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Comparative Study of Pulsed Electric Field and Thermal Processing of Apple Juice with Particular Consideration of Juice Quality and Enzyme Deactivation

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As an alternative to thermal pasteurization, pulsed electric fields (PEF) were applied to apple juices on laboratory and pilot plant scale, investigating the effects on juice quality. PEF application still falls under the EU Novel Food Regulation. Consequently, extensive investigation of quality parameters is a prerequisite to prove substantial equivalence of juices resulting from the novel process and conventional production, respectively. Juice composition was not affected by PEF treatment. However, browning of the juices provided evidence of residual enzyme activities. On laboratory scale, complete deactivation of peroxidase (POD) and polyphenoloxidase (PPO) was achieved when PEF treatment and preheating of the juices to 60 °C were combined. Under these conditions, a synergistic effect of heat and PEF was observed. On pilot plant scale, maximum PPO deactivation of 48% was achieved when the juices were preheated to 40 °C and PEF-treated at 30 kV/cm (100 kJ/kg). Thus, minimally processed juices resulted from PEF processing, when applied without additional conventional thermal preservation. Since this product type was characterized by residual native enzyme activities and nondetectable levels of 5-hydroxymethylfurfural, also when preheating up to 40 °C was included, it ranged between fresh and pasteurized juices regarding consumers' expectation of freshness and shelf life. Consistent with comparable iron contents among all juice samples, no electrode corrosion was observed under the PEF conditions applied.

KEYWORDS: Apple juice; juice quality; peroxidase; polyphenoloxidase; preservation; pulsed electric fields (PEF)

INTRODUCTION

The growing demand for minimally processed foods has supported the interest in nonthermal processing methods, such as γ (*1*), ultraviolet (*2*), or microwave (*3*) irradiation, as well as dense phase CO_2 (*4, 5*), high pressure (*6, 7*), and electrodialysis (*8*) processes. Treatment with pulsed electric fields (PEF) for preservation of liquid food is one of the most promising technologies that might replace traditional thermal pasteurization (*9, 10*). So far, studies of PEF application in juice technology have mainly focused on microorganisms and the deactivation of the microbial flora such as mesophilic bacteria, molds, and yeasts (*11*−*15*). The lethal effect of PEF is based on the irreversible formation of pores in the cell membranes. It mainly depends on electric field strength, pulse duration, and treatment temperature.

Because PEF treatment is a novel process for food preservation, assessment of the substantial equivalence to conventionally processed juices is a prerequisite. While PEF-treated products

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are already available on the US market (*16*), their legal status in Europe is still a matter of pending discussion. The composition of juices originating from PEF treatment of apple mash has recently been investigated (*17, 18*), and some data are also available for PEF application in food preservation (*19*−*25*). As observed by Qin et al. (*26*), the shelf life of apple juice after PEF treatment (36 kV/cm, 25 μs) was 3 weeks and juices did not show apparent changes in their physicochemical and sensory properties due to PEF application. Similarly, PEF processing (40 kV/cm, 97 ms) was suitable to inactivate endogenous microorganisms, thus extending the shelf life of orange juice while reducing the loss of major quality-determining parameters, such as ascorbic acid, flavor, and color (*27*). This resulted in a better acceptance relative to thermally processed orange juice. However, with respect to the substantial equivalence of PEF and thermally preserved juices, as required by EC Regulation no. 258/97, there is still a need for more detailed comparative data.

Furthermore, thermal preservation not only reduces microbial loads but also includes deactivation of enzymes. In apple juices, enzymes catalyzing browning reactions, such as polyphenoloxidase (PPO) and peroxidase (POD), as well as enzymes affecting viscosity and cloud stability, such as pectinmethylesterase and polygalacturonase, are of particular interest. However, reports of the effects of PEF on enzymes differ widely and are even contradictory, with enzyme deactivation rates ranging between 0% and 97% (*28*). Since experimental data concerning energy input and other treatment conditions are not always fully available, a comparison of the studies is difficult. The deactivation effect has recently been associated with conformational changes of the enzymes, such as reduction of the α-helix fractions (*29*). While most investigations on deactivation kinetics dealt with isolated enzymes on a laboratory scale (*29*−*32*), studies considering the complex food matrix of real-life samples under semi-industrial conditions are scarce (*22, 23*). However, such studies would be needed to establish PEF treatment as a nonthermal preservation process suitable for the food industry.

Therefore, the objective of this work was to compare the effects of PEF treatment and conventional thermal pasteurization of apple juices both on laboratory and pilot-plant scale. One focus was on juice composition and physicochemical quality, since substantial equivalence of the juices has to be proved. Second, the shelf life extending effect of juice pasteurization was to be evaluated in terms of residual activities of PPO and POD. Finally, secondary technical effects implied by PEF treatments, such as electrode corrosion and radical formation, were to be considered.

MATERIALS AND METHODS

Solvents and Reagents. Tropolone, L-proline, and 4-methylcatechol were purchased from Fluka (Buchs, Switzerland) and polyelectrolyte Poly-Dadmac solution from Mütek (Herrsching, Germany). The reference compounds for HPLC analysis, (−)-epicatechin, *p*-coumaric acid, chlorogenic acid, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-rutinoside, procyanidin B2, phloretin, and phloridzin dihydrate, were obtained from Extrasynthèse (Lyon, France). Standard amino acids were from Phenomenex (Torrance, CA). As references for minerals, certified mixed standard solutions from CPI International (Amsterdam, The Netherlands) were used. All other solvents and reagents were of analytical or HPLC grade and were purchased from VWR (Darmstadt, Germany). Deionized water was used for all analyses.

PEF System. PEF treatments were carried out in a continuous PEF system designed and constructed at the Technical University of Berlin. A pulse modulation system of ScandiNova (Uppsala, Sweden) was used to generate rectangular pulses. Pulse width was set in the range of 3 to 8 μs. Two different treatment chambers were applied for continuous laboratory and pilot plant scale experiments, respectively. In both cases, colinear configuration was realized by using a central high-voltage electrode and two grounded electrodes made of stainless steel and separated by acetal isolators (*33*). The electrode gap of the PEF chamber used for laboratory experiments was 4 mm, while that of the chamber applied on a pilot plant scale was 10 mm. The apple juice was conveyed through a central drilling with an internal diameter of 4 and 10 mm, respectively.

Juice Processing on Laboratory Scale. For enzyme deactivation studies on laboratory scale, apples were dejuiced with a Profi Juicer (Gastroback, Hollenstedt, Germany) immediately prior to PEF treatment. Apples cv. "Braeburn", purchased in July 2006 from a grocery in Berlin, were used due to the limited availability of cider varieties. For each processing temperature, ∼2 L of juice was freshly produced. For the studies of PPO and POD deactivation, it was important to exclude protein precipitation as a side effect of polyphenol oxidation. Therefore, ascorbic acid was added as antioxidant in a dosage of 500 mg/kg, which is 2.5-fold higher than the generally applied dosage in the fruit juice industry. Due to the low endogenous POD activities of the apples, a fungal POD (Sigma, St. Louis, MO) was added (>850 U/L) to surpass the detection limit. At this stage of processing, an aliquot of fresh juice (∼200 mL of the last juice batch) was immediately subjected to thermal deactivation studies on laboratory scale, as described below. For PEF processing of each batch, the juice was gently stirred and subsequently pumped through a coil by means of a peristaltic hose pump (model 323DU, Watson Marlow Bredel Pumps, Cornwall, England). The coil was heated in an oil bath (Haake Messtechnik, Karlsruhe, Germany) to adjust the inlet temperature T_{in} prior to the PEF treatment. T_{in} was varied between 20 and 60 °C. Juice temperature was monitored at the exit of the PEF treatment chamber, using a fiber optic thermometer FT 1110C (Takaoka Electric, Tokyo, Japan). The juices were cooled immediately after the PEF treatment by passing a coil in a water bath (model cc3-k6, Huber Kältemaschinen, Offenburg, Germany). Samples were frozen in liquid nitrogen and stored at −80 °C until analysis of enzyme activities. The flow rate during PEF processing was set at 5 L/h. Rectangular pulses with a pulse width of 3 μs were used. Electric field strengths of 15, 25, and 35 kV/cm, respectively, were applied. Depending on the frequency settings, energy inputs ranged between 8.5 and 65.5 kJ/kg.

Juice Processing on Pilot Plant Scale. For PEF preservation on a pilot plant scale, apple juice freshly prepared from fruits cv. "Winterrambur" harvested in Oktober 2006 (Weeg, Glantal, Germany), i.e., a typical raw material in juice processing, was used. Per batch, 220 kg of apples were dejuiced, using a hydraulic horizontal filter press system HPL 200 (Bucher-Guyer AG Foodtech, Niederweningen, Switzerland). Prior to preservation, coarse particles were removed with a separator (SAVR 3036, Westfalia, Oelde, Germany). Mimicking industrial juice processing, ascorbic acid was added in a typical dosage of 200 mg/L to prevent browning resulting from oxidative reactions. Juice samples collected at this stage of processing were used as control for each batch (control a, b).

Prior to each processing cycle, the PEF system was sanitized with 3% sodium hydroxide solution at 60 °C, followed by rinsing with a peracetic acid−hydrogen peroxide solution (5 g/kg) (TensidChemie, Muggensturm, Germany) at ambient temperature. The parameters of the PEF treatments are listed in **Table 1**. For each variant, approximately 50 L of juice was used. Variant 4 served as a multipass experiment, where the same juice sample passed the treatment chamber twice. The inlet temperature (T_{in}) of each processing variant was adjusted, using a heating coil. When T_{in} had been reached, the juice was pumped through the PEF chamber and subsequently cooled in a plate heat exchanger. The juice temperature at the exit of the PEF chamber (T_{out}) was monitored to determine the temperature rise caused by dissipation of electric energy. The juices were filled in sterilized glass bottles (0.5 L). Since aseptic packaging could not be realized, storage of the juices at 4 °C was limited to 10 days to prevent microbial spoilage. Samples

Table 1. Process Parameters of Pulsed Electric Field (PEF) Treatments for Apple Juice Preservation on Pilot Plant Scale[a]

PEF variants	T_{in} [°C]	E [kV/cm]	V [kg/h]	I [A]	W_{pulse} [J]	f [Hz]	W_{total} [kJ/kg]	T_{out} [°C]
PEF 1	20	30	65	120	9	260	130	51
PEF 2	30	30	50	132	9.65	175	121	59
PEF 3	40	30	62.5	142	10.1	173	100	63
PEF 4a	30	30	50	133	9.6	143	100	54
PEF 4b	30	30	55	126	9.2	170	100	54

[a] Process variant PEF 4 was a multipass experiment comprising two PEF cycles (4a/4b). T_{in}: inlet temperature. E: electric field strength. V: flow rate. I: electric current. W_{pulse}: energy per pulse. f: frequency. W_{total}: total energy input. T_{out}: outlet temperature.

were collected immediately after processing (day 0) and after 1, 3, and 10 days, frozen in liquid nitrogen, and stored at −80 °C until analysis of enzyme activities and juice quality.

Determination of Peroxidase and Polyphenoloxidase Activities. POD activity was determined as described earlier (*34*) with minor modifications. Aliquots of 0.4 mL of apple juice were added to 1.1 mL of McIlvaine buffer (pH 6.5) that consisted of 30% 0.1 M citric acid and 70% 0.2 M disodium phosphate and contained 12 mmol/L tropolone and 3.3 mmol/L H_2O_2. For quantification of the enzyme activity, the increase in absorbance of the yellow reaction product was recorded at 418 nm ($\varepsilon = 2075$ L mol^{-1} cm^{-1}) every 15 s for 15 min at 25 °C. POD activity was calculated from the slope within the initial linear range of the absorbance−time curve, partly occurring after a short lag-phase. For blank correction, the slopes of a sample blank (0.4 mL water instead of juice) and a reagent blank (1.1 mL McIlvaine buffer without tropolone and H_2O_2) were subtracted. Enzyme activity (EA) was expressed in μkat/L of juice. The residual activity (RA) was calculated according to eq 1, where EA_0 and EA_t were the activities before and after the preservation step, respectively.

$$RA = (EA_t/EA_0) \times 100\% \quad (1)$$

PPO activity was determined by using a slightly modified protocol described previously (*34*). The reaction mixture consisted of 1.5 mL of reaction buffer [0.5 mmol/L sodium dodecyl sulfate in McIlvaine buffer (pH 6.5) consisting of 30% 0.1 mol/L citric acid and 70% 0.2 M disodium phosphate], 0.2 mL of L-proline in reaction buffer, and 0.1 mL of apple juice. The reaction was started by adding 0.2 mL of 25 mM 4-methylcatechol into the reaction buffer. The formation of the pink proline−catechol adduct was recorded by absorbance measurements at 525 nm ($\varepsilon = 1550$ L mol^{-1} cm^{-1}) every 15 s for 6 min at 25 °C. PPO activity was quantified as described above for POD.

Examination of Thermal Deactivation of Peroxidase and Polyphenoloxidase. To investigate enzyme deactivation caused by thermal impact during PEF application, freshly produced juice samples, placed into glass capillaries with an inner diameter of 0.98 mm (Kleinfeld Labortechnik, Stötefeld, Germany), were heated in a water bath at temperatures between 40 and 70 °C for 5 to 120 s (*35*), then cooled immediately in ice−water. From the enzyme activities, decimal deactivation time (D-value) at temperature T and the temperature increase implying decimal reduction of D (z-value) were calculated for PPO and POD according to eqs 2 and 3 based on a conventional first-order model, with EA_t being the enzyme activity in μkat/L at time t, EA_0 the initial enzyme activity in μkat/L, t the treatment time in min, z in °C, and T_1 and T_2 the temperatures in °C, corresponding to decimal reduction times D_1 and D_2 in min.

$$D = t/(\log EA_0 - \log EA_t) \quad (2)$$

$$z = (T_2 - T_1)/(\log D_1 - \log D_2) \quad (3)$$

Analysis of Composition and Physicochemical Quality of the Fruit Juices. Total soluble solids (TSS), pH value, density, and total acidity (TA), the latter calculated as citric acid, were determined according to IFU methods (*36*). L-Malic acid, glucose, fructose, sucrose, and sorbitol were quantified by enzymatic-spectrophotometric methods, using respective test kits (r-biopharm, Darmstadt, Germany).

For the determination of sodium, potassium, magnesium, calcium, and iron contents, the samples were centrifuged at 50000 × g for 60 min to remove coarse particles, followed by dilution with 4 mol/L nitric acid (1:1, v/v) to a final concentration of 2 mol/L nitric acid. After external calibration with certified mixed standard solutions, minerals were measured by inductively coupled plasma optical emission spectrometry (ICP-OES), using radial torch viewing (VISTA Pro radial, Varian, Mulgrave, Australia). A concentric glass nebulizer and a double-pass glass cyclonic spray chamber were used for sample injection.

Contents of individual phenolic compounds and 5-hydroxymethylfurfural (HMF) were determined by HPLC as described previously (*17*). The Folin−Ciocalteu reagent was used for quantifying total phenolics photometrically, the TEAC and the FRAP assays for the determination of antioxidant capacity, as described earlier (*17, 18*).

On the basis of external calibration, free amino acids were quantified by gas chromatography with flame ionization detection (FID), using the EZ:faast cleanup and derivatization kit (Phenomenex, Torrance, CA, USA) (*37*). The juices were centrifuged at 14500 rpm for 10 min before cleanup and derivatization. GC analysis was carried out on a CP 9001 gas chromatograph (Chrompack, Middleburg, The Netherlands), with the FID operated at 320 °C. The split ratio was 1:15 and helium was used as carrier gas. The oven temperature was increased from 110 to 320 °C within 7 min, followed by 1 min of isothermal hold.

Proteins of PEF-treated apple juices, including controls and thermally pasteurized ones, were analyzed by SDS-PAGE, using a Multiphore electrophoresis system (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Juice samples were centrifuged (10 min, 14500 rpm). The supernatants were mixed (1:3, v/v) with a nonreducing sample buffer (pH 7.5, 50 mM Tris-HCl, 35 mM SDS, 0.01% bromophenol blue) and heated at 95 °C for 5 min. Aliquots of 20 μL were applied on a gradient gel 8−18% (GE Healthcare Bio-Sciences AB). Electrode buffer strips (GE Healthcare Bio-Sciences AB) were used. Proteins were detected by silver staining (*38*) with the addition of a marker SM0661 (Fermentas, Burlington, Ontario, Canada) to identify the molecular weights of the protein bands.

Color analysis (CIE $L^*a^*b^*C^*h^\circ$) was performed by using a UV/vis spectrometer Lambda 20 (Perkin-Elmer, Überlingen, Germany), controlled by the UVWinLab V 2.85.04 and Wincol V 2.05 color softwares (Perkin-Elmer Instruments, Norwalk, CT). The cloudy juices were centrifuged (14500 rpm, 10 min) before analysis. Illuminant D_{65} and a 10° observer angle were set. Furthermore, browning indices (BI) of the centrifuged juice samples were calculated according to eq 4 from their absorbance at 420 (A_{420nm}) and 700 nm (A_{700nm}), respectively.

$$BI = A_{420nm} - A_{700nm} \quad (4)$$

The charge levels of preserved apple juices and controls were measured with a PCD 02 particle charge detector (Mütek) that was controlled by the Mütek PCD titration 1.2 software and combined with a Titrino 702 SM (Metrohm, Herisau, Switzerland). Aliquots of 2 mL of juice and 10 mL of dilution medium were titrated with a polyelectrolyte solution (0.001 mol/L Poly-Dadmac). As blank, the dilution medium, consisting of 24 g/L glucose, 64 g/L fructose, 17 g/L sucrose, 7.4 g/L malic acid, and 0.09 g/L citric acid, and adjusted to pH 3.5, was used. The specific particle charge Q (in C/L) was calculated according to eq 5 from the difference ΔV of the polyelectrolyte volumes V (in mL) required for sample and blank titration, the polyelectrolyte concentration c (in mol/L), the sample volume v (in mL), and the Faraday constant ($F = 96485.553$ C/mol).

$$Q = \frac{\Delta V c F}{v} \quad (5)$$

Statistical Analysis. By using the GLM procedure of SAS 9.1 (SAS Institute, Cary, NC, USA), the data were subjected to analysis of variance (one-way ANOVA) and subsequent multiple pairwise comparison of means (Tukey or Duncan tests) to identify significant differences ($p \leq 0.05$) between treatments.

RESULTS AND DISCUSSION

Overall Juice Composition following PEF Treatments on Pilot Plant Scale. TSS, TA, pH, density, and the contents of

Table 2. Overall Composition of Fresh Juices (controls) and Those Preserved by Pulsed Electric Fields (PEF) and Conventional Pasteurization (past.)[a]

variants	pH	TSS [°Brix]	density [g/cm^3]	TA [g/L]	sucrose [g/L]	glucose [g/L]	fructose [g/L]	L-malic acid [g/L]	D-sorbitol [g/L]
control a	3.54[b]	12.63[b]	1.05[b]	4.90[b]	42.01[b]	13.71[b]	53.39[b]	5.98[b]	6.55[b]
PEF 2	3.53	12.63	1.05	4.87	41.41	13.92	53.00	6.25	6.72
PEF 4a	3.53	12.65	1.05	4.88	42.96	14.38	54.24	6.10	6.70
PEF 4b	3.53	12.63	1.05	4.87	42.06	14.18	53.61	5.80	6.72
control b	3.55	12.66	1.05	4.85	38.95	13.94	53.60	6.11	6.43
PEF 1	3.54	12.69	1.05	4.85	39.29	14.11	54.05	6.44	6.20
PEF 3	3.54	12.38	1.05	4.88	39.29	14.11	53.68	6.40	6.42
past.	3.50	12.76	1.05	4.96	39.90	15.65	56.07	6.38	6.67

variants	Na [mg/L]	K [mg/L]	Mg [mg/L]	Ca [mg/L]	Fe [mg/L]
control a	8.7[c] ± 0.0 b	617.3[c] ± 3.3 ab	20.8[c] ± 0.1 a	16.0[c] ± 0.2 b	0.21[c] ± 0.02 a
PEF 2	18.3 ± 0.7 a	621.6 ± 0.4 a	21.1 ± 0.0 a	16.5 ± 0.1 ab	0.22 ± 0.01 a
PEF 4a	9.1 ± 0.1 c	613.8 ± 1.2 ab	21.0 ± 0.1 a	16.4 ± 0.1 ab	0.28 ± 0.04 a
PEF 4b	11.6 ± 0.1 a	606.3 ± 3.0 b	20.9 ± 0.1 a	16.9 ± 0.1 a	0.23 ± 0.01 a
control b	4.0 ± 0.7 x	625.8 ± 3.8 x	20.4 ± 0.1 x	18.4 ± 0.2 xy	0.17 ± 0.00 z
PEF 1	3.2 ± 0.0 x	618.1 ± 3.4 x	20.3 ± 0.1 x	19.0 ± 0.3 x	0.17 ± 0.00 z
PEF 3	4.1 ± 0.0 x	620.6 ± 0.5 x	20.4 ± 0.0 x	18.8 ± 0.1 xy	0.24 ± 0.00 y
past.	2.7 ± 0.0 x	616.3 ± 4.3 x	20.4 ± 0.1 x	17.9 ± 0.1 y	0.25 ± 0.00 x

[a] Process parameters of PEF variants 1−4, cf. Table 1. [b] Means of two determinations (CV < 5%). TSS: total soluble solids. TA: total acidity. [c] Means and standard errors of two determinations. Values with the same letters a−c and x−z, respectively, within one row (vertical) are not significantly different ($p < 0.05$). Na: sodium. K: potassium. Mg: magnesium. Ca: calcium. Fe: iron.

Table 3. Amino Acid Composition of Fresh Juices (control) and Those Preserved by Pulsed Electric Fields (PEF) and Conventional Pasteurization (past.)[a]

	amino acid content [mg/L]								
	aspartic acid	asparagine	glutamic acid	serine	threonine	proline	valine	glycine	isoleucine
control a	107.2[b] ± 1.58 a	90.9 ± 0.61 a	91.4 ± 1.54 a	15.4 ± 0.47 a	5.1 ± 0.08 a	5.4 ± 0.08a	2.5 ± 0.12a	1.1 ± 0.04a	2.5 ± 0.01a
PEF 2	97.0 ± 12.66 a	93.5 ± 0.44 a	83.0 ± 12.30 a	15.9 ± 0.16 a	5.2 ± 0.04 a	5.4 ± 0.15a	2.6 ± 0.06a	1.2 ± 0.10a	2.6 ± 0.03a
PEF 4a	101.4 ± 2.17 a	86.0 ± 2.79 a	91.7 ± 2.01 a	15.3 ± 0.61 a	5.3 ± 0.18 a	5.2 ± 0.00a	2.5 ± 0.05a	1.2 ± 0.09a	2.3 ± 0.04b
PEF 4b	106.6 ± 1.73 a	94.0 ± 1.56 a	96.8 ± 1.46 a	15.5 ± 0.01 a	4.9 ± 0.01 a	5.2 ± 0.03a	2.5 ± 0.01a	1.1 ± 0.06a	2.3 ± 0.03b
control b	77.9 ± 1.33 x	48.2 ± 0.63 x	66.0 ± 3.84 y	11.8 ± 0.39 x	4.6 ± 0.06 x	5.0 ± 0.01x	2.3 ± 0.09x	0.9 ± 0.08x	2.5 ± 0.03x
PEF 1	77.5 ± 2.13 x	51.2 ± 0.61 x	69.0 ± 0.07 xy	11.9 ± 0.39 x	4.7 ± 0.14 x	4.8 ± 0.01x	2.2 ± 0.12x	1.0 ± 0.02x	2.6 ± 0.01x
PEF 3	78.1 ± 2.37 x	51.4 ± 3.21 x	86.4 ± 3.31 x	11.7 ± 0.44 x	4.5 ± 0.02 x	4.7 ± 0.01x	2.2 ± 0.14x	0.9 ± 0.00x	2.4 ± 0.14x
past.	80.3 ± 0.89 x	68.6 ± 1.21 y	59.8 ± 4.59 y	13.1 ± 0.16 x	4.9 ± 0.05 x	5.3 ± 0.28x	2.3 ± 0.01x	1.0 ± 0.05x	2.5 ± 0.04x

[a] Process parameters of PEF variants 1−4: cf. Table 1. [b] Means and standard errors of two determinations. Values with the same letters a−b and x−y, respectively, within one row (vertical) are not significantly different ($p < 0.05$).

sugars, sorbitol, L-malic acid, and minerals of the cloudy apple juices before and after the preservation steps are shown in **Table 2**. It becomes evident that the preservation method did not affect the major constituents of apple juices. Solely the glucose and fructose contents of the thermally preserved juice differed from those of the control, consistent with its slightly higher TSS (12.8 °Brix). Three of the tested minerals in preserved and control samples were below the ranges given by the AIJN Code of Practice (*39*) for potassium (900−1500 mg/L), magnesium (40−75 mg/L), and calcium (30−120 mg/L), respectively. The iron content, which is an important evaluation parameter for PEF application as discussed below, was approximately 0.22 mg/L, thus being far below the maximum level of 5 mg/L for apple juices (*39*). Apart from the sodium contents differing among variants, all chemical parameters were in good agreement. Consistent with the findings obtained on pilot plant scale, juice quality was not affected by PEF application on laboratory scale, as demonstrated in terms of TSS, TA, pH, density, and the contents of sugars and L-malic acid (data not shown).

The contents of selected amino acids are displayed in **Table 3**. Even though the assessment of apple juices based on their amino acid spectrum is limited (*39*), amino acid profiles and contents are often used for authenticity control. Generally, asparagine is considered the predominant amino acid in apple juices, followed by aspartic acid, with their sum amounting to approximately 80% of total free amino acids (*39*). Since in this study both preserved juices and controls had similarly high glutamic acid contents, the relative total amounts of asparagine and aspartic acid only ranged between 50% and 60%. Nevertheless, all amino acids included in this study met the AIJN-values of apple juices (*39*). No process-dependent deviation of amino acid levels occurred among differently treated juices.

In the present study, potential changes of proteins were explored by a comparative electrophoretic approach. Separation of proteins by SDS-PAGE is shown in **Figure 1** for differently preserved apple juices. No differences of the protein pattern were observed among the proteins of control, PEF-treated, and thermally preserved juices, respectively, regarding their molecular weight distribution. Solely, the protein band at ∼35 kDa was no longer detectable after thermal pasteurization. Thus, even at maximum energy input through PEF (130 kJ/kg) with an electric field strength of 30 kV/cm, the molecular weight of proteins remained unchanged. These findings are in contrast to β-lactoglobulin and egg white, where formation of protein aggregates by covalent bonds was demonstrated by electrophoresis after exposure to up to 10 pulses in the order of milliseconds with an electric field strength of 12.5 kV/cm and energy inputs of 1.631 and 2.465 J/mL (*40*). However, in the present study, pulse duration (3 μs) and total treatment time (max 78 μs) were considerably lower. Nevertheless, food safety and substantial equivalence are prerequisites for commercialization according to the EU food regulation (EC 258/97). In apple

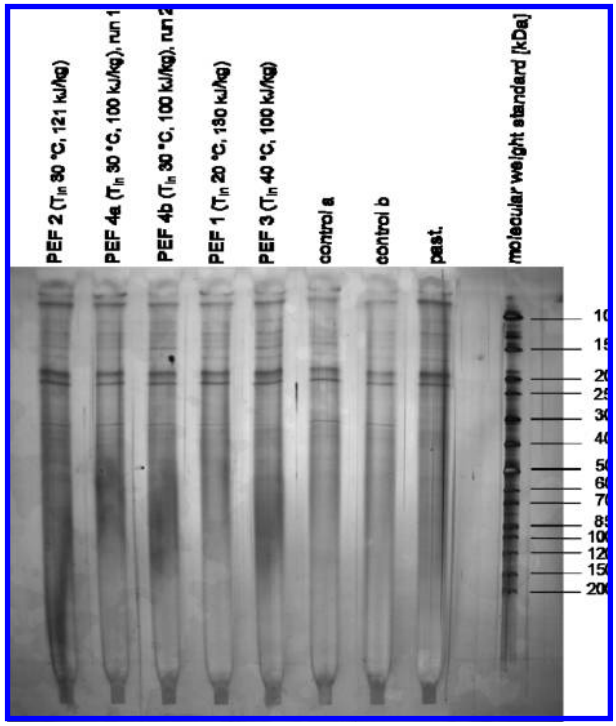

Figure 1. SDS-PAGE of fresh juices (control) and those preserved by pulsed electric fields (PEF) and thermal pasteurization (past.). Molecular weights of a molecular standard are marked in the last band.

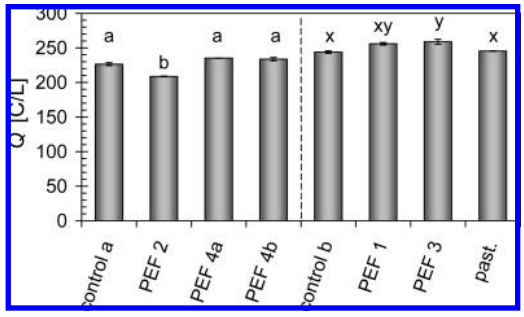

Figure 2. Specific particle charges *Q* of fresh juices (control) and those preserved by pulsed electric fields (PEF) and thermal pasteurization (past.). Significant differences among preserved juices and respective controls are indicated by different letters. Process parameters of PEF variants 1−4: cf. Table 1.

juice production, conventional thermal pasteurization with hot-filling was proven crucial for reducing overall allergenic activity to a minimum (*41*). Since uniformity of the protein pattern in PEF-treated juices and respective controls was demonstrated by SDS-PAGE (**Figure 1**), immunologic investigations, similar to the qualitative and quantitative approach reported by Wigotzki (*41*), would be required to prove whether PEF treatments are as effective as conventional thermal pasteurization in reducing the allergenic potency of apple juices.

Due to electric dissipation, electrolytic reactions at the electrodes might occur, thus changing the total charge of PEF-treated apple juices. Dependent on pH, cloud particles of apple juices are usually negatively charged (*42*). The specific charges of the cloudy juices ranged between 209 and 259 C/L (**Figure 2**). Whereas the juice, obtained after PEF application at 30 °C with an energy input of 121 kJ/kg (PEF 2), had a significantly lower particle charge than the respective control sample (control a), PEF treatment at 40 °C with an energy input of 100 kJ/kg (PEF 3) resulted in a juice with a significantly higher particle charge relative to its control (control b). Thus, no uniform trend was observed.

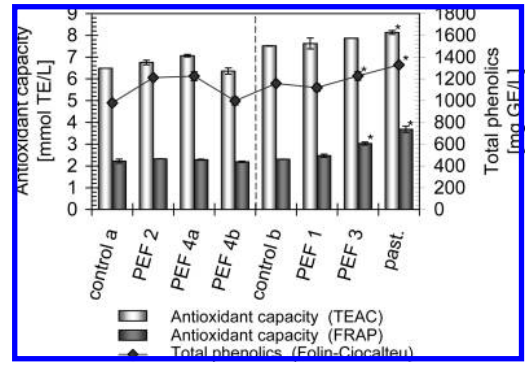

Figure 3. Total phenolic contents [in gallic acid equivalents (GE)/L] and antioxidant capacity [in mmol Trolox equivalents (TE)/L] of fresh juices (control) and those preserved by pulsed electric fields (PEF) and thermal pasteurization (past). Asterisks (∗) indicate values with significant difference relative to respective controls. Process parameters of PEF variants 1−4: cf. Table 1.

Polyphenolics and Antioxidant Capacity of Juices after PEF Treatment on Pilot Plant Scale. Phenolic acids, such as caffeic and *p*-coumaric acids esterified with quinic acid, flavanol monomers, di- and oligomers, quercetin glycosides, and dihydrochalcones are the main phenolic compounds of apple juices (*43*). Total polyphenolics of preserved juices and controls, as determined by the Folin−Ciocalteu method, are displayed in **Figure 3**. Compared with the sum of the individual phenolic compounds (424 ± 15 mg/L), as quantified by HPLC analysis, spectrophotometric results from the Folin−Ciocalteu assay revealed an overestimation of approximately 170%. This well-known phenomenon is caused by the interference of other reducing compounds with the unspecific Folin−Ciocalteu reagent (*44*). As revealed by HPLC, the main phenolic compounds of the juices were chlorogenic (65%) and *p*-coumaroylquinic acids (20%). The individual phenolic compounds were not affected by PEF preservation (data not shown). The significantly higher levels of total phenolics in the juice after thermal pasteurization and PEF variant 3, respectively, might be attributed to interfering substances, since their phenolic levels obtained by HPLC were in the same range as those of the control (data not shown). Ascorbic acid, which also contributes to the Folin−Ciocalteu result, was not detected in either of the juice variants. Thus, the observation of Aguilar-Rosas et al. (*24*) reporting losses of total phenols in apple juices of 14.5% and 32.2% after PEF treatment and thermal pasteurization, respectively, was not confirmed.

As shown by **Figure 3**, the antioxidant capacity of apple juices, which is mainly ascribed to polyphenolics and ascorbic acid, followed a trend similar to that of the total polyphenolics content. This is not surprising, since the Folin−Ciocalteu assay, like the TEAC and the FRAP assays, is based on electron transfer reactions, and ascorbic acid was absent in all variants. After thermal pasteurization, a significantly higher antioxidant capacity was observed. Likewise, the FRAP value of PEF variant 3 (T_{in} = 40 °C, W = 100 kJ/kg) significantly exceeded that of the respective control. Besides, the antioxidant capacity of the juices was not affected by the different treatment conditions.

Color Quality of the Bottled Juices Treated by PEF on a Pilot Plant Scale. Color analysis proved to be a challenge, since browning of the samples was observed already during the centrifugation step prior to photometric determination. While visual appearance of the sealed bottles did not differ, browning started immediately after opening of the bottles, when the juices

Table 4. Browning Indices (BI), Brightness (L^*), and Chroma (C^*) of Fresh Juices (control) and Those Preserved by Pulsed Electric Fields (PEF) (electric field strength $E = 30$ kV/cm) and Conventional Pasteurization (past.) after 1, 3, and 10 days of Storage at 4 °C, respectively[a]

	storage time		
	day 1	day 3	day 10
		BI	
control a	1.08[b] ± 0.05 b	1.02[b] ± 0.03 b	1.05[b] ± 0.06 b
PEF 2	0.65 ± 0.04 c	1.29 ± 0.05 a	1.38 ± 0.02 a
PEF 4a	1.29 ± 0.02 a	1.30 ± 0.08 a	1.26 ± 0.05 ab
PEF 4b	1.34 ± 0.02 a	1.11 ± 0.03 ab	1.28 ± 0.01 ab
control b	1.26 ± 0.03 xy	1.23 ± 0.03 x	1.09 ± 0.05 y
PEF 1	1.16 ± 0.05 y	1.35 ± 0.05 x	1.50 ± 0.02 x
PEF 3	1.43 ± 0.02 x	1.26 ± 0.05 x	1.39 ± 0.02 x
past.	0.47 ± 0.00 z	0.45 ± 0.00 y	0.44 ± 0.00 z
		L^*	
control a	67.57[c] ± 0.19 b	73.51[c] ± 2.02 a	70.89[c] ± 0.47 a
PEF 2	76.34 ± 1.17 a	70.71 ± 0.86 a	68.58 ± 0.20 ab
PEF 4a	65.29 ± 0.63 b	70.24 ± 0.64 a	69.20 ± 0.17 ab
PEF 4b	64.64 ± 0.55 b	72.64 ± 0.88 a	68.21 ± 0.73 b
control b	66.30 ± 0.57 y	72.08 ± 0.98 x	72.65 ± 0.33 y
PEF 1	68.82 ± 0.47 x	70.09 ± 0.52 x	67.58 ± 0.61 z
PEF 3	63.53 ± 0.46 z	69.85 ± 0.61 x	67.12 ± 0.18 z
past.	82.04 ± 0.06 w	82.41 ± 0.21 y	82.28 ± 0.12 x
		C^*	
control a	61.19[c] ± 0.59 ab	54.82[c] ± 3.89 b	61.85[c] ± 0.82 c
PEF 2	51.58 ± 3.52 b	65.32 ± 0.83 a	68.77 ± 0.04 a
PEF 4a	66.32 ± 0.41 a	65.38 ± 0.90 a	67.89 ± 0.55 a
PEF 4b	67.02 ± 0.70 a	62.18 ± 1.65 ab	65.50 ± 0.39 b
control b	67.17 ± 0.90 x	64.49 ± 2.77 x	64.50 ± 0.61 y
PEF 1	67.92 ± 1.05 x	68.38 ± 1.33 x	73.03 ± 0.20 w
PEF 3	68.93 ± 0.46 x	64.37 ± 0.51 x	68.82 ± 0.10 x
past.	27.26 ± 0.03 y	25.91 ± 0.11 y	25.30 ± 0.05 z

[a] Process parameters of PEF variants 1−4: cf. Table 1. Values with the same letters a−c and w−z, respectively, within one row (vertical) are not significantly different ($p < 0.05$). [b] Means and standard errors of four or six determinations. [c] Means and standard errors of two or four determinations.

came into contact with atmospheric oxygen. Browning (BI), brightness (L^*), and chroma (C^*) are compiled in **Table 4**. Only after thermal pasteurization of the juice, BI did not exceed 0.5, whereas it ranged from 0.65 to 1.50 for the PEF-treated juices and the controls. The L^* value was 82 for the thermally preserved sample, thus exceeding significantly those of controls and PEF-treated juices, which varied from 64 to 77. Chroma was ~26 for the pasteurized juice, whereas C^* of controls and PEF-treated juices was between 52 and 73. Undesirable color changes of apple juices are usually due to enzymatic browning caused by POD and PPO (*45*), where phenolic compounds serve as substrates of oxidative enzymes to yield *o*-quinones which polymerize and give rise to the formation of brown pigments (*46*).

Effects of PEF Treatments on Enzyme Deactivation on Laboratory Scale. Prior to the pilot plant experiments, deactivation of PPO and POD in apple juice was assessed on a laboratory scale. Since the electrical conductivities of the apple juices were quite low (~1.22 mS), only low energy inputs between 8.5 and 65.5 kJ/kg were reached. In **Figure 4**, the residual activities of POD and PPO after PEF application with electric field strengths of 15, 25, and 35 kV/cm, respectively, are displayed. At 20 and 40 °C, residual POD activities varied between 85 and 106%, irrespective of the electric field strengths and energy inputs applied. In contrast, a marked loss of POD activity was observed, when PEF application was at 60 °C with energy inputs W above ~30 kJ/kg (**Figure 4A**). Complete POD deactivation was accomplished at an energy input of ~65 kJ/kg. The electric

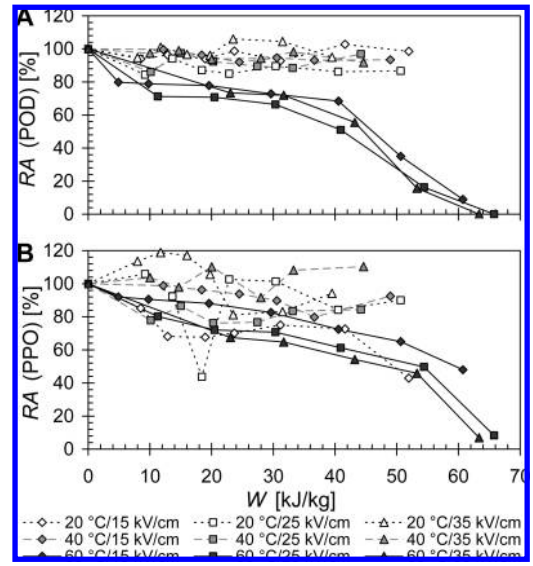

Figure 4. Residual activities (RA) of (**A**) peroxidase (POD) and (**B**) polyphenoloxidase (PPO) after application of pulsed electric fields (PEF) on laboratory scale at electric field strengths of 15, 25, and 35 kV/cm and inlet temperatures of 20, 40, and 60 °C, respectively. Standard error ≤6%.

field strength seemed to be less important. Analogous to POD, PPO activities were only reduced by a combination of PEF application with the maximum inlet temperature studied (60 °C; **Figure 4B**). However, the data obtained varied within a wide range for the inlet temperatures of 20 and 40 °C, respectively, which may be ascribed to matrix effects. Apple PPO is usually bound to membranes (*47, 48*). Since turbidity of the apple juice used in this study was not uniform, the genuine PPO activities varied between 0.6 and 2.8 μkat/L, thus explaining the great variation of PPO deactivation, as shown in **Figure 4B**. Van Loey et al. (*28*) even reported an increase in PPO activity of PEF-treated apple juices, probably caused by the improved release of PPO due to the destruction of cell membranes. Residual pectin methylesterase (PE) activities in orange juice following PEF treatments between 25 and 35 kV/cm at 200 Hz ranged from >90% to ~20% depending on processing time (*32*), where great standard deviations also occurred probably due to matrix effects. Maximum PE deactivation of ~80% required PEF treatment at 35 kV/cm for an extended exposure of 1500 μs, with the temperature not exceeding 37.5 °C.

Due to energy dissipation, the temperature of the PEF-treated juices rose, depending on the energy input applied. To distinguish between thermal effects on enzymes and their deactivation specifically caused by the electric field, the contribution of heat to total enzyme deactivation was estimated. In a separate experiment, thermal deactivation of POD and PPO was characterized by quantification of D- and z-values (eqs 2 and 3). D-values for POD and PPO at 60 °C were 2.5 and 9.6 min, respectively, while z-values of 15.8 and 16.3 °C were obtained. POD, generally considered the most heat-stable enzyme, is a well-established indicator of appropriate heat treatments in vegetable processing. Poor thermal stability of POD may partly be ascribed to heating at the acidic conditions of the fruit juices. For PEF treatments, Zhong et al. (*29*) reported that PPO was more susceptible than POD. In the present study, a fungal POD-preparation had been added due to the low native POD activity. Thus, unlike isolated enzyme preparations, the genuine apple

Table 5. Residual Activities (RA) of Polyphenoloxidase and Peroxidase in Apple Juices after Pulsed Electric Field Treatments at 60 °C on Laboratory Scale with Different Electric Field Strengths (E), Energy Inputs (W), and Outlet Temperatures (T_{out}) and Quantification of the Deactivation Effect Specifically Caused by Pulsed Electric Fields (ΔRA) by Subtraction of the Calculated Relative Contribution of Heat (RA_{therm}) from Total Residual Activities (RA)

			polyphenoloxidase			peroxidase		
E [kV/cm]	W [kJ/kg]	T_{out} [°C]	RA [%]	RA_{therm} [%]	ΔRA [%]	RA [%]	RA_{therm} [%]	ΔRA [%]
15	4.9	60.8	92.0[a] ± 2.9 ab	98.5	6.4	79.8 ± 1.7 b	94.2	14.3
15	9.8	61.6	90.6 ± 1.8 bc	98.3	7.7	78.9 ± 4.7 b	93.5	14.6
15	19.6	63.6	88.1 ± 0.9 bc	97.7	9.7	77.8 ± 0.8 bc	91.3	13.5
15	29.6	65.5	82.6 ± 0.9 c	97.0	14.4	72.8 ± 0.7 bc	88.7	16.0
15	40.6	67.7	72.5 ± 2.3 d	96.0	23.5	68.4 ± 1.5 c	84.8	16.4
15	50.7	70.0	65.0 ± 0.6 d	94.5	29.4	35.0 ± 0.5 d	79.5	44.5
15	60.7	72.2	48.0 ± 0.5 e	92.5	44.5	9.0 ± 0.2 e	72.9	63.9
25	11.3	63.0	80.2 ± 2.8 b	97.9	17.7	71.3 ± 1.7 b	92.0	20.8
25	20.5	64.2	72.1 ± 1.6 bc	97.5	25.4	70.7 ± 1.3 b	90.6	19.9
25	30.3	66.1	70.7 ± 0.7 c	96.8	26.1	66.4 ± 0.4 b	87.8	21.4
25	40.9	69.1	61.2 ± 1.8 d	95.1	33.9	51.0 ± 1.6 c	81.8	30.8
25	54.4	71.4	49.7 ± 1.3 e	93.3	43.5	16.3 ± 1.2 d	75.5	59.2
25	65.8	73.9	8.4 ± 1.9 f	90.6	82.2	0.0 ± 0.4 e	66.7	66.7
35	23.0	65.5	67.5 ± 1.2 b	97.0	29.5	73.4 ± 2.5 b	88.7	15.4
35	31.7	66.7	64.7 ± 0.8 bc	96.5	31.8	72.0 ± 2.7 b	86.8	14.7
35	43.2	69.1	54.2 ± 1.6 bc	95.1	40.9	55.6 ± 2.1 c	81.8	26.2
35	53.3	71.5	45.8 ± 0.9 d	93.2	47.4	15.7 ± 0.4 d	75.2	59.5
35	63.4	73.9	6.9 ± 1.2 e	90.6	83.7	0.0 ± 0.6 e	66.7	66.7

[a] Means and standard errors of three determinations. Values with the same letters within one column are not significantly different ($p < 0.05$).

PPO was probably additionally protected by the fruit matrix and therefore less prone to deactivation by the preservation steps applied.

Table 5 summarizes the effects of thermal preservation and PEF treatments at an inlet temperature of 60 °C. This temperature was chosen, since thermal deactivation effects were most notable at temperatures ≥60 °C. T_{out} after PEF application was in the range expected for the energy inputs involved, with a maximal increase of 13.9 °C, corresponding to an energy input W of 66 kJ/kg at an electric field strength E of 25 kV/cm. Cooling was completed within 8 s. Enzyme deactivation was not exclusively attributed to thermal effects, since POD and PPO activity retention (RA_{therm}), as calculated from thermal D- and z-values, exceeded total retention (RA) resulting from deactivation by PEF application at elevated temperatures. With ΔRA, the deactivation effect specifically caused by PEF was calculated as the difference between total residual activity (RA) and the theoretically estimated one (eq 2) that resulted from heat-induced deactivation at the outlet temperature (RA_{therm}). A synergistic effect between inlet temperature and PEF application became evident. Also Espachs-Barroso et al. (*49*) concluded that PEF application is more efficient to reduce pectinmethylesterase activity, when carried out between 55 and 65 °C. Consistent with their report, our data provide evidence that complete deactivation of PPO and POD by PEF requires combination with high temperature. Temperature rise may be realized either by high PEF energy or by preheating of the juices. Thus, an efficient processing concept should include a combination of moderate heat and PEF application, using the electrical energy dissipated during the PEF treatment for preheating (*50*).

However, according to Van Loey et al. (*28*), enzyme deactivation following PEF application is completely ascribed to thermal effects. In their studies, enzyme deactivation did not exceed 10%, except for prolonged processing time (1000 pulses, 1 Hz, $E = 10-30$ kV/cm). Loss of enzyme activity was probably caused by electrochemical reactions at the electrode surfaces. In the present study, only the global heating due to PEF processing was determined, since accurate temperature registration during the short-time pulses was not feasible. Thus, it is worth mentioning that so-called hot-spots might be responsible for partial enzyme deactivation, supporting the assumption of Van Loey et al. (*28*).

Table 6. Residual Polyphenoloxidase (PPO) Activities of Apple Juices, Preserved by Pulsed Electric Fields (PEF) or Conventional Pasteurization (past.), during Storage over 10 days at 4 °C[a]

	residual PPO activity [%]			
variant	day 0	day 1	day 3	day 10
PEF 1	77[b] ± 3.5 a y	71 ± 5.4 b x	85 ± 4.4 a z	83 ± 3.6 a z
PEF 2	63 ± 3.2 c y	99 ± 4.9 a x	63 ± 4.0 b y	59 ± 3.9 bc z
PEF 3	52 ± 2.1 d xy	47 ± 4.8 d y	48 ± 3.1 c y	57 ± 3.9 c y
PEF 4a	77 ± 3.6 a x	77 ± 2.2 b x	79 ± 5.2 a x	80 ± 4.6 a x
PEF 4b	68 ± 3.3 b y	72 ± 1.5 bc y	81 ± 6.1 a x	65 ± 2.6 b y
past.	n.d.	n.d.	n.d.	n.d.

[a] Process parameters of PEF variants 1−4: cf. Table 1. n.d., not detected. [b] Means and standard errors of six determinations. Values with the same letters within one column (a, b, c, d) and one line (x, y, z), respectively, are not significantly different ($p < 0.05$).

Effects of PEF Treatments on Enzyme Deactivation on Pilot Plant Scale. In the control juices, PPO activity amounted to 4521 ± 165 μkat/L, whereas POD activity was close to the detection limit, precluding the determination of PEF-induced POD deactivation. Therefore, evaluation of preservation effects was based on PPO activity. As demonstrated in **Table 6**, all PEF treatments resulted in a decrease of PPO activity. Most efficient deactivation, with 52% of the initial PPO activity being retained, was achieved at an energy input of 100 kJ/kg (PEF 3) and an inlet temperature of 40 °C. Since the maximum temperature T_{out} did not exceed 63 °C (**Table 1**) and the juices were cooled immediately after the PEF treatments, global thermal deactivation effects were minimized.

Unlike the laboratory scale experiments, partial enzyme deactivation on pilot plant scale was achieved with inlet temperatures as low as 20 °C (PEF 1). Since the greatest activity loss was observed at the highest inlet temperature (PEF 3), a synergistic effect of PEF application and temperature, as already observed on laboratory scale, was confirmed by the pilot plant experiments.

Since regeneration of enzymes may occur during storage, the juices were stored for 10 days at 4 °C. Within this period, PPO did not regenerate (**Table 6**). For pectinmethylesterase, a 90% reduction in orange juice due to PEF application was reported (*15*). No enzyme reactivation was observed by the authors during storage for 112 days at 4 °C and 56 days at 22 °C.

Technical Effects of PEF Processing. The high currents passing the electrode−liquid interface in a PEF chamber may cause electrochemical reactions (*51*). Up to now, very little attention has been paid to this problem, which might affect food quality. Radicals may be generated due to release of metal ions into the samples through electrode corrosion, electrolysis, or bond cleavage during PEF exposure. In this study, the maximum energy input per pulse was 10 J (**Table 1**), which is far below the dissociation energy of chemical bonds (e.g., dissociation energies of C−H and O−H bonds are 416 and 463 kJ/mol, respectively). Therefore, bond cleavage is very unlikely to occur.

Decreasing antioxidant capacity in the radical scavenging assay would have been a first indication of radical formation in the juices. However, measuring radical formation, e.g., of the ascorbyl radical, would be a promising alternative to detect the effects of the energy input directly. Therefore, regarding product safety, exclusion of radical formation in future investigations, using more specific electron spin resonance (ESR) methods, would be helpful. Since PEF treatments can trigger electrochemical reaction products with bactericidal properties, formation of mutagens may also occur. For grape juice subjected to PEF treatment, the occurrence of reactive oxygen species or other oxidative mutagens was reported (*52*). However, the energy input applied with 300 pulses at a field strength of 26.7 kV/cm was approximately ten times higher than that in the present study.

Morren et al. (*51*) described the reduction of electrode corrosion through pulse modulation. Minimization was achieved by reducing the pulse duration. Also Roodenburg et al. (*53, 54*) dealt with the metal release of stainless steel electrodes due to PEF treatment. The main elements of stainless steel, iron, chromium, nickel, and manganese, were dissolved in an aqueous sodium chloride solution due to repeated passage through a PEF chamber. However, the metal concentrations found in PEF-treated orange juice did not exceed the maximum values allowed for fruit juices and those given by the EU Drinking Water for Human Consumption Directive. Furthermore, the authors described that the metal release during the use of stainless steel tubing without PEF treatment was in the same order of magnitude as the metal release due to PEF application. In the present study, PEF treatment had no adverse effect on the iron content of the juices, especially relative to thermal pasteurization, which is in accordance with the results reported by Roodenburg et al. (*54*).

Since HMF is generally accepted as an indicator of heat treatments, its content was determined to compare PEF with conventional thermal treatments. According to the AIJN Code of Practice (*39*), HMF in apple juice should not exceed 20 mg/L. In pasteurized juice, 2.23 ± 0.06 mg/L HMF was found, whereas HMF was not detected in the PEF-treated juices. Since HMF formation indicates heat application, its presence could also be indicative of thermally induced sensory changes. However, this aspect was not part of our study.

In conclusion, the results obtained in the present study clearly demonstrate that PEF does not affect the composition of apple juices under the conditions applied, consistent with previous findings (*19, 24, 25*). Hence, this aspect supported PEF applicability with respect to the requirements set by the EU Novel Food Regulation (EC 258/97). However, since residual enzyme activities strongly affected juice color, applicability of PEF as a nonthermal alternative to heat pasteurization is limited. Therefore, similar to freshly squeezed fruit juices, additional requirements for packaging, storage, and distribution, like chilling and packing in small units under oxygen exclusion, are necessary. Analyses concerning food hygiene were not included in our study. However, extensive reduction of microbial counts at the field strength and energy inputs applied has been reported (*12, 15*). The different deactivation behavior of genuine PPO and an added fungal POD preparation demonstrated the need to use real-life samples to evaluate the applicability of the PEF process for nonthermal food preservation. Due to the synergistic effect of PEF application and heat, a so-called minimal process, ensuring juice stability by combining low temperatures and PEF treatment might be feasible to achieve prolonged shelf life, while reducing the thermally induced negative impact, such as HMF formation.

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