF resistant as well, since no complete inactivation of PME has been reported. Nonetheless, PEF-treated juices had comparable cloud stability during storage with thermally pasteurized juices (Yeom et al., 2000; Hodgins et al., 2002).

In contrast and in spite of using long treatment times, Van Loey et al. (2001) reported less than 10% inactivation of commercial tomato and orange PME in distilled water after PEF treatment at different conditions. The treatment was carried out at electric field strengths of 10 to 30 kV/cm, two pulse width values (5 and 40 microseconds), two pulse frequencies (1 and 100 Hz), and up to 1000 pulses depending on the field strength applied. Orange PME dissolved in freshly squeezed orange juice and buffer with the same pH (3.7) and electrical conductivity as orange juice was also treated with electric field strength of up to 35 kV/cm and 1000 pulses of one microsecond at 1 or 2 Hz. The maximum inactivation obtained was again 10%. In fact, increased PME activity was observed at most processing conditions in orange juice perhaps due to cell damage and release of intracellular PME. The authors concluded that in the absence of thermal effects, PEF does not cause significant enzyme inactivation (Van Loey et al., 2001). Although the specific energy input values are not explicitly stated for each experimental condition, the maximum energy input used in this study was about 500 kJ/kg, which is relatively low compared to the specific energy inputs used in other studies (see Table 1).

Table 1 Summary of representative studies on the effect of PEF on PMEs from different sources

Source	Equipment	Medium	Pulse characteristics[1]	T_{max} [°C]	% inactivation	*References*
Tomato	Batch unit, with exponential decay pulses, aluminium electrodes, d = 1–4mm	8.8% (w/v) NaCl (extraction solution)	E = 24 kV/cm, τ = 0.02 ms, t = 8000 μs, Q≈44 MJ/L	15°C	93.8%	*(Giner et al. 2000)*
Tomato	Batch: two types of chambers, one with two stainless cylinders connected by a plastic tubing (D = 11.4 mm, d = 3 mm) a plastic cuvette with parallel aluminium electrodes (A = 2 cm^2, d = 2 mm)	Distilled water	E = 10–30 kV/cm, τ = 5 μs, 40 μs, N = 1–1000, t_{max} at 10 kV/cm = 40 ms t_{max} at 20 kV/cm = 4000 μs t_{max} at 30 kV/cm = 400 μs	ambient	≤10% inactivation	*(Van Loey et al. 2001)*
Tomato	Batch, two parallel aluminium electrodes (A = 2 cm^2, d = 2 mm)	Tris-HCl buffer (pH 7.0)	Monopolar square wave pulses, E = 16.8 kV/cm, t = 1600 μs, f = 0.5 Hz, w = 4 μs, Q = 1067 kJ/kg	43.4°C	87% inactivation	*(Espachs-Barroso et al. 2006)*
Tomato	OSU 4-F, continuous (60ml/min), square wave pulses, stainless steel electrodes with d = 0.29 cm V = 0.012 ml	juice	35 kV/cm, τ = 4 μs, t = 1500 μs, f = 100 Hz	35 °C	82% inactivation	*(Aguilo-Aguayo et al. 2008b)*
Orange	Batch: two types of chambers, one with two stainless cylinders connected by a plastic tubing (D = 1.14 cm, d = 3 mm) and a plastic cuvette with parallel aluminium electrodes (A = 2 cm^2, d = 2 mm)	Distilled water	E = 10–30 kV/cm τ = 5 μs, 40 μs N = 1–1000 t_{max} at 10 kV/cm = 40 ms t t_{max} at 20 kV/cm = 4000 μs t_{max} at 30 kV/cm = 400 μs	ambient	≤10% inactivation	*(Van Loey et al. 2001)*
		McIlvaine buffer (pH 3.7) Orange juice (pH 3.7)			≤10% inactivation Increased PME activity in most cases	
Orange	OSU-3C, continuous, stainless steel tubular electrodes, d = 1 cm, 6 co-field tubular treatment chambers connected in series, square wave monopolar pulses	Orange juice	E = 35 kV/cm, t = 59 μs τ = 1.4 μs	60.1°C	90% inactivation	*(Yeom et al. 2000)*
Orange	OSU 3-C, continuous (0.31 ml/s), stainless steel tubular electrodes, d = 0.2 cm, square wave pulse, 6 co-field tubular treatment chambers connected in series, cooling coils after each pair of chambers submerged in water bath	Orange juice	E = 25 kV/cm, τ = 2.0 ms, t = 250 ms (most probably 2.0 μs and 250 μs respectively)	~64°C	90% inactivation	*(Yeom et al. 2002)*
Orange	Batch, stainless steel electrodes (D = 16.5 cm), d = 3 mm, V = 49.6 ml	Acidified orange juice (pH 3.5)	E = 80 kV/cm, N = 20, f = 0.333 Hz, τ = 2–3 μs, t = 40 to 60 μs	44°C	92.7%, the result is only an estimate	*(Hodgins et al. 2002)*
Orange	OSU 4-F, continuous (60 ml/min), square wave pulses, stainless steel electrodes d = 0.29 cm, V = 0.012 cm^3	Orange juice	E = 35 kV/cm, τ = 4 μs, bipolar, t = 1500 μs, f = 200 Hz, Q = 8085 kJ/L	37.5°C	78.1%	*(Elez-Martinez et al. 2007)*
Orange	OSU 4-D, continuous (60 ml/min), stainless steel tubular electrodes (D = 0.23 cm), d = 0.293 cm, 6 co-field tubular treatment chambers serially connected, cooling coils after each pair of chambers submerged in water bath	Orange juice	E = 25 kV/cm, t = 330 μs, τ = 2 μs, square wave bipolar pulse	72°C	90.6%	*(Sentandreu et al. 2006)*

Orange	Batch, two parallel aluminium electrodes ($A = 2\ cm^2$), d = 2 mm	Tris-HCl buffer (pH 7.0)	Monopolar square wave pulses, E = 17.5 kV/cm, t = 400 μs, f = 5 Hz, τ = 4 μs, Q = 277 kJ/kg	67.2°C, thermal effect accounted for	87% inactivation	*(Espachs-Barroso et al. 2006)*
Orange	Continuous pilot scale, co-linear chamber, d = 20 mm	Orange juice	Monopolar pulses, E = 23 kV/cm, f = 90 Hz, 130 L/hr, Q = 76±4 kJ/L	Tin = 38°C, Tout = 58°C	34% inactivation	*(Vervoort et al. 2011)*
Red grape fruit	Continuous, two parallel stainless steel electrodes (160 mm×5 mm), d = 2 mm, with a heating bath and a cooling bath maintained at 1°C	Red grapefruit juice	Monopolar pulses, f = 15 Hz, τ = 1 μs, E = 20,30,40 kV/cm, t = 25, 50, 100 μs, Tin = 23, 35, 50°C	60 and 72°C at 30 and 40 kV/cm and 100 μs	96.8% inactivation (Tin = 50, 40 kV/cm, 100 μs)	*(Riener et al. 2009)*
Clementine	OSU 4-D, continuous (60 ml/min), stainless steel tubular electrodes (D = 0.23 cm), d = 0.293 cm, 6 co-field tubular treatment chambers serially connected, cooling coils after each pair of chambers submerged in water bath	Clementine juice	E = 25 kV/cm, t = 330 μs, τ = 2 μs, square wave bipolar pulse	72°C	88.3%	*(Sentandreu et al. 2006)*
Ortanique fruits	OSU 4-D, continuous (60 ml/min), stainless steel tubular electrodes (D = 0.23 cm), d = 0.293 cm, 6 co-field tubular treatment chambers serial connected, cooling coils after each pair of chambers submerged in water bath	Ortanique fruit juice	E = 25 kV/cm, t = 330 μs, τ = 2 μs, square wave bipolar pulse	72°C	85.2%	*(Sentandreu et al. 2006)*
Orange – carrot blend	OSU 4-D, continuous (60 ml/min), stainless steel tubular electrodes (D = 0.23 cm), d = 0.293 cm, 6 co-field tubular treatment chambers serial connected, cooling coils after each pair of chambers submerged in water bath	Orange-carrot juice blend	E = 25 kV/cm, t = 340 μs	63°C	81.4%	*(Rodrigo et al. 2003)*
Orange – carrot blend	OSU 4-D, continuous (60 ml/min), stainless steel tubular electrodes (D = 0.23 cm), d = 0.293 cm, 6 co-field tubular treatment chambers serial connected, cooling coils after each pair of chambers submerged in water bath	Orange-carrot juice blend	E = 25 kV/cm, t = 330 μs, τ = 2.5 μs, f = 904 Hz	70°C	81%	*(Rivas et al. 2006)*
Banana	Batch, two parallel aluminium electrodes ($A = 2\ cm^2$), d = 2 mm gap	Tris-HCl buffer (pH 7.0)	Monopolar square wave pulses, E = 16.8 kV/cm, t = 1600 μs, f = 0.5 Hz, τ = 4 μs, Q = 1067 kJ/kg	43.4°C	45% inactivation	*(Espachs-Barroso et al. 2006)*
Carrot	Batch, two parallel aluminium electrodes ($A = 2\ cm^2$), d = 2 mm	Tris-HCl buffer (pH 7.0)	Monopolar square wave pulses, E = 15 kV/cm, t = 600 μs, f = 5 Hz, τ = 4 μs, Q = 309 kJ/kg	67.2°C, thermal effect accounted for	83% inactivation	*(Espachs-Barroso et al. 2006)*
Commercial pectolytic preparation	Batch, two circular stainless steel electrodes in parallel (D = 3.4 cm), d = 1.5 cm, V = 13.62 ml, air cooling, exponential decay pulses	water	E = 38 kV/cm, t = 340 μs Q∼1250 kJ/L	37.2°C	86.8% inactivation	*(Giner et al. 2005b)*
Commercial pectolytic preparation	Batch, parallel aluminium plate electrodes, d = 0.1–0.4 cm, exponential decay pulses, cooling every 5 pulses	water	E = 20 kV/cm, t = 8000 μs, τ = 0.04 ms, Q = 10909 kJ/L	25°C	97.9% inactivation	*(Giner et al. 2005a)*

1: E: electric field strength, τ: pulse width, t: total treatment time, N: number of pulses, Q: total energy input per unit volume, f: frequency, d: electrode gap, D: pipe diameter, A: area, V: volume

Regardless, most of the reported PEF inactivation effects cannot be attributed solely to a thermal effect. In the studies by Giner et al. (2000, 2005b); Hodgins et al. (2002), and Elez-Martinez et al. (2007), the reported maximum temperature the samples reached were below 44°C, which cannot cause thermal inactivation of the studied enzymes (see Table 1). In the cases where the temperature of the samples reached 60°C or higher, the residence time at that temperature was very short (1.5 and 9 seconds) (Yeom et al. 2000; Sentandreu et al., 2006). For instance, in the study by Yeom et al. (2000) on PEF processing of orange juice, the residence time at the maximal temperature of 60.1°C was reported to be nine seconds. According to Tajchakavit and Ramaswamy (1995), the *D* value for the thermal inactivation of orange PME at 60°C is 153 seconds (Tajchakavit and Ramaswamy, 1995). Thus, the observed 90% inactivation of PME may not be solely caused by the thermal effect. In order to determine the contribution of heat, Yeom et al. (2002) compared PEF treatment of orange juice at 35 kV/cm for an extremely long time of 184 milliseconds of orange juice with thermal treatment of similar time–temperature profile. The PEF treatment resulted in 83.2% inactivation of PME, whereas the thermal treatment caused only 24.1% inactivation (Yeom et al., 2002). Espachs-Barroso et al. (2006) studied the thermal and PEF inactivation of PMEs from carrot, tomato, banana, and orange in Tris-HCl buffer (pH 7.0) with the same objective. At low frequency (0.5 Hz) and pulses of four microseconds at 17.5 kV/cm, the temperature did not rise above 43.4°C, whereas treatments at a higher frequency (5 Hz) caused an increase to a maximum of 67.2°C after 400 microseconds, although temperature measurements could only be conducted between pulses and not during the pulse treatment. These authors calculated the contribution of the thermal effect during high-frequency PEF treatment using the thermal inactivation kinetic parameters and the temperature evolution during PEF treatment. PEF treatment at different conditions caused inactivation of all the PMEs to a different extent. PEF (16.8 kV/cm, 0.5 Hz, $t = 1600$ microseconds) caused a maximum inactivation of 45% and 87% of banana and tomato PME, respectively, at the lower frequency condition where the temperature did not exceed 43.4°C. Subtracting the thermal effect, a maximum of 87% inactivation of orange PME was observed after PEF treatment at 17.5 kV/cm for 400 microseconds and 5 Hz. A maximum of 83% inactivation of carrot PME was obtained after PEF treatment at 15 kV/cm for 600 microseconds and 5 Hz after taking into account the thermal effect (Espachs-Barroso et al., 2006). Nevertheless, it should be noted that the thermal effect in the aforementioned studies and others is accounted on the basis of temperature measurements between pulses or at the outlet of the PEF chamber. The instantaneous temperature increase during pulsing and localized hot spots in the treatment chamber may contribute to what is considered as PEF-induced inactivation of enzymes (Schilling et al., 2008; Jaeger et al., 2009; Buckow et al., 2010). In the simulation study mentioned earlier by Jaeger et al. (2009), temperature hot spots with peak temperatures in excess of 80°C were predicted compared to the 61°C outlet temperature in a co-linear PEF chamber indicating that the outlet temperature is not a reliable indicator of the temperature history of the product, although that to a certain extent depends on the configuration of the treatment chamber and the resulting flow and electric field distribution (Buckow et al., 2011).

Polygalacturonase (PG)

There are few studies on the effect of PEF processing on the activity of polygalacturonase. Only 12% inactivation of tomato PG was observed after PEF treatment of tomato juice at 35 kV/cm for 1500 microseconds using bipolar pulses of four microseconds width applied at 100 Hz. The treatment temperature was maintained below 35°C (Aguilo-Aguayo et al., 2008b). On the other hand, 98% inactivation of fungal PG was observed in a commercial pectolytic preparation after a very long treatment of 32.4 milliseconds at 10.28 kV/cm with a specific energy input of 22.56 MJ/L. The reported maximum temperature during the treatment was 25°C (Giner et al., 2003). In another study on the same enzyme preparation, a maximum of only 76.5% inactivation was observed with PEF treatment at higher intensity (38 kV/cm) and considerably shorter but still long treatment time (1.1 milliseconds). The temperature was maintained below 25°C by the integrated cooling system of the continuous treatment unit. Interestingly, treatments at milder conditions caused activation of the enzyme. About 10% increase in PG activity was observed following treatment at 15 kV/cm for 300 microseconds (Giner-Segui et al., 2006). Based on this result, it can be deduced that the reported increased apple juice yield following PEF treatment (Toepfl et al., 2006; Schilling et al., 2007) may be in part due to the activation of endogenous PG.

Polyphenol Oxidase (PPO)

A number of studies have been performed on the effect of PEF treatment on PPOs from different plant sources including apple (Giner et al., 2001; Van Loey et al., 2001), peach (Giner et al., 2002), pear (Giner et al., 2001), and mushroom (Van Loey et al., 2001; Yang et al., 2004; Zhong et al., 2007). The results are summarized in Table 2. Depending on the PEF treatment conditions and enzyme source, different levels of PPO inactivation have been reported. Giner et al. (2001) observed a maximum of 97% and 62% inactivation of apple and pear PPO in crude extracts following six milliseconds PEF treatment at 24.6 kV/cm and 22.6 kV/cm, respectively. In both cases, the reported bulk temperature during the treatment did not exceed 15°C, which would rule out thermal effect (Giner et al., 2001). Similarly, the same group reported that PEF treatment of 24.3 kV/cm for five milliseconds in bipolar mode caused 70% inactivation of peach PPO (Giner et al., 2002).

Ho et al. (1997) observed a maximum of 40% inactivation of purified mushroom PPO in buffer after PEF treatment at 45 kV/cm for 60 microseconds using exponential decay pulses with instant charge reversal. Increasing the treatment time to 200 microseconds or the electric field strength above 45 kV/cm

Table 2 Summary of representative studies on the effect of PEF on PPOs from different sources

Source	Equipment	Medium	Pulse characteristics[1]	T_{max}[°C]	% inactivation	*References*
Apple	Batch, parallel aluminium plate electrodes, d = 0.1–0.4 cm, exponential decay pulses, cooling every 5 pulses	Extraction buffer (pH 6.5)	E = 24.6 kV/cm, t = 6 ms, τ = 0.02 ms, bipolar	15°C	96.85% inactivation	*(Giner et al. 2001)*
Apple	Batch: two types of chambers, one with two stainless cylinders connected by a plastic tubing (D = 1.14 cm, d = 3 mm) and the second a plastic cuvette with parallel aluminium electrodes (A = 2 cm^2, d = 2 mm))	Phosphate buffer (pH 6)	E = 10–30 kV/cm τ = 5 μs, 40 μs N = 1–1000 t_{max} at 10 kV/cm = 40 ms t t_{max} at 20 kV/cm = 4000 μs t_{max} at 30 kV/cm = 400 μs	ambient	≤10% inactivation	*(Van Loey et al. 2001)*
			N = 1000, f = 1Hz, E = 10 kV/cm, t = 40 ms		21% inactivation	
		McIlvaine buffer (pH 3.8)	E = 7 kV/cm, 40 ms, f = 10 Hz	60°C	32% inactivation	
		Apple juice (pH 3.8)	E = 10–30 kV/cm τ = 5 μs, 40 μs N = 1–1000		Increased activity	
Apple	Continuous: colinear configuration with central high voltage electrode and two grounded electrodes separated by acetal isolators. Laboratory scale: D = 4 mm	Apple juice with 500 mg/kg ascorbic acid	τ = 3 μs, E = 15–35 kV/cm, Q = 8.5–65.5 kJ/kg T_{inlet} = 20 to 60 °C	51–73.9°C	93.1% inactivation at 35 kV/cm, 63.4 kJ/kg and Toutlet = 73.9°C	*(Schilling et al. 2008)*
		Apple juice with 200 mg/L ascorbic acid	τ = 3 μs, E = 30 kV/cm, Q = 100–130 kJ/kg T_{inlet} = 20 to 40 °C	51–63°C	48% inactivation at Tinlet = 40 °C, 100 kJ/kg and Toutlet = 63 °C	
	Pilot scale: D = 10 mm					
Apple	Continuous, two parallel stainless steel electrodes (160 mm×5 mm), d = 2 mm, with a heating bath and a cooling bath maintained at 1 °C	Apple juice	f = 15 Hz, τ = 1 μs E = 20,30,40 kV/cm t = 25, 50, 100 μs Tin = 23, 35, 50 °C	Tmax = 72 °C at 40 kV/cm Tmax = 65°C at 30 kV/cm	71% and 66.6% inactivation at 30 and 40 kV/cm, Tin = 50 °C, t = 100 μs	*(Riener et al. 2008a)*
Mushroom	Batch unit, Vmax = 148 ml, exponential decay pulse with instant charge reversal, two parallel cylindrical stainless steel electrodes (D = 16.5 cm), d = 0.3 cm	Buffer (pH 6.5)	E∼45 kv/cm t = 60 μs, τ = 2 μs, f = 0.5 Hz	20°C	40% inactivation	*(Ho et al. 1997)*

(Continued on next page)

Table 2 Summary of representative studies on the effect of PEF on PPOs from different sources *(Continued)*

Source	Equipment	Medium	Pulse characteristics[1]	Tmax[°C]	% inactivation	*References*
Mushroom	Batch: two types of chambers, one with two stainless cylinders connected by a plastic tubing (D = 1.14 cm, d = 3 mm) and the second a plastic cuvette with parallel aluminium electrodes (A = 2 cm^2, d = 2 mm))	Distilled water Phosphate buffer (pH 6) McIlvaine buffer (pH 4,5,7)	E = 10–30 kV/cm τ = 5 μs, 40 μs N = 1–1000 t_{max} at 10 kV/cm = 40 ms t_{max} at 20 kV/cm = 4000 μs, t_{max} at 30 kV/cm = 400 μs	ambient	≤10% inactivation	*(Van Loey et al. 2001)*
Mushroom	Continuous, stainless steel tubular electrodes (D = 0.23 cm), d = 0.292 cm, 6 co-field tubular treatment chambers serially connected, cooling coils after each pair of chambers submerged in water bath	Potassium phosphate buffer with NaCl (pH 6.4)	E = 33.6 kV/cm, τ = 2 μs, t = 126 μs, f = 800 Hz	26°C	38.2% inactivation	*(Yang et al. 2004)*
Mushroom	Continuous (52.5 ml/min), V = 6 ml, exponential decay pulse, round parallel stainless steel electrodes (D = 2.76 cm), d = 1 cm, with cooling coil immersed in ice-water bath	Phosphate buffer	f = 10 Hz, τ = 1.4 μs E = 25 kV/cm, t = 744 μs	40°C	76.2% inactivation	*(Zhong et al. 2007)*
Mushroom	Micro batch Electroporation cuvette, parallel aluminium electrodes, d = 2 mm,	Phosphate buffer (pH 6.5)	f = 1 Hz, E = 0–35 kV/cm, Q = 0 to 265 kJ/kg 300 pulses	Tmax = 30 °C	No inactivation	*(Moritz et al. 2012)*
Peach	Batch, parallel aluminium plate electrodes, d = 0.1–0.4 cm, exponential decay pulses, cooling every 5 pulses	Extraction buffer (pH 4.5)	E = 24.3 kV/cm, t = 5 ms, τ = 0.02 ms, bipolar	25°C	70% inactivation	*(Giner et al. 2002)*
Pear	Batch, parallel aluminium plate electrodes, d = 0.1–0.4 cm, exponential decay pulses, cooling every 5 pulses	Extraction buffer (pH 6.5)	E = 22.6 kV/cm, t = 6 ms, τ = 0.02 ms, bipolar	15°C	62% inactivation	*(Giner et al. 2001)*
White grape (cv. Parellada)	OSU 4-F, continuous (7.8 ml/s), square wave pulses, bipolar, stainless steel electrodes with d = 0.29 cm, V = 0.012 cm^3	White grape juice	E = 25–35 kV/cm, f = 200–1000 Hz, t = 1–5000 μs, τ = 4 μs	35–40°C	Complete inactivation at 30 kV/cm, 600 Hz, 5000 μs	*(Marsells-Fontanet and Martin-Belloso 2007)*

1: E: electric field strength, τ: pulse width, t: total treatment time, N: number of pulses, Q: total energy input per unit volume, f: frequency, d: electrode gap, D: pipe diameter, A:area, V: volume

did not significantly change the degree of enzyme inactivation (Ho et al., 1997). Nearly, the same level of inactivation was observed by Yang et al. (2004) who reported 38% inactivation of purified mushroom PPO dissolved in buffer after PEF treatment at 33.6 kV/cm for 126 microseconds with the temperature reaching a maximum of about 26°C. Thermal treatment simulating the temperature profile during the PEF treatment was also carried out resulting in 17.4% inactivation of the enzyme (Yang et al., 2004). In another study, 76.2% inactivation of mushroom PPO in buffer was obtained after a longer PEF treatment of 744 microseconds at 25 kV/cm (Zhong et al., 2007), which is significantly higher than the aforementioned studies. Investigation of the enzyme using far-UV CD revealed that the inactivation was accompanied by changes in the secondary structure of the enzyme (Zhong et al., 2007). Schilling et al. (2008) observed 93.1% inactivation of apple PPO in apple juice when the juice was subjected to PEF treatment at 35 kV/cm and 63.4 kJ/kg with an inlet temperature of 60°C and a corresponding outlet temperature of 73.9°C. The estimated thermal inactivation under the same time–temperature profile was 9.4%. PEF treatments at inlet temperatures of 20 to 40°C and 30 kV/cm resulted in a significant PPO inactivation at all inlet temperatures with maximum inactivation at 40°C and a corresponding outlet temperature of 63°C and a higher-specific energy input of 100 kJ/kg (Schilling et al., 2008). Similarly, Riener et al. (2008a) observed 66.6% inactivation of PPO in apple juice after PEF treatment at 30 kV/cm for 100 microseconds of the juice pre-heated to 50°C. The temperature of the sample after the treatment was 72°C. Thermal pasteurization of the juice at 72°C for 26 seconds resulted in 46% inactivation of PPO. Considering the fact that the residence time of the juice in the PEF chamber was 6.6 seconds, the authors concluded that part of the observed inactivation is due to the effect of PEF. PEF treatments under the same condition and after pre-heating to lower temperatures (23 and 35°C) resulted in lower inactivation levels of 40.6% and 50% with the temperature increasing by 15°C in both cases after the PEF treatment (Riener et al., 2008a).

Contrary to what is reported in the aforementioned studies, Van Loey et al. (2001) observed less than 10% inactivation of purified mushroom PPO dissolved in water after PEF treatment at a range of conditions. Varying the pH and conductivity of the medium as well as protein concentration did not affect the sensitivity of the enzyme toward PEF. The maximum inactivation of 32% was observed for purified apple PPO dissolved in buffer of the same conductivity and pH as apple juice after a long PEF treatment at low intensity (7 kV/cm, 40 milliseconds, 10 Hz). The inactivation was attributed to thermal effects as the temperature increased to 60°C during the treatment (Van Loey et al., 2001). PEF treatment of freshly squeezed apple juice resulted in increased PPO activity probably due to PPO release from PEF-damaged cells (Van Loey et al., 2001). The results of the different studies show that PEF at the right condition causes substantial PPO inactivation. However, almost all these studies were performed in buffer solutions as a medium and often at impractical processing conditions. Thus, the results cannot be extrapolated to real food systems since the medium properties affect the sensitivity of PPO to PEF treatment. Therefore, more work needs to be done in real food systems and at practical PEF processing conditions, that is, at electric field intensity of 20–40 kV/cm, processing times less than 200 microseconds, and at specific energy input sufficient for pasteurization of the product, which can be optimized through processing at mild temperature conditions.

Peroxidase (POD)

Depending on the source and the PEF process, different levels of PEF inactivation of PODs have been reported. Ho et al. (1997) observed a maximum of about 30% inactivation of soybean POD in phosphate buffer (pH 6.0) following PEF treatment at 73.3 kV/cm for 60 microseconds using exponential decay pulses with instant charge reversal. The processing temperature was reported to be 20°C. Increasing the treatment time to 200 microseconds did not have a significant effect on the degree of inactivation at the same condition (Ho et al., 1997). Substantially, less inactivation was reported by Yang et al. (2004) who studied the effect of PEF treatment on the activity of soybean POD dissolved in potassium phosphate buffer (pH 6.4). They observed a maximum inactivation of 18.1% at PEF treatment of 34.9 kV/cm for 126 microseconds. The maximum temperature during the treatment was reported to be 42°C (Yang et al., 2004).

On the other hand, Elez-Martinez et al. (2006) found orange POD to be susceptible to PEF inactivation, although they used a much more intensive process compared to what was used for soybean POD. They reported complete inactivation of POD in orange juice after treatment at 35 kV/cm for 1500 microseconds. Bipolar PEF pulses of four microseconds width at a pulse frequency of 200 Hz were used in the study. According to the authors, the temperature was maintained below 35°C (Elez-Martinez et al., 2006). Likewise, Schilling et al. (2008) observed complete inactivation of POD in apple juice (where fungal POD was added to boost the endogenous POD activity) after PEF treatment at 25 kV/cm, 65 kJ/kg, and inlet temperature of 60°C with a corresponding outlet temperature of 73.9°C. Thermal inactivation under the same temperature condition was estimated to be 33.3%. At lower inlet temperatures (20 and 40°C), the observed inactivation was less than 15% regardless of the electric field strength (15–35 kV/cm) or specific energy input applied (Schilling et al., 2008). On the other hand, Riener et al. (2008a) observed less sensitivity of apple POD to PEF inactivation with 65% inactivation after PEF treatment at 30 kV/cm for 100 microseconds of apple juice preheated to 50°C with an outlet temperature of 72°C. Thermal pasteurization at 72°C for 26 seconds resulted in 48% inactivation and the authors concluded that part of the inactivation is PEF mediated since the residence time in the PEF chamber was only 6.6 seconds. Preheating to lower temperatures of 23 and 35°C (with corresponding outlet temperatures of 38 and 50°C) resulted in

lower levels of inactivation (about 36% in both cases) (Riener et al., 2008a).

Tomato POD showed a similar susceptibility toward PEF inactivation with 97% inactivation after PEF treatment of tomato juice at 35 kV/cm for 1500 microseconds using four microseconds of bipolar pulses at 100 Hz. The temperature during the treatment was reported to be less than 35°C (Aguilo-Aguayo et al., 2008b). White grape POD was found to be less susceptible to PEF inactivation with a maximum of 50% inactivation after PEF treatment of grape juice at 30 kV/cm and a long treatment of three milliseconds using four microseconds of bipolar pulse at 600 Hz. The same inactivation level was observed after a slightly longer treatment time of five milliseconds at 25 kV/cm and 1000 Hz. The maximum temperature of the juice was reported to be between 35 and 40°C (Marsells-Fontanet and Martin-Belloso, 2007). In contrast, Vervoort et al. (2011) observed only 30% inactivation of POD in orange juice after PEF treatment (23 kV/cm, 90 Hz, and 76 kJ/L) and inlet and outlet temperatures of 38 and 58°C, which was sufficient for pasteurization of the juice. The lower level of inactivation in this case might be due to the lower-specific energy load used in the study.

Van Loey et al. (2001) reported a maximum of 10% inactivation of horseradish POD dissolved in water following PEF treatment in a range of conditions. Changing the pH of the medium affected the stability of POD toward PEF treatment. When the pH of the medium was decreased from seven to four, a maximum inactivation of 60% was obtained after 1000 pulses of one microsecond width and frequency of 1 Hz treatment regardless of the electric field strength (10–30 kV/cm). A further 10% decrease in POD activity was observed after two hours of storage at room temperature following the treatment. Varying the concentration of the enzyme in the range of 1 to 5 mg/mL (and thereby the conductivity from 10 to 90 μS/cm) did not affect the sensitivity of the enzyme to PEF. A maximum of 10% inactivation was observed under these conditions. Changing the conductivity of the medium at constant protein concentration in the range from one to four mS/cm (which results in an increase of the specific energy input from 380 to 850 J/kg for one microsecond pulses at 20 kV/cm) also did not have a substantial effect on the sensitivity of the enzyme toward PEF (Van Loey et al., 2001).

Zhong et al. (2005) observed 34.7% inactivation of horseradish POD after PEF treatment at 22 kV/cm for 1861.5 microseconds using exponential decay pulses. The temperature during the treatment did not exceed 40°C. Further decrease in the activity of the PEF-treated enzyme was observed during storage at 4°C, although the decrease was not as high as in the study by Van Loey et al. (2001), perhaps due to the lower storage temperature. The inactivation was accompanied by changes in the conformation of the enzyme as observed by far-UV circular dichroism and fluorescence spectroscopy (Zhong et al., 2005). In a subsequent study using parallel electrodes instead of the co-axial ones used in the previous study, the same group observed a similar 32.2% inactivation of horseradish POD in acetate buffer after PEF treatment of 25 kV/cm for 1740 microseconds (Zhong et al., 2007).

Lipoxygenase (LOX)

There are not many reports on the effects of PEF on LOX. Van Loey et al. (2001) studied the effects of PEF processing on purified soybean lipoxygenase in model systems as well as green peas LOX in green pea juice. They observed a maximum of 64% inactivation of soybean LOX after prolonged treatments of 1000 pulses at 1 Hz using 5 and 40 microseconds pulses at 10 kV/cm (with treatment times equivalent to 5 and 40 milliseconds). They attributed this observation to a small off-shot current associated with the PEF equipment rather than the PEF treatment, as no more than 10% enzyme inactivation was observed in a range of other conditions. Varying the protein concentration and thereby the conductivity of the medium did not have any significant effect on the sensitivity of the enzyme to PEF treatment. Similarly, no inactivation of LOX was observed after PEF treatment of green pea juice in a range of conditions (Van Loey et al., 2001).

Min et al. (2003b) observed 54% inactivation of LOX in cold break tomato juice following PEF treatment at 40 kV/cm for 57 microseconds using a commercial-scale PEF equipment (400 Liters/hour). The equipment consisted of six serially connected co-field PEF chambers with heat exchangers between each pair of PEF chambers so that the temperature at the inlet of each pair of chambers was maintained at 45°C. A further 20% decrease in the activity of LOX was observed during storage at 4°C (Min et al., 2003b). Aguilo-Aguayo et al. (2008a) observed a maximum of 44.2% inactivation of LOX in strawberry juice after PEF treatment of 35 kV/cm for 1000 microseconds and energy input of 5512 kJ/L at pulse frequency of 150 Hz and four microseconds of pulse width using monopolar square wave pulses. Applying bipolar pulses of seven microseconds width at 250 Hz at the same electric field intensity and for the same treatment time resulted in a 14.1% increase in activity. A bench scale PEF unit (OSU-4F) consisting of eight co-field flow tubular chambers with each pair connected to a coiled tube submerged in ice-water was used in the experiment and the temperature did not exceed 40°C (Aguilo-Aguayo et al., 2008a).

Riener et al. (2008b) studied the effect of PEF processing on LOX in soya milk. They studied the inactivation of the enzyme after preheating soya milk samples (23, 35, and 50°C) and subjecting them to PEF treatment at 20 to 40 kV/cm for 25 to 100 microseconds. The maximum inactivation of 84.5% was observed after PEF treatment at 40 kV/cm for 100 microseconds of the sample preheated to 50°C with an outlet temperature of 69°C. 21.5% of the inactivation occurred during the preheating of the soya milk to 50°C. Lower levels of inactivation were observed in samples preheated to lower temperatures (Riener et al., 2008b).

β-glucosidase

A limited inactivation of β-glucosidase was observed in strawberry juice after PEF treatment at 35 kV/cm for 1000

microseconds and a specific energy input of 5512 kJ/L at different pulse frequencies (50–250 Hz), pulse widths (one to seven microseconds), and pulse polarities (bipolar and monopolar) with the temperature maintained below 40°C. The maximum inactivation was 26.8% at 250 Hz and seven-microsecond pulse width using bipolar pulses. Other conditions resulted in increased activity of up to 12.5%, although the same electric field strength (35 kV/cm), treatment time (1000 microseconds), and specific energy input (5512 kJ/L) were used (Aguilo-Aguayo et al., 2008a).

Factors that Affect PEF Inactivation of Enzymes

There is a wide variation in literature on the effect of PEF on food quality-related enzymes. This is expected as the inactivation effect of PEF depends on the processing condition (electric field strength, E, total energy input, Q, temperature, etc.) which is closely related to the design of the PEF system, the physical properties of the medium (electrical conductivity, model vs. real food systems, pH, viscosity, etc.), and the enzyme and its source. There are not many studies in literature which compare the stability of enzymes during PEF processing under similar processing conditions. In addition, based on the available information in literature, it is hard to rank enzymes in terms of their stability toward PEF inactivation since the stability of enzymes also depends on their source. For instance, POD was found to be more susceptible to PEF inactivation than PPO in tomato juice (Aguilo-Aguayo et al., 2008b) and apple juice (Schilling et al., 2008), whereas the opposite was observed in the case of grape juice (Marsells-Fontanet and Martin-Belloso, 2007).

Similarly, there are not many studies that systematically compared the sensitivity of enzymes from different sources under exactly the same processing conditions. Nevertheless, differences in the sensitivity of the same enzyme from different sources to PEF inactivation have been reported. Espachs-Barroso et al. (2006) observed that banana PME was the most resistant to PEF inactivation followed by tomato PME, carrot PME, and orange PME in that order (see Table 1). For instance, a specific energy input of 1067 kJ/kg was required for 87% inactivation of tomato PME, whereas the same level of orange PME inactivation was achieved with a specific energy input of only 277 kJ/kg (Espachs-Barroso et al., 2006).

PEF Processing Parameters

The critical processing parameters that affect the rate and level of enzyme inactivation are electric field strength, treatment time and specific energy input (Giner et al., 2000, 2005b; Espachs-Barroso et al., 2006; Elez-Martinez et al., 2007; Yeom et al. 2000; Yeom et al. 2002), pulse frequency (Espachs-Barroso et al., 2006; Elez-Martinez et al. 2007), pulse width (Elez-Martinez et al., 2007), and process temperature. It should be noted that many of these parameters are interdependent. For instance, the higher the electric field strength or the treatment time, the higher the specific energy input. Increased rate and level of PME (Giner et al., 2000, 2005b; Yeom et al. 2002; Espachs-Barroso et al., 2006), PG (Giner et al. 2003; Giner-Segui et al. 2006), PPO (Ho et al., 1997; Giner et al., 2001, 2002; Zhong et al., 2007), and POD (Yang et al., 2004; Zhong et al., 2005, 2007) inactivation have been observed with an increase in electric field strength, treatment time, and thus the specific energy input.

Increasing the pulse frequency (Espachs-Barroso et al., 2006; Elez-Martinez et al., 2007) or the pulse width (Elez-Martinez et al., 2007) while maintaining constant energy density also resulted in higher enzyme inactivation. Increasing the pulse frequency while maintaining the total energy input resulted in higher inactivation of orange POD (Elez-Martinez et al., 2006), PMEs from orange (Espachs-Barroso et al., 2006; Elez-Martinez et al., 2007), banana, tomato, and carrot (Espachs-Barroso et al., 2006). Espachs-Barroso et al. (2006) observed a higher increase in temperature at higher-pulse frequency treatments ($\leq$43.4°C at 0.5 Hz compared to a maximum of 67.2°C at 5 Hz). Thus, they attributed the effects of higher pulse frequency to temperature increase since less time is available for energy dissipation and temperature equilibration at higher frequency leading to higher inactivation due to the synergistic inactivation effects of temperature and PEF (Espachs-Barroso et al., 2006). Other studies did not report temperature increases with increasing pulse frequency. Likewise, there are studies which reported increased PEF inactivation of enzymes with increase in pulse width (Giner et al., 2000, 2001, 2002, 2003; Elez-Martinez et al., 2007). Increasing pulse width at constant-specific energy input also resulted in higher inactivation of orange PME (Elez-Martinez et al., 2007). Another group reported that increasing the pulse width at constant number of pulses resulted in higher inactivation of PG in commercial pectinase preparation (Giner et al., 2003), tomato PME (Giner et al., 2000), and PPOs from peach (Giner et al., 2002), apple, and pear (Giner et al., 2001). However, those increases are certainly due to increased PEF treatment time as a result of the increased pulse width and perhaps increased temperature due to decreased time for energy dissipation. Pulse polarity (bipolar vs. monopolar) has also been reported to have an effect on PEF inactivation of enzymes (Giner et al., 2002; Elez-Martinez et al., 2007). Bipolar pulses resulted in a higher level of orange PME inactivation at the same total treatment time compared to monopolar pulses (Elez-Martinez et al., 2007). Similar results were reported for peach PPO (Giner et al., 2002), whereas no significant effect of pulse polarity was observed in the case of tomato PME (Giner et al., 2000). The opposite was reported for orange POD with monopolar pulses resulting in higher inactivation compared to bipolar pulses under the same PEF treatment conditions (Elez-Martinez et al., 2006).

Temperature

In general, Ohmic heating causes a temperature increase during PEF processing, which can play a significant role in PEF

inactivation of enzymes. A closer examination of the literature reveals that excessively high-specific energy inputs and treatment times much longer than the practical limit (>200 microseconds) are usually required to get substantial inactivation of enzymes by PEF treatment at room temperature conditions (see Tables 1 and 2). For instance, a specific energy input of 44 MJ/L and 8000 microseconds treatment time have been used to achieve 93.8% inactivation of PME at the reported maximum temperature of 15°C (Giner et al., 2000). Despite this fact, there are not many studies which investigated the effect of temperature on PEF inactivation of enzymes in a systematic manner. Yeom et al. (2002) investigated the effects of temperature on PEF inactivation of PME in orange juice at 25 kV/cm by submerging the tubes connecting a pair of PEF treatment chambers (see Table 1 for details about the treatment chamber) in a water bath maintained at 10 to 50°C. The inactivation effect of PEF increased with increased water bath temperature. About 90% inactivation of orange PME was reported by PEF treatment at 25 kV/cm for 250 milliseconds (based on our knowledge of the treatment system, most probably 250 microseconds) in a 50°C ($T_{max} = \sim 66$°C) water bath, whereas only 36.3% inactivation was observed when the treatment was carried out in a 10 to 20°C water bath ($T_{max} = \sim 48$°C) under the same PEF setting indicating synergistic inactivation effects of mild heat and PEF (Yeom et al., 2002). Espachs-Barroso et al. (2006) observed higher inactivation of PMEs from different sources at 5 Hz compared to 0.5 Hz pulse frequency. A higher temperature increase was observed at 5 Hz (maximum of 67.2°C) compared to 0.5 Hz (maximum of 43.4°C). This was attributed to the synergistic inactivation effect of PEF and temperature (Espachs-Barroso et al., 2006). Schilling et al. (2008) also reported synergistic effects of temperature and PEF on the inactivation of enzymes. They observed limited inactivation of POD and PPO in apple juice when the inlet temperature was 20 to 40°C at 15 to 35 kV/cm and specific energy inputs between 8.5 and 65.5 kJ/kg. Increasing the inlet temperature to 60°C resulted in complete inactivation of POD and 93.1% inactivation of PPO at 35 kV/cm and specific energy input of 63.4 kJ/kg. The contributions of heat based on the global heating effect were only 33.3% and 9.4% for POD and PPO, respectively (Schilling et al., 2008), clearly indicating synergistic effects of mild heat and PEF. Similarly, 28.6 and 16% increase in the level of inactivation of POD and PPO, respectively, in apple juice was observed with increase in pretreatment temperature of the juice from 23 to 50°C (with corresponding final temperatures of 38 to 65°C at 30 kV/cm and 100 microseconds treatment time) (Riener et al., 2008a). Analogous effects of preheating temperatures were observed by the same group on the inactivation of soya bean LOX (Riener et al., 2008b) and orange PME (Riener et al., 2009) after PEF treatment of soya milk and orange juice, respectively. The synergistic effect of mild heat and PEF was attributed to an increased mobility of charged groups at higher temperatures, which affects electrostatic interactions and the stability of proteins including enzymes (Yang et al., 2004).

Medium Properties

The stability of enzymes depends on the matrix in which they are suspended. In the case of PEF processing, change of matrix can have dual consequences; it may alter the stability of the enzyme as well as the PEF treatment parameters under the same applied voltage condition since physicochemical properties such as electrical conductivity may change. With change in conductivity, the energy per pulse delivered to the product changes possibly resulting in changes in the level of inactivation. Yang et al. (2004) studied the effect of electrical conductivity of the medium on the PEF inactivation of mushroom PPO in phosphate buffer (pH 6.5). The electrical conductivity was adjusted by varying the concentration of NaCl in the solution. A significant increase in the inactivation of PPO was observed at field strength higher than 20 kV/cm, when the conductivity increased from 0.112 S/m to 0.342 S/m. For instance, about 5% inactivation was observed after 126 microseconds treatment at 35 kV/cm and 1.12 mS/cm while the inactivation was more than 40% at 3.42 mS/cm under the same PEF condition. The authors attributed the increased inactivation due to the presence of more free ions in the solution at higher conductivity, which may enhance, suppress, or disrupt electrostatic interactions during PEF treatment thereby affecting the stability of the enzyme (Yang et al., 2004). However, the increase in conductivity was accompanied by an increase in the maximum temperature during the treatment, which may have contributed to the higher inactivation at higher conductivity. The increase in temperature per pair of PEF treatment chambers was 13°C at 44 kV/cm and 1.12 mS/cm, whereas a 26°C increase in temperature was observed at 34.2 kV/cm and 3.42 mS/cm (Yang et al., 2004). In contrast, increasing the medium conductivity from 1 to 4 mS/cm did not have significant effect on the inactivation of mushroom PPO and horseradish POD with less than 15% inactivation of the enzymes after PEF treatment at 6 to 20 kV/cm, pulse widths from 1 microseconds (at 20 kV/cm) to 40 microseconds (at 6–8 kV/cm) and the number of pulses varying from 100 to 400. The increase in conductivity from 1 to 4 mS/cm was accompanied by increases in the specific energy input from 380 to 850 J/kg for 1 microseconds pulses at 20 kV/cm and from 1600 to 3950 J/kg for 40 microseconds pulses at 8 kV/cm (Van Loey et al., 2001). The same authors compared the stability of PPO and PME in apple juice and orange juice, respectively, with model systems of similar pH and conductivity. They observed up to 32% inactivation of PPO and 10% inactivation of PME in the model systems while no inactivation and some increase in activity was observed in the juices (Van Loey et al., 2001).

Van Loey et al. (2001) investigated the effect of pH on the PEF inactivation of mushroom PPO and horseradish POD in model systems. Varying the pH from 4 to 7 did not have significant effect on the stability of PPO under the studied condition. On the other hand, the pH of the medium had significant effect on the sensitivity of POD to inactivation by PEF. About 60% inactivation of POD was observed after 1000 microseconds

treatment at 1 Hz regardless of the field intensity (10–30 kV/cm) in McIlvaine buffer of pH 4 with further 30% decrease in activity during two hours storage at room temperature while less than 10% inactivation of the enzyme was observed in distilled water. Increasing the pH had similar effect on pepsin with 30% more inactivation at pH 6.4 compared to pH 2.1 (Yang et al., 2004). Reversible changes in pH may also occur during PEF treatment of nonbuffered solutions (Saulis et al., 2005; Meneses et al., 2011). This may cause significant inactivation of susceptible enzymes as observed in the case of mushroom PPO in the study mentioned earlier (Meneses et al., 2011).

Enzyme Inactivation Kinetics

Enzyme inactivation kinetic data and predictive inactivation models are useful for the design and optimization of PEF processes, which are sufficient for enzymatic stabilization of products without over processing. There are not many studies on the inactivation kinetics of enzymes during PEF processing. The existing data shows first-order inactivation of many enzymes during PEF processing as in the case of thermal processing. First-order inactivation models described the kinetics of inactivation of tomato PME (Giner et al., 2000), fungal PME (Giner et al., 2005b), and PG (Giner et al., 2003) in commercial enzyme preparations, PPO's from peach (Giner et al., 2002), mushroom (Zhong et al., 2007), apple, and pear (Giner et al., 2001), and horseradish POD (Zhong et al., 2007). A first-order fractional conversion model described the rate of inactivation of POD (Elez-Martinez et al., 2006) and PME (Elez-Martinez et al., 2007) in orange juice with the stable fraction dependent on the electric field intensity. Perhaps the different PME and POD isoenzymes in orange have different stability toward PEF. The Weibull distribution model has also been used successfully to describe the PEF inactivation of these enzymes as well as fungal PME in a commercial pectinase preparation (Giner et al., 2005a).

Different empirical models have been used to relate the rate and level of inactivation with the PEF processing parameters such as electric field strength and specific energy input. Exponential models were successfully used to describe the dependence of PEF inactivation rate constants of enzymes including tomato PME (Giner et al., 2000), PPOs from peach (Giner et al., 2002), apple, and pear (Giner et al., 2001), fungal PG (Giner et al., 2003), and horseradish (Zhong et al., 2007) and orange POD (Elez-Martinez et al., 2006) to the applied electric field strength. On the other hand, linear dependence of the inactivation rate constant on the electric field strength was observed in the case of fungal PME in a commercial enzyme preparation (Giner et al., 2005b). Exponential decay equations have also been used to describe the relationship of residual activity following PEF processing with the specific energy input quite successfully for a number of enzymes including tomato PME (Giner et al., 2000), fungal PG (Giner et al., 2003), and PPOs from peach, pear, and apple (Giner et al., 2001, 2002). Other equations that have been used in this context are Fermi's and Hülsheger's models (Giner et al., 2000, 2005a; Giner-Segui et al., 2006; Elez-Martinez et al., 2007), both of which described well the dependence of PEF enzyme inactivation on the electric field strength.

CONCLUSION

From the foregoing discussion, it is clear that PEF is a promising preservation technology for liquid foods. Better quality retention in PEF-processed products compared to thermal processing has been observed in many cases. However, PEF has often little or a limited effect on enzymes at processing conditions sufficient for microbial inactivation (50–1000 kJ/kg). This is advantageous in preservation applications where microbial inactivation while retaining bioactive proteins is the desired objective. On the other hand, quality degradation may arise from the residual activity of enzymes, which to a certain extent can be controlled through appropriate packaging and refrigerated storage.

At sufficiently high-specific energy input (e.g. >1000 kJ/kg), PEF causes significant inactivation of enzymes at ambient and mild temperature conditions. The available data in the literature suggests that both electrochemical effects and Ohmic heating contribute to the observed inactivation. The relative contribution of each depends on the design of the PEF treatment chambers as well as the treatment conditions. Substantial PEF-induced inactivation of all the major plant quality-related enzymes including PME (up to 97%), PG (up to 76.5%), PPO (up to 97%), POD (up to 97%), and LOX (up to 64%) have been reported at room temperature condition, although excessively large specific energy inputs were required (1066–44000 kJ/L). Nevertheless, significant synergy is observed between mild heat and PEF resulting in the inactivation of these enzymes at reasonable energy inputs. The discussions in the forgoing sections give an indication that substantial enzyme inactivation under reasonable PEF processing conditions (63–309 kJ/L) can be achieved when PEF treatment is combined with mild-processing temperatures. Therefore, combination with mild heat appears to offer a good prospective future for PEF pasteurization of plant-based food products allowing both microbial and enzyme inactivation at practically feasible processing conditions that give reasonable shelf life especially if combined with suitable packaging and storage conditions.

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