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Review

Effects of high electric field pulses on enzymes

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The application of high electric field pulses (HELP) in food processing offers potential both as a preservation technology, and as an adjunct to other processes, such as drying, extraction. In respect of the use of pulsed electric fields as preservation technology, the impact of this technology on food quality degrading enzymes is of particular interest. This paper presents a detailed literature review on the effects of pulsed electric fields on enzymes, complemented with recent results on the impact of this HELP-technology on enzymes, obtained within EU project 'High electric field pulses: food safety, quality and critical process parameters' (contract FAIR-CT97-3044).

Literature review

Some enzymes are positively utilized during food processing for recovery of by-products, for developing new food products, for achieving higher rates and levels of extraction, or for improving food quality in terms of, e.g. flavour. On the other hand, enzymes might also have detrimental effects on food quality. Food quality defects can be caused by enzymes naturally present in the food, or by enzymes produced by certain micro-organisms. Hence, besides microbial inactivation, preservation technologies aim at inactivating enzymes with deteriorative action. Because of the beneficial and/or detrimental effects of enzymes, control of enzymatic activity is required in many food processing steps. Either one wants to promote the beneficial effects of the enzyme by enhancing the enzymatic activity during processing; in this case knowledge of enzyme stability under the relevant processing conditions is required for process design (e.g. thermostability, resistance towards acid environments). Alternatively, in case of detrimental enzymatic action, one wants to eliminate or retard the enzymatic reaction by, e.g. enzyme inactivation or use of inhibitors.

The use of high electric field pulses (HELP) is being investigated as a new technology for food preservation because HELP can be applied to inactivate vegetative micro-organisms with no or limited increase in food temperature (Barbosa-Canovas, Pothakamury, Palou, & Swanson, 1998; Wouters & Smelt, 1997). However, in comparison with the extensive research devoted to the destruction of microorganisms by HELP, there is only limited information available concerning the effects of pulsed electric fields on enzyme activity, indicating varying results (Table 1).

Grahl and Märkl (1996) investigated, in addition to the inactivation of microorganisms, the effect of exponential decaying high voltage pulses ($E = 21.5$ kV/cm) on alkaline phosphatase, lactoperoxidase and lipase in raw milk. The treatment chamber was composed of two plain parallel carbon electrodes ($A = 50$ cm^2, $d = 0.5$ cm). *Alkaline phosphatase* and *lactoperoxidase* did not show any large-scale inactivation under the HELP conditions tested. HELP-induced inactivation of *lipase* up to 60% was only established at high energy input (>200 kJ/l).

Barsotti, Dumay, Mu, Diaz, and Cheftel (2001) investigated the effect of high voltage pulses on *lactate dehydrogenase*, a tetrameric enzyme stabilised in part by electrostatic interactions. After applying 200 exponential decaying pulses of 31.6 kV/cm and 0.96 μs at 1.1 Hz and 30 °C, no loss in lactate dehydrogenase activity was observed.

However, other studies indicate that some enzymes that are detrimental for food quality during storage can be inactivated using high voltage electric pulses.

Vega-Mercado, Powers, Barbosa-Canovas, and Swanson (1995) investigated the effect of pulsed electric fields on *plasmin* (or alkaline protease, an indigenous

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Table 1. Literature review on the effects of pulsed electric fields on enzymes

Enzyme	Operating conditions	Medium	pulse characteristics	T (°C)	% inact.	Ref.
Plasmin (bovine milk)	Continuous (45 ml/min) electrodes: parallel, stainless steel, cooled $V = 8$ cm^3, $d = 0.6$ cm	SMUF 100 μg plasmin/ml pH 6.11 0.056 M	$E = 30$ kV/cm, $w = 2$ μs #$= 50$, $f = 0.1$ Hz	15	90	Qin, Pothakamury, Barbosa-Canovas, and Swanson, 1996; Vega-Mercado, Powers, Barbosa-Canovas, and Swanson, 1995
Protease (*Pseudomonas fluorescens* M3/6)	Continuous (0.5 l/min) electrodes: coaxial, stainless steel $V = 28.5$ cm^3, $d = 0.6$ cm	Skim milk	$E = 15$ kV/cm, $w = 2$ μs #$= 98$, $f = 2$ Hz	50	60	Vega-Mercado, Powers, Barbosa-Canovas, and Swanson, 1995; Vega-Mercado, Powers, Barbosa-Canovas, and Swanson *et al.*, 1995
		TSB with yeast extract	$E = 18$ kV/cm, $w = 2$ μs #$= 20$, $f = 0.25$ Hz	22	80	
		Casein–Tris buffer	$E = 15$ kV/cm, $w = 2$ μs #$= 98$, $f = 2$ Hz	50	0	
Alkaline phosphatase (bovine milk)	Batch electrodes: $d = 0.1$ cm	Raw milk	$E = 18.8$ kV/cm $w = 400$ μs #$= 70$	22	60	Barbosa-Canovas *et al.*, 1998; Castro *et al.*, 1994
		Milk 2% fat			60	
		Skim milk			65	
Alkaline phosphatase (bovine milk)	Batch electrodes: $d = 0.1$ cm	Raw milk	$E = 13.2$ kV/cm #$= 70$	44	96	Barbosa-Canovas, Qin, and Swanson, 1996
Alkaline phosphatase (bovine intestinal mucosa)	Batch electrodes: parallel, stainless steel $V = 49.5$ cm^3, $d = 0.3$ cm	Buffer (pH 9.8) + 1 M diethanolamine + 0.5 mM MgCl	Exp. decay with instant charge reversal $E = 80$ kV/cm, $w = 2$ μs #$= 30$, $f = 0.5$ Hz	20	5	Ho *et al.*, 1997
Alkaline phosphatase (milk)	Batch electrodes: parallel plain, carbon $V = 25$ cm^3, $d = 0.5$ cm	Raw milk	Exp. decay $E = 21.5$ kV/cm Total energy input up to 400 kJ/l	NR	0	Grahl and Märkl, 1996
Lactoperoxidase (milk)	Batch electrodes: parallel plain, carbon $V = 25$ cm^3, $d = 0.5$ cm	Raw milk	Exp. decay $E = 21.5$ kV/cm Total energy input up to 400 kJ/l	NR	0	Grahl and Märkl, 1996
Lipase (milk)	Batch electrodes: parallel plain, carbon $V = 25$ cm^3, $d = 0.5$ cm	Raw milk	Exp. decay $E = 21.5$ kV/cm Total energy input ≈400 kJ/l	NR	60	Grahl and Märkl, 1996
Lipase (wheat germ)	Batch electrodes: parallel, stainless steel $V = 49.5$ cm^3, $d = 0.3$ cm	Deionized water	Exp. decay with instant charge reversal $E = 87$ kV/cm, $w = 2$ μs #$= 30$, $f = 0.5$ Hz	20	85	Ho *et al.*, 1997

(*continued on next page*)

Table 1 (*continued*)

Enzyme	Operating conditions	Medium	pulse characteristics	*T* (°C)	% inact.	Ref.
Glucose-oxidase (*Aspergillus niger*)	Batch electrodes: parallel, stainless steel $V = 49.5$ cm^3, $d = 0.3$ cm	buffer (pH 5.1) + 50 mM Na-acetate	Exp. decay with instant charge reversal $E = 50$ kV/cm, $w = 2$ μs #$= 30$, $f = 0.5$ Hz	20	75	Ho *et al.*, 1997
α-Amylase (*Bacillus licheniformis*)	Batch electrodes: parallel, stainless steel $V = 49.5$ cm^3, $d = 0.3$ cm	Deionized water	Exp. decay with instant charge reversal $E = 80$ kV/cm, $w = 2$ μs #$= 30$, $f = 0.5$ Hz	20	85	Ho *et al.*, 1997
Peroxidase (soybean)	Batch electrodes: parallel, stainless steel $V = 49.5$ cm^3, $d = 0.3$ cm	Buffer (pH 6.0) + 100 mM K-phosphate	Exp. decay with instant charge reversal $E = 73$ kV/cm, $w = 2$ μs #$= 30$, $f = 0.5$ Hz	20	27	Ho *et al.*, 1997
Polyphenoloxidase (mushroom)	Batch electrodes: parallel, stainless steel $V = 49.5$ cm^3, $d = 0.3$ cm	Buffer (pH 6.5) + 50 mM K-phosphate	Exp. decay with instant charge reversal $E = 50$ kV/cm, $w = 2$ μs #$= 30$, $f = 0.5$ Hz	20	40	Ho *et al.*, 1997
Polyphenoloxidase (apple)	NR	McIlvaine buffer (pH 6.5) + 1M NaCl + 5% w/v PVPP	Bipolar exp. decay $E = 24$ kV/cm, $w = 20$ μs #$= 300$, $f =$ nr	15	97	Giner *et al.*, 1997
Polyphenoloxidase (peach)	NR	NR	Bipolar exp. decay $E = 24$ kV/cm, $w = 20$ μs #$= 400$, $f =$ nr	25	70	Giner, Gimeno, Rosell *et al.*, 1999
Polyphenoloxidase (pear)	NR	NR	Bipolar exp. decay $E = 22$ kV/cm, $w = 20$ μs #$= 300$, $f =$ nr	25	62	Giner, Gimeno, Rosell *et al.*, 1999
Papain (papaya)	Continuous (46.2 ml/min) electrodes: 4 co-field flow tubular TC intermediate cooling	1 mM EDTA	Square wave $E = 20$–50 kV/cm, $w = 4$ μs #$= 500$, $f = 1500$ Hz	35	85 (after 24 h storage at 4°C)	Yeom *et al.*, 1999
Pectinmethylesterase (orange)	Continuous (98 l/h) electrodes: co-field tubular stainless steel diam = 0.635 cm $d = 1$ cm	Freshly squeezed, frozen and thawed Valencia orange juice	$E = 35$ kV/cm, $w = 1.4$ μs $f = 600$ Hz Treatment time = 59 μs	60	88	Yeom *et al.*, 2000
Lactate dehydrogenase (beef heart)	Batch electrodes: parallel (horizontal) stainless steel $d = 0.5$ cm, $V = 5.7$ mL	20 mM K-phosphate buffer pH 7.2 273 ohm cm	Exp. decay $E = 31.6$ kV/cm, $w = 0.96$ μs #$= 200$, $f = 1.1$ Hz	30	0	Barsotti *et al.*, 2000

NR, not reported.

enzyme in bovine milk) in simulated milk ultrafiltrate (SMUF, pH 6.11, 0.056 M) at 100 μg plasmin/ml. A continuous flow through chamber with built-in heat exchangers in the parallel stainless steel electrodes (gap = 0.6 cm) was used to apply 10–50 pulses of 2 μs and an electric field in the range from 15 to 45 kV/cm at 0.1 Hz. An aliquot of 15 ml of SMUF was circulated in a closed loop system at a flow rate of 45 ml/min. Processing temperature was 10 or 15 °C measured using thermocouples attached to the surface of the heat exchanger. Plasmin activity decreased 90% after applying 50 pulses at both 30 and 45 kV/cm and 15 °C, which can be considered a nonthermal process compared to the same degree of plasmin inactivation at 40 °C for 15 min. Analysis of variance demonstrated that number of pulses, electric field and temperature were significant in the inactivation of plasmin at the 99% confidence level. HELP-treated plasmin solutions showed no significant changes in activity after 24 h storage at 4 °C, which suggested a permanent inactivation of the enzyme when exposed to pulsed electric fields. The inactivation mechanism of plasmin by HELP may, according to the authors, be explained in terms of charge and configuration changes due to the electrostatic nature of plasmin.

The same research group investigated the inactivation of an extracellular *protease* from *Pseudomonas fluorescens M3/6* by pulsed electric fields in three media (Vega-Mercado, Powers, Barbosa-Canovas, Swanson, & Luedecke, 1995; Vega-Mercado, Powers, Martin-Belloso, Luedecke, Barbosa-Canovas, & Swanson, 1997). A continuous treatment chamber ($V = 28.5$ mL, gap = 0.6 cm) was connected to a peristaltic pump to provide a constant flow rate of 500 ml/min. Inactivation of the protease varied with the intensity of the electric field, the number of pulses and the presence of substrate. An 80% reduction in protease activity was attained in tryptic soy broth with yeast extract after 20 pulses of 2 μs and 18 kV/cm at 0.25 Hz. When the protease was treated in sterilized skim milk, pulsed electric fields of 14 and 15 kV/cm and pulsing rates of 1 and 2 Hz achieved 40 and 60% inactivation after, respectively, 32 and 98 pulses of 2 μs. However, the protease treated in skim milk with a 25 kV/cm electric field at 0.6 Hz increased in proteolytic activity. Experiments using casein–Tris buffer instead of skim milk showed no significant inactivation of the protease. It was suggested by the authors that casein as a substrate protected the protease from inactivation by HELP. A complex formed between casein and protease probably provides a more stable conformation than free protease, thus preventing the enzyme from unfolding by the presence of casein. These observations indicate the potential influence of the environmental conditions on the stability of enzymes towards HELP processing and indicate the need for and the importance of experimental studies towards the effects of HELP on enzymes in real food systems, besides studies in model systems.

Castro, Swanson, Barbosa-Canovas, and Meyer (1994) investigated the effect of HELP-processing on *alkaline phosphatase* in three types of milk. The activity of alkaline phosphatase was reduced by 60% in raw and 2% fat milk and by 65% in non-fat milk after 70 pulses of 400 μs, which is a relatively high pulse width, and 18.8 kV/cm at 22 °C (Barbosa-Canovas *et al.*, 1998). While native alkaline phosphatase resists proteolysis by trypsin, it became sensitive after application of electric pulses. Using polyacrylamide gel electrophoresis of untreated and HELP-treated alkaline phosphatase samples, it was demonstrated that HELP does not hydrolyze alkaline phosphatase.

Ho, Mittal, and Cross (1997) studied the effects of pulsed electric fields on several enzymes in aqueous solution using a pilot scale pulser ($C = 0.12$ μF), generating exponential decay pulses of 2 μs with instant charge reversal at a fixed pulse frequency of 0.5 Hz. A horizontally positioned batch treatment chamber ($V = 49.5$ mL) consisted of two circular parallel stainless steel electrodes at a distance of 0.3 cm. The electric field strength was varied from 13 to 87 kV/cm. The electrical conductivity of the enzyme solutions varied from 0.4 to 16 mS/cm. The initial sample temperature was 20 °C. No change in temperature or pH was noticed when measured after HELP treatment.

Besides for lysozyme and pepsin, enzyme activity decreased with increase in field intensity, the degree of inactivation varying from enzyme to enzyme. Bovine *alkaline phosphatase*, dissolved in a buffer containing 1M diethanolamine and 0.5 mM magnesiumchloride (pH 9.8), was subjected to 30 pulses of 40, 60 and 80 kV/cm. Under all conditions tested, a slight 5% reduction in alkaline phosphatase activity could be noticed. *Peroxidase* from soybean, dissolved in a 0.1 M potassiumphosphate buffer (pH 6), was treated for 30 and 100 pulses at different field strengths. It was noticed that field strength had a significant influence on activity retention, whereas number of pulses had no effect at the 5% significance level. A moderate 30% reduction was obtained by applying 100 pulses of 73 kV/cm. *Polyphenoloxidase* from mushroom, dissolved in 50 mM potassiumphosphate buffer (pH 6.5), was subjected to 30 exponential decay pulses with instant charge reversal of 20, 50 and of 80 kV/cm. Polyphenoloxidase showed a moderate 40% reduction in enzyme activity after 30 pulses of 80 kV/cm, as was observed for peroxidase. *Lipase* from wheat germ, dissolved in de-ionized water, was subjected to 30 pulses of different field strength. Lipase activity decreased with increase in applied field strength. An 85% reduction in lipase activity was observed after 30 pulses of 87 kV/cm. *Glucose oxidase* from *Aspergillus niger*, dissolved in a buffer containing 50 mM sodiumacetaat (pH 5.1), was subjected to 30

pulses of field intensities in the range from 17 to 63 kV/cm. Increasing the field strength increased the degree of inactivation, although no further inactivation was obtained by increasing from 50 to 63 kV/cm. Glucose oxidase exhibited a reduction in activity of 75% after 30 pulses at 50 kV/cm. *Alpha-amylase* from *Bacillus licheniformis*, dissolved in de-ionized water, was subjected to 30 pulses of field intensities in the range from 20 kV/cm up to maximally 80 kV/cm. Again it could be noticed that a higher field strength resulted in a higher degree of α-amylase inactivation. 30 pulses of 80 kV/cm resulted in an activity decrease of 85%.

In case of lysozyme and pepsin, high electric field pulses (30 pulses at 13–80 kV/cm) appeared to have a stimulatory effect. *Lysozyme* inactivated up to 60% after 30 pulses of 13.5 kV/cm, but only up to 20% after 30 pulses at 50 kV/cm. From 50 kV/cm on, a further increase in electric field strength resulted in a lower residual lysozyme activity. Under all experimental HELP-conditions tested, *pepsin* activity increased as compared to an untreated sample. A maximum activity increase of 260% was observed after 30 pulses at 40 kV/cm.

Yeom, Zhang, and Dunne (1999) studied the inactivation of *papain*, a cysteine protease in papaya, by square wave high voltage pulses of 4 μs and 20–50 kV/cm at a frequency of 1.5 kHz. The number of pulses varied from 200 to 500. 150 ml of papain solution was continuously circulated at a flow rate of 0.77 ml/s through a series of four co-field flow tubular treatment chambers, with intermediate cooling at 10 °C between each pair of chambers. Temperature did not increase over 35 °C during HELP treatment. The HELP treatments tested had no significant effect on the papain activity at the 5% level when the activity was measured immediately after HELP treatment. However, significant irreversible papain inactivation was observed in HELP-treated papain after 24 and 48 h storage at 4 °C. A HELP treatment of 500 pulses in the range from 20 to 50 kV/cm resulted in about 85% decrease in papain activity determined after 24 h storage at 4 °C, regardless of the electric field strength applied. A higher number of pulses at 50 kV/cm caused higher reduction of papain activity. The authors tried to elucidate the mechanism of papain inactivation by HELP and concluded that oxidation of the papain active site, namely a cysteine residue, was not the major cause of inactivation, but that the inactivation of papain was related to the loss of α-helix structure in papain induced by HELP.

Giner and co-workers (Giner, Gimeno, Ortega, Barbosa-Canovas, & Martin-Belloso, 1999; Giner, Gimeno, Rosell, Barbosa-Canovas, & Martin-Belloso, 1999; Giner, Rauret-Arino, Barbosa-Canovas, & Martin-Belloso, 1997) investigated the effect of HELP treatments on *polyphenoloxidase* extracted from apples, peaches and pears. HELP treatments were carried out using a laboratory scale pulse generator, yielding exponential decay pulses, in mono- and bipolar mode, of 20–100 μs duration at 3–24 kV/cm. The temperature never exceeded 25 °C, though no specifications on the temperature measurement and control are given. Apple polyphenoloxidase was reduced by 97% after 300 bipolar pulses of 20 μs and 24 kV/cm. Increasing the field strength or the number of pulses, increased the degree of inactivation. For peach polyphenoloxidase, 70% reduction in activity was maximally achieved at 24 kV/cm in bipolar mode. At constant electric field strength, peach PPO inactivation by HELP could be related to the total treatment time by a first order kinetic model. For pear polyphenoloxidase a maximal reduction in activity of 62% was achieved after 300 pulses at 22.3 kV/cm. At constant electric field strength, pear PPO inactivation by HELP could be related to the total treatment time by a zero order kinetic model.

In their study on the effects of pulsed electric fields on the quality of orange juice, Yeom, Streaker, Zhang, and Min (2000) investigated the effect of HELP on *pectinmethylesterase* activity. The HELP equipment consisted of a series of co-field tubular stainless steel treatment chambers (diameter = 0.635 cm, gap = 1 cm) where Valencia orange juice was pumped through at a flow rate of 98 l/h. Pulses of 1.4 μs and 35 kV/cm were fired at 600 Hz. The total treatment time was 59 μs. The inlet temperature was 24 °C and the outlet temperature after the treatment chamber was 60 °C, recorded by RTD probes placed in short outlet T-pieces. The HELP treatment at 35 kV/cm for 59 μs decreased irreversibly 88% of pectinmethylesterase activity.

The observed effects of HELP on enzymes by different research groups appear to depend, besides on the enzyme, on the characteristics of the HELP system used and on the electric process parameters. Because the technology has not yet been standardized, HELP process and equipment specifications are varying from study to study, sometimes they are even not reported in detail, which makes a comparison of results between different studies very difficult, sometimes impossible and it should anyway be done very carefully, taking into account all of the experimental conditions such as electric field strength, number of pulses, wave shape, pulse width and batch, step-wise or continuous circulation processing. The importance of adequate temperature control/registration during HELP treatment, especially for studies in small batch treatment chambers, is often neglected so that no distinction can be made between thermal and high-voltage effects on enzymes. A theoretical temperature increase can be estimated from the total energy input, assuming that all the electrical energy delivered is dissipated as heat in the sample, which is, however, not the case. The rate of heat dissipation towards the environment depends to a large extent on the treatment chamber design and the thermal conductivities of the materials used.

Most of the work on enzyme inactivation by HELP has been carried out in model food systems such as distilled water, buffer solutions, simulated milk ultrafiltrate and other media but do not investigate enzyme inactivation in real food products. The aim of the present study was to determine the effects of HELP on several food quality related enzymes, increasing the complexity of the medium from simple model systems to real food products.

Materials and methods

Pulsed electric field system

Monopolar square-wave high electric field pulses were delivered to the sample using a flexible labscale HELP-equipment. The equipment consists of a high voltage charging unit (max. output of 20 kV and 20 mA, FUG, Germany) to charge a 2 μF capacitor (Maxwell, CA, USA). The capacitor is partially discharged over the treatment chamber and a 15 ohm series resistor (Cesiwid, NY, USA) by sending a TTL signal delivered from a function generator (Tektronix, AFG 320, OR, USA) to an IGBT on/off switch (Behlke, HTS101-80-FI, Germany), protected by two diodes (Behlke, FDA 100-80, Germany). The switching system is limited to 10 kV and 800 A. A 75 MHz high voltage probe (Tektronix, P6015A, OR, USA) connected to a 100 MHz digital oscilloscope (Tektronix, TDS 220, OR, USA) is used for data acquisition of the (voltage-time) signal at the treatment chamber.

Two types of treatment chambers were used: (i) two polished stainless steel cylinders (diameter of 1.14 cm, gap of 3 mm) connected by a plastic tubing with a fine hole to fill and remove the liquid enzymic sample, and (ii) a plastic cuvette with two parallel aluminium electrodes with a surface of 2 cm^2 and 2 mm distance between the electrodes. For each sample-process combination, a fibre optic temperature sensor (Nortech, Canada) was inserted in the treatment chamber during pulsing to have an idea on the temperature history of the sample, and hence to be able to take into account any thermal effects.

Enzyme systems

The sensitivity of several food related enzymes towards HELP processing has been investigated: lipoxygenase (LOX), polyphenoloxidase (PPO), peroxidase (POD), pectinmethylesterase (PME), alkaline phosphatase (ALP) and lactoperoxidase (LPER). The complexity of the medium has been systematically increased from distilled water over buffer solutions at different pH values and electric conductivities to real liquid food extracts or juices. Specifications on the selected enzymes, the mediums used, and the methods for activity reading are given in Table 2. For all experiments, the residual enzyme activity was measured within 10 min after the HELP treatment and compared to the enzyme activity of a non-treated enzyme sample.

Results and discussion

Model systems

The sensitivity of LOX, PPO, PME, and POD, dissolved in distilled water, towards HELP processing has been tested at different field strengths (10, 20 and 30 kV/cm), two different pulse widths (5 and 40 μs), two pulse frequencies (1 and 100 Hz) and a number of pulses from 1 to 1000, depending on the field strength applied (1–10 pulses at 30 kV/cm, 10–100 pulses at 20 kV/cm and 100–1000 pulses at 10 kV/cm).

Within the range of experimental conditions tested, none of the enzymes could be inactivated more than 10% by high voltage pulses. Except after longer processing times resulting from a high number of pulses at low pulse frequency (i.e. after 1000 pulses at 1 Hz), inactivation of LOX and PPO of 64 and 21%, respectively, was noticed. It has, however, been experimentally proven that the significant inactivations of LOX and PPO under these conditions were not caused by the high voltage pulses, but by an artefact, namely a small off-state current of the IGBT switch. This off-state current varies linearily with the voltage level charged on the capacitor, ranging from 15 μA at 500 V to 260 μA at 9000 V. Since LOX and PPO are both oxidoreductases that contain respectively iron and copper atoms at their active site, electrochemical reactions taking place at the electrode surfaces during the longer treatment times might cause the observed inactivation of the enzymes.

Influence of medium properties

Because of the stability of the selected food quality related enzymes towards HELP-processing, sensitisation of PPO, POD and LOX, has been endeavoured by changes in medium properties, in especially pH, protein content and medium conductivity.

The influence of pH on the stability of PPO and POD towards HELP processing has been investigated in the pH range from 4 to 7. HELP experiments were performed at field strengths from 2 to 31 kV/cm, pulse widths from 1 to 40 μs and the number of pulses varying from 10 to 1000. It was verified that the medium pH did not change after the HELP treatment. Changes in pH did not influence the stability of PPO towards HELP processing, whereas a pH decrease to 4 decreased the stability of POD towards HELP processing. POD was maximally inactivated to 60% after 1000 pulses of 1 μs at 1 Hz, regardless of the electric field strength. During storage at room temperature after the HELP treatment, a further decrease in POD activity was noticed up to 10% residual activity after 2 h storage at room temperature.

Several HELP-experiments were performed to investigate the influence of protein content, thereby changing the medium conductivity and the energy content per pulse, on the activity retention of LOX, PPO and POD. PPO and POD were dissolved in distilled water at con-

centrations between 1 and 5 mg/ml (conductivity varying from 10 to 90 μS/cm). In case of LOX, HELP-treatments were performed on samples with enzyme concentrations up to 15 mg/ml, yielding a conductivity of 1.07 mS/cm at room temperature. HELP-treatments applied were 100 pulses with pulse widths of 5 and 40 μs at 1 Hz and an electric field strength of 10 and 20 kV/cm. Within the range of enzyme concentrations tested no significant influence of the protein content on the HELP sensitivity of the enzymes tested was observed. The activity after HELP treatment was maximally reduced with 10%.

The influence of medium conductivity at constant protein content on the HELP stability of POD and PPO was evaluated in the range from 1 to 4 mS/cm. HELP experiments were performed at field strengths from 6 to 20 kV/cm, pulse widths from 1 μs (at 20 kV/cm) to 40 μs (at 6–8 kV/cm) and the number of pulses varying from 100 to 400. By increasing the medium conductivity from 1 to 4 mS/cm, the energy density per pulse increased from about 380 to 850 J/kg for 1 μs pulses at 20 kV/cm and from 1600 to 3950 J/kg for 40 μs pulses at 8 kV/cm. For both POD and for PPO, the medium conductivity did not influence the enzyme stability towards HELP processing, and a maximal reduction in enzyme activity of 15% was obtained.

Real food systems

Finally, the effect of HELP on the selected food relevant enzymes has been investigated in liquid real food products: LOX in pea juice, PPO in apple juice, pectinmethylesterase (PME) in orange juice, and lactoperoxidase (LPO) and alkaline phosphatase (AP) in milk.

The effect of HELP on lipoxygenase in green pea juice (squeezed and centrifuged for 15 min at 12,000 *g*) was investigated at field strengths from 2.5 to 20 kV/cm, a pulse width of 1 μs and number of pulses from 100 to 400 at a pulse frequency of 1 Hz. After the HELP conditions tested, lipoxygenase in pea juice was not inactivated.

The effect of HELP on polyphenoloxidase, extracted from apples, was investigated both in freshly squeezed apple juice, as well as in buffer solutions with the same pH and electrical conductivity as freshly squeezed apple

Table 2. Overview of enzymic systems tested and activity reading methods

Enzyme	Origin	Medium	Conductivity at room temperature	Activity assay
Lipoxygenase	Soybean (Sigma)	Distilled water 53,600 Units/ml	60–80 μS/cm	Spectrophotometric at 234 nm and 25°C substrate: linoleic acid
	Green peas	Green pea juice	5.95 mS/cm	Polarographic at pH 6 and 25°C substrate: linoleic acid
Polyphenoloxidase	Mushroom (Sigma)	Distilled water 352 Units/ml	12 μS/cm	Spectrophotometric at 411 nm and 23°C substrate: catechol
		Phosphate buffer (pH 6) 352 Units/ml	1–4 mS/cm	
		Mc Ilvaine buffer pH 4–5–7	2 mS/cm	
	Apples (cv. Golden delicious)	Phosphate buffer (pH 6)	1.02 mS/cm	Spectrophotometric at 411 nm and 23°C substrate: catechol
		Mc Ilvaine buffer (pH 3.8)	2.12 mS/cm	
		Apple juice (pH 3.8)	2.12 mS/cm	
Pectinmethylesterase	Tomato (Sigma)	Distilled water 40.2 Units/ml	50 μS/cm	Titrimetric at pH 7 and 22°C substrate: 0.35% apple pectin
	Orange peel (Sigma)	Distilled water 105 Units/ml	1.08 mS/cm	Titrimetric at pH 7 and 22°C substrate: 0.35% apple pectin
		Mc Ilvaine buffer (pH 3.7)	4 mS/cm	
		Orange juice (pH 3.7)	4 mS/cm	
Peroxidase	Horseradish (Sigma)	Distilled water 9.6 Units/mL	6.5 μS/cm	Spectrophotometric at 510 nm and 25°C substrate: phenol + hydrogen peroxide + 4-amino-antipyrine
		Phosphate buffer (pH 7) 352 Units/ml	1–4 mS/cm	
		Mc Ilvaine buffer zpH 4–5–7	2 mS/cm	
alkaline phosphatase	Bovine milk	Raw milk	4.46 mS/cm	Spectrophotometric at 425 nm substrate: *p*-nitrophenylphosphate
lactoperoxidase	Bovine milk	Raw milk	4.46 mS/cm	Spectrophotometric at 412 nm substrate: ABTS + hydrogen peroxide

juice. Samples were treated for up to 1000 pulses of 1 μs and 31 kV/cm or of 40 μs and 7 kV/cm at 1 Hz. Even treatments of 1000 pulses of 1 μs at 31 kV/cm and 1 Hz did not result in more than 10% inactivation of polyphenoloxidase. By increasing pulse frequency up to 10 Hz, PPO could be inactivated (e.g. 32% activity decrease after 1000 pulses of 40 μs at 7 kV/cm and 10 Hz). However, the higher pulse frequency resulted in a temperature increase of the treated solution up to 60 °C and the observed inactivation of PPO was caused by thermal effects. HELP treatment of freshly squeezed apple juice resulted mostly in increased polyphenoloxidase activity in the juice after the HELP treatment which can probably be explained by the release of PPO from cells, who were intact before the treatment and damaged by the application of HELP.

In analogy with apple polyphenoloxidase, pectinmethylesterase was extracted from oranges and dissolved in freshly squeezed orange juice and in buffer solutions of the same pH and electrical conductivity as orange juice. Orange pectinmethylesterase could in none of the systems tested be inactivated to more than 10% by a HELP treatment (e.g. 1000 pulses of 1 μs at 1 or 2 Hz and field strength up to 35 kV/cm). HELP treatment of orange juice resulted mostly in an increased pectinmethylesterase activity, probably due to cell permeabilisation and release of intracellular pectinmethylesterase.

The susceptibility of alkaline phosphatase in raw milk towards HELP processing was evaluated in the range from 6.7 to 20 kV/cm by subjecting raw milk samples to 200 pulses of 2 μs at 1 Hz. At the conditions tested, no decrease in alkaline phosphatase activity after HELP treatment was noticed. Increasing the pulse width of the 200 pulses from 1 μs to 40 μs at 10 kV/cm, resulted in a 74% inactivation of alkaline phosphatase in raw milk. Recording of the temperature evolution during the HELP treatment, revealed that under these HELP conditions, the sample temperature increased up to 70°C. Taking into account the thermostability characteristics of alkaline phosphatase in raw milk, it can be concluded that the observed inactivation after HELP treatment can be mainly attributed to thermal effects. When no temperature increase during HELP processing was observed, alkaline phosphatase activity was not decreased due to HELP processing.

In addition to alkaline phosphatase, it was tested whether lactoperoxidase, a natural antimicrobial component in raw milk, was inactivated by HELP processing. Raw milk was subjected to up to 100 pulses of 5 μs and 19 kV/cm at 1Hz and tested for lactoperoxidase activity. Lactoperoxidase in raw milk could not be inactivated under the HELP-conditions tested. Even after energy inputs of 500 kJ/kg, no decrease in lactoperoxidase activity was observed.

Conclusions

Under the HELP conditions tested, the selected enzymes, namely lipoxygenase, polyphenoloxidase, pectin-methylesterase, and peroxidase in distilled water are resistant towards HELP processing. Because of this HELP stability, sensitisation of the enzymes has been endeavoured by changes in medium properties in terms of pH, protein content, and medium conductivity. None of these changes could decrease the HELP stability of the enzymes. Afterwards, the research on the effect of HELP on enzymes has evolved from simple model systems to more complex liquid dairy or fruit and vegetable products. In case significant inactivations in enzyme activity were noticed, it could be concluded based on the temperature registration during and immediately after HELP processing, that these inactivations are caused by thermal effects. When it was ascertained that their was no temperature increase during HELP processing, none of the enzyme activities could be decreased significantly by HELP processing.

As compared to inactivation studies of vegetative micro-organisms by HELP, enzymes are generally more resistant to electric pulses than vegetative microorganisms. The limited effects of HELP on food related enzymes can represent a restriction for the use of HELP as food preservation method that can be overcome by combining HELP with other preservation factors (e.g. mild heat treatment, cooled storage) to control enzymatic activity during storage. On the other hand, knowing that some enzymes are positively used in the food industry, HELP can be applied for non-thermal microbial decontamination not at the expense of biological activity.

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References

Barbosa-Canovas, G. V., Pothakamury, U. R., Palou, E., & Swanson, B. G. (1998). Biological effects and applications of pulsed electric fields for the preservation of foods. In G. V. Barbosa-Canovas, U. R. Pothakamury, E. Palou, & B. G. Swanson (Eds.), *Nonthermal preservation of foods* (pp. 73–112). New York: Marcel Dekker.

Barbosa-Canovas, G. V., Qin, B. L., & Swanson, B. G. (1996). Biological effects induces by pulsed electric fields of high intensity. In M. Rodrigo, A. Martinez, S. M. Fiszman, C. Rodrigo, & A. Mateu (Eds.), *Tecnologias avanzadas en esterilizacion y seguridad de alimentos y otros productos* (pp. 151–165). Spain: Grafo Impresores, S.L.

Barsotti, L., Dumay, E., Mu, T. H., Diaz, M. D. F., & Cheftel, J. C. (2001). Effects of high voltage electric pulses on protein-based food constituents and structures. *Trends in Food Science and Technology*, this issue.

Castro, A. J., Swanson, B. G., Barbosa-Canovas, G. V., & Meyer, R. (1994). *Pulsed electric field modification of milk alkaline phosphatase activity*. PhD thesis, Washington State University.

Giner, J., Gimeno, V., Ortega, M., Barbosa-Canovas, G. V., & Martin-Belloso, O. (1999). Inhibition of peach polyphenoloxidase by pulsed electric fields. In *Proceedings of European Conference on Emerging Food Science and Nutrition*, p. 109, Tampere, Finland, 22–24 November.

Giner, J., Gimeno, V., Rosell, J., Barbosa-Canovas, G. V., & Martin-Belloso, O. (1999). Inhibition of pear polyphenoloxidase by pulsed electric fields. In *Proceedings of European Conference on Emerging Food Science and Nutrition*, p. 110, Tampere, Finland, 22–24 November.

Giner, J., Rauret-Arino, A., Barbosa-Canovas, G. V., & Martin-Belloso, O. (1997). Inactivation of polyphenoloxidase by pulsed electric fields. In *Proceedings IFT Annual Meeting*, p. 19, Orlando, USA.

Grahl, T., & Märkl, H. (1996). Killing of microorganisms by pulsed electric fields. *Appl. Microbiol. Biotechnol., 45*, 148–157.

Ho, S. Y., Mittal, G. S., & Cross, J. D. (1997). Effects of high field electric pulses on the activity of selected enzymes. *J. Food Eng., 31*, 69–84.

Qin, B. L., Pothakamury, U. R., Barbosa-Canovas, G. V., & Swanson, B. G. (1996). Nonthermal pasteurization of liquid foods using high-intensity pulsed electric fields. *Critical Reviews in Food Science and Nutrition, 36*, 603–627.

Vega-Mercado, H., Powers, J. R., Barbosa-Canovas, G. V., & Swanson, B. G. (1995). Plasmin inactivation with pulsed electric fields. *Journal of Food Sci., 60*, 1143–1146.

Vega-Mercado, H., Powers, J. R., Barbosa-Canovas, G. V., Swanson, B. G., & Luedecke, L. (1995). Inactivation of a protease from *Pseudomonas fluorescens* M3/6 using high voltage pulsed electric fields. In *Proceedings IFT Annual Meeting*, p. 267, California, USA.

Vega-Mercado, H., Powers, J. R., Martin-Belloso, O., Luedecke, L., Barbosa-Canovas, G. V., & Swanson, B. G. (1997). Effect of pulsed electric fields on the susceptibility of proteins to proteolysis and the inactivation of an extracellular protease from *Pseudomonas fluorescens* M3/6. In R. Jowitt, ed. *Engineering & Food at ICEF 7: Part I* (pp. C73–C76). Sheffield Academic Press Ltd, Sheffield, UK.

Wouters, P. C., & Smelt, J. P. P. M. (1997). Inactivation of microorganisms with pulsed electric fields: potential for food preservation. *Food Biotechnology, 11*, 193–229.

Yeom, H. W., Streaker, C. B., Zhang, Q. H., & Min, D. B. (2000). Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurisation. *Journal of Agricultural and Food Chemistry, 48*, 4597–4605.

Yeom, H. W., Zhang, Q. H., & Dunne, C. P. (1999). Inactivation of papain by pulsed electric fields in a continuous system. *Food Chemistry, 67*, 53–59.