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Effect of pulsed electric fields on the endogenous microflora and physico-chemical properties of porcine blood plasma

Einfluss gepulster elektrischer Felder auf die endogene Mikroflora und physikalisch-chemische Parameter von Schweineblutplasma

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Summary

Objective: Blood plasma is a quickly perishable product. The aim of this study was to determine the impact of pulsed electric fields (PEF) on the inactivation of the endogenous microflora and on physico-chemical parameters of porcine blood plasma.
Methods: PEF application was performed at an initial temperature of 30 °C, electric field strength of 12 kV/cm, a frequency of 169 Hz which corresponds to a specific energy input of 113 kJ/kg and for a total treatment time of 130 µs. To determine the initial amounts of bacteria in the raw material and to investigate the effect of PEF on the inactivation of the endogenous microflora, samples were taken before and after PEF treatment, as well as on storage days 4, 8 and 14.
Results: We found a 1.44 $\log_{10}$ CFU/mL reduction in total aerobic plate count (TPC; $p<0.001$). For *Pseudomonas* spp. and *Enterobacteriaceae* subsequent to PEF application, counts were below the detection limit ($p<0.0001$ and $p<0.05$, respectively). A storage experiment showed significantly lower TP ($p<0.01$), *Pseudomonas* spp. ($p<0.01$) and *Enterobacteriaceae* ($p<0.001$) counts in the PEF treated plasma after storage for 14 days at 3 °C. There was no difference between native and PEF treated blood plasma samples concerning values of protein, ash, pH, electrical conductivity and a_w.
Conclusion: In conclusion, PEF application and low temperature storage at 3 °C resulted in a strongly retarded growth of microorganisms in the PEF treated plasma samples, extending its shelf-life to 14 days based on strictest criteria for microbiological acceptability (5 x 10^4 CFU/mL for TPC, 5 x 10^3 CFU/mL for *Pseudomonas* spp., 5 x 10^2 CFU/mL for *Enterobacteriaceae,* 5 x 10^2 CFU/mL for coagulase-positive staphylococci and 1 x 10^2 CFU/mL for sulphite-reducing anaerobic bacteria). Thus, PEF treatment may be considered as an appropriate method for reducing microorganisms in porcine blood plasma and for extending shelf life.

Keywords: Blood plasma, pulsed electric fields, microbial inactivation, physico-chemical properties, microbial shelf life

Zusammenfassung

Zielsetzung: Blutplasma ist ein schnell verderbliches Produkt. Ziel dieser Studie war daher, den Einfluss gepulster elektrischer Felder (PEF) auf die Inaktivierung der endogenen Mikroflora, sowie auf physikalisch-chemische Parameter von Blutplasma zu untersuchen.
Methoden: Die PEF Anwendung wurde bei einer initialen Temperatur von 30 °C, einer elektrischen Feldstärke von 12 kV/cm, einer Frequenz von 169 Hz (entspricht einem spezifischen Energieeintrag von 113 kJ/kg) und einer totalen Behandlungszeit von 130 µs durchgeführt. Es wurden Proben im Rohmaterial, vor und nach der PEF-Behandlung sowie an den Lagerungstagen 4, 8 und 14 gewonnen. Anhand der gewonnenen Proben konnte der Anfangskeimgehalt und der Einfluss der PEF-Behandlung auf die Inaktivierung der endogenen Mikroflora des Blutplasmas untersucht werden.
Ergebnisse: Die PEF-Behandlung führte zu einer Reduktion der Gesamtkeimzahl (TPC) um 1.44 $\log_{10}$ KbE/ml; $p<0.001$). Die Keimzahlen für *Pseudomonas* spp. und *Enterobacteriaceae* lagen im Anschluss an die PEF Anwendung unterhalb der Nachweisgrenze ($p<0.0001$ bzw. $p<0.05$). Das Lagerungsexperiment zeigte signifikant niedrigere Gesamtkeimzahlen ($p<0.01$), sowie signifikant niedrigere Keimzahlen an *Pseudomonas* spp. ($p<0.01$) und *Enterobacteriaceae* ($p<0.001$) im PEF-behandelten Plasma nach einer Lagerung für 14 Tage bei 3 °C. Bezüglich der Parameter Protein, Asche, pH, elektrische Leitfähigkeit und aw gab es keine signifikanten Unterschiede zwischen PEF-behandeltem und nativem Blutplasma.
Schlussfolgerung: PEF-Anwendung und eine Lagerung bei 3 °C führen zu einem deutlich verzögerten Wachstum von Mikroorganismen. Die Haltbarkeit kann unter Berücksichtigung strikter Kriterien für die mikrobiologische Akzeptabilität (TPC: 5 x 10^4 CFU/ml, Pseudomonas spp.: 5 x 10^3 CFU/ml, Enterobacteriacea: 5 x 10^2 CFU/ml, Koagulase-

positive Staphylokokken: 5 x 10^2 CFU/ml, Sulfit-reduzierende-anaerobe-Bakterien: 1 x 10^2 CFU/ml) bis zu 14 Tage verlängert werden.
Somit kann die PEF-Behandlung als geeignete Methode zur Reduktion von natürlich vorkommenden Mikroorganismen und zur Haltbarkeitsverlängerung von Schweineblutplasma genannt werden.

Schlüsselwörter: Blutplasma, Mikrobiologische Inaktivierung, Haltbarkeit, physikalisch-chemische Parameter

Introduction

Slaughterhouses as well as the processing of meat generate a significant amount of solid and liquid by-products (Toldra et al. 2012) like whole blood and blood plasma, which are valuable due to their high biological value and excellent functional properties (Saguer et al, 2007; Toldrà et al. 2004).

Due to its neutral to slightly alkaline pH and high a_w-value blood plasma is a quickly perishable product (Stöppler and Promberger, 1991; Nowak and von Mueffling, 2006). Blood plasma stored at 3 °C reaches unacceptable microbiological limits of 10^7 CFU/mL within 7 days of storage (Nowak and von Mueffling, 2006; Stiebing, 1985).

The use of pulsed electric fields (PEF) represents an emerging food processing technology for liquid food. Microorganisms could be inactivated at lower temperatures compared with conventional heat treatment technologies (Amiali et al., 2007; Saldaña et al., 2011; Cregenzán-Alberti et al., 2015) During PEF processing, high voltage electric fields are applied in short electric pulses to a product located in a treatment chamber placed between two conductive electrodes (Heinz et al., 2002). PEF has been shown to disrupt the cell membrane by forming pores (electroporation) which increases the permeability of the cells (Sale and Hamilton,1967; Marx et al., 2011; Tao, 2015). The loss of viability is correlated with the sum of nonpermanent and permanent membrane permeabilization (García et al., 2007). Generally, PEF application at higher temperatures proved to be more effective than PEF at lower temperatures (Aronsson and Ronner, 2001; Sepulveda et al., 2005; Heinz et al., 2003; Moody et al., 2014). Thus, PEF technology may be effectively used as an enhanced mild thermal preservation method (Moody et al., 2014).

Only few investigations have analyzed the impact of PEF on the shelf life and safety or on sensory parameters of blood or blood plasma (Kiessling and Töpfl, 2012; Boulaaba et al., 2014a; Boulaaba et al., 2014b). In a previous study we focused on PEF effects on blood plasma inoculated with *Pseudomonas (P.) fragi, Escherichia (E.) coli* (K12) and *Staphylococcus (S.) xylosus* (Boulaaba et al., 2014a). The objective of the present work was to investigate the effect of PEF on the inactivation of naturally occurring microorganisms and shelf life, as well as changes in physico-chemical properties of porcine blood plasma.

Material and methods

Plasma collection

Porcine blood plasma was received from Sonac Loenen B.V, (Loenen gld, the Netherlands) as previously described by Boulaaba, Kiessling (2014a). In short, plasma was separated by centrifuging blood using a continuous disc centrifuge (GEA Westfalia separator, Oelde, Germany). Plasma was packed in 20-liter bag-in-box-systems and transported to the German Institute of Food Technologies e. V., Quakenbrück, Germany (DIL). It was stored under cooling conditions (≤3 °C) and PEF treated on the following day with the continuous pilot plant ELEA® HVP5.

Sample collection

Sampling and PEF processing from 10 batches took place over the time period of nine months. Two untreated (native) and two PEF-treated samples from each blood plasma batch were packaged aseptically in sterile polypropylene tubes (250mL capacity). They were transported in a cooler (2.5 ± 0.5 °C) to the laboratory of the Institute for Food Quality and Food Safety, University of Veterinary Medicine Hannover, Germany. The temperature profile was recorded as previously described for whole porcine blood (Boulaaba et al., 2014b). The samples were stored in the laboratory in a refrigerated room at ≤3 °C prior to examination which started on the same day. Five plasma batches were PEF processed to analyze microbial shelf life. For this purpose, PEF treated and native control samples were stored in sterile 1 L glass bottles at ≤3 °C for 14 days. Samples were periodically taken for microbial growth after selected storage times (days 4, 8, and 14 after PEF treatments).

PEF treatments

The PEF system used was a pilot plant high intensity electric field pulser (ELEA® HVP-5) manufactured by the German Institute of Food Technologies (DIL e. V., Quakenbrück, Germany). Experimental setup for PEF treatments consisted of a reservoir, a pump, two heat exchangers and two treatment chambers (Figure 1). The coaxial continuous treatment chambers were composed of two TITAN electrodes separated by a gap of 7 mm. The apparatus generated approximately square waveform pulses of a width of 20 µs.

Based on the investigations of a previous study (Boulaaba et al., 2014a), we selected the most appropriate treatment condition to perform analysis, including the impact of PEF on inoculated microorganisms and the endogenous microflora of blood plasma as well as on physico-chemical parameters and gelling properties. We used electric field strength of 12 kV/cm for treatment times of 132 µs. The applied pulse frequencies of 168 Hz correspond to an energy input of 113 kJ/kg. Pulse width was 20 µs and the flow rate was fixed at 50 L/h. Blood plasma with a temperature ≤3 °C was filled in a storage container in form of a funnel. Via the included eccentric screw pump plasma was transported through a plate heat exchanger. Thus, to increase sensitivity of the membrane structure (Kiessling and Töpfl, 2012; Aronsson et al., 2001), the blood plasma was tempered to 30 ± 0.5 °C shortly before PEF application; outlet temperature was 58 °C. Directly after PEF treatment, blood plasma

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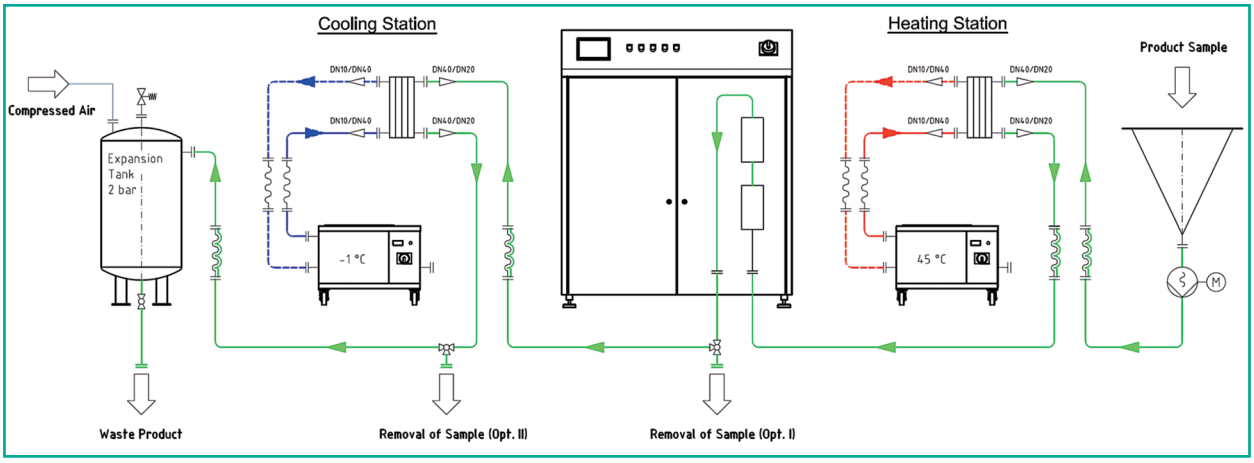

FIGURE 1: *Schematic view of the test set-up.*

was cooled down to 21 °C by a second plate heat exchanger. Samples for microbiological and physico-chemical investigations were collected at the return line of the system output by means of a three-way cock. Samples were stored on crushed ice until examination. Operating pressure was 2 bar.

Endogenous microflora

To determine the initial amounts of bacteria in the raw material and to investigate the effect of PEF on the inactivation of the endogenous microflora, microbiological examinations were performed. Samples were taken on the day of PEF application before and after PEF treatment (10 plasma batches with two repetitions each), as well as on storage days 4, 8 and 14 (5 plasma batches with two repetitions each). The microbiological examination of total plate count (TPC), *Pseudomonas* spp., *Enterobacteriaceae,* coagulase-positive staphylococci, *Bacillus* spp. and sulphite-reducing anaerobic bacteria was done as previously described for whole porcine blood by Boulaaba, Egen (2014b). In short, TPC was determined using the pour plate technique on plate count agar. The enumeration of presumptive *Pseudomonas* bacteria was conducted by the spread plate method on Glutamat Starch Phenolred Agar and reading with oxidase test (Bactident® Oxidase, Merck). *Enterobacteriaceae* was enumerated on VRBG Agar using the overlay method. Furthermore, coagulase-positive staphylococci were determined using the Baird-Parker Agar and confirmed with coagulase test (Bactident® Staph plus, Merck). Sulfite-reducing anaerobic bacteria were conducted using the Sodium Sulfit Ferricitrat Agar in tubes. After incubation of sample dilution for 10 min at 80 °C, the enumeration of *Bacillus* spp. was conducted by the spread plate method using the standard nutrient agar I. Results for the inactivation of the described microorganisms are expressed in logarithmic units. Microbial counts below the detection limit were integrated in the calculation with 5 CFU/mL (TPC, sulphite-reducing anaerobic bacteria) or 50 CFU/mL (others). Generally, the survival ratio of microorganisms S was defined as:

$$S = \log_{10} (N_t/N_0)$$

Chemical and physical determinations

All chemical and physical properties were analyzed in samples collected shortly before and after PEF application (10 plasma batches with two repetitions each). Samples were analyzed for raw protein, ash, hemoglobin, pH, electrical conductivity and a_w as already described for whole porcine blood by Boulaaba, Egen (2014b). In short, raw protein (in percentage) was determined using the Kjeldahl method (Kjeldatherm and Vapodest analyzer Gerhardt, Bonn, Germany). The amount of ash (in percentage) was determined by drying plasma in an oven following by heating in a muffle furnace. Hemoglobin was detected photometrically with Merckotest Hemoglobine (Merck Diagnostica, Darmstadt, Germany) and measurement of color gradiation (Photometer Heλios ß, Unicam, Cambridge, UK). The pH values and electrical conductivity were determined using a battery-powered pH meter (Portamess® 651-2, Knick, Elektronische Messgeräte, Berlin, Germany) and an electrical conductivity measuring instrument (GMH 3410, Greisinger electronic GmbH, Regenstauf, Germany). The water activity (a_w) value was analyzed using a cryometric device (AWK-10, Nagy, Gäufelden, Germany). The connected computer recorded the temperatures and calculated the a_w value (MESA software, Nagy).

Statistical analysis

Data were processed with Excel® (Microsoft™) and analyzed statistically with SAS® Enterprise guide® version 4.3 (Statistic Analyzing Systems, SAS Institute Inc. Cary, NC, USA). Microbial counts were transformed into $\log_{10}$ values. Means and standard deviations were calculated with PROC UNIVARIATE. Residuals were tested with PROC UNIVARIATE for normal distribution before further analysis. The level of significance of the study was set at $\alpha = 5$ %. To determine the effects of PEF on endogenous microflora and physico-chemical parameters, we used the student's t (PROC MEANS) and in the case of skewed data sets the signed-rank test (PROC UNIVARIATE). Analysis of variance for repeated measurement was performed to determine variations among native and treated samples during storage (PROC GLM REPEATED). In case of significance, means of groups on different storage days were analyzed with the Tukey-test or, regarding skewed data sets, with the Wilcoxon two-sample test (PROC NPAR1WAY WILCOXON).

Results and discussion

Effect of PEF on the endogenous microflora

The initial Total Plate Count (TPC) of the blood plasma used for PEF application amounted to 3.62 ± 0.89 $\log_{10}$ CFU/mL. Mainly *Pseudomonas* spp. and *Enterobacteriaceae* were detected in the native plasma samples (table 1). Sulphite-reducing anaerobic bacteria and coagulase-positive staphy-

TABLE 1: *Inactivation of naturally occurring microorganisms in porcine blood plasma by PEF treatment (n = 10 plasma batches).*

	Native (± sd) lg CFU/mL	PEF[1)] (± sd) lg CFU/mL	*p* value
TPC	3.62 ± 0.89[A]	2.18 ± 0.72[B]	<0.001
Enterobacteriaceae	3.22 ± 1.03[A]	<2[2)B]	<0.05
Pseudomonas spp.	4.23 ± 1.04[A]	<2[2)B]	<0.0001
Bacillus spp.	<2[2)]	<2[2)]	ns
Coagulase-positive staphylococci	2.01 ± 0.69[A]	<2[2)A]	ns
Sulphite-reducing anaerobic bacteria	0.91 ± 0.46[3)A]	0.73 ± 0.10[3)A]	ns

sd = standard deviation; ns = no significance; [1)] PEF treated with the following treatment conditions: 12 kV/cm, 168 Hz, 113 kJ/kg, treatment time 132 µs, pulse width 20 µs. [2)] detection limit <100 CFU/mL; [3)] detection limit <10 CFU/mL; Means with different superscripts in capital letters (A and B) in each row differ significantly

lococci were detected in two native plasma batches (2.06 and 1.40 $\log_{10}$ CFU/mL), and in four native plasma batches (3.91, 2.24, 1.94 and 1.85 $\log_{10}$ CFU/mL), respectively. *Bacillus* spp. was not verifiable. These findings are mainly in agreement with previous studies, which found similar microorganisms predominating in blood plasma (Saguer et al., 2007; Stöppler and Promberger, 1991; Nowak and von Mueffling, 2006; Stiebing, 1985; Otto, 1983; Dàvila et al, 2006; Haeger et al., 1984).

With the chosen parameters (2.3), PEF treatment led to a significant reduction in TPC (p<0.001), *Pseudomonas* spp. (p<0.0001) and *Enterobacteriaceae* (p<0.05). Moreover, the bacterial count for *Enterobacteriaceae* and *Pseudomonas* spp. in blood plasma fell below the detection limit (Figure 2).

In a previous study by Boulaaba, Egen (2014b), whole porcine blood was PEF-treated with a realized energy input of 114 kJ/kg and significant inactivation was shown for TPC and *Pseudomonas* spp. but not for *Enterobacteriaceae.* In general, reduction was more effective in blood plasma than in whole blood, probably due to the content of erythrocytes in whole blood and differences in pH. Blood plasma showed with pH 7.7 higher pH values than whole blood with approx. pH 7.15 (Nowak and von Mueffling 2006; Boulaaba et al., 2014b). It is known that the pH of the treated medium influences the resistance of microorganisms against PEF (García et al., 2007; Saldaña et al., 2012; Somolinos et al., 2010). However, differences of 0.55 pH units in whole blood and plasma in our study were not as pronounced as in the examinations of the mentioned studies.

It could be shown that gram-negative microorganisms were more sensitive to PEF treatment than gram-positive microorganisms (Aronsson and Ronner, 2001; Mazurek et al., 2002). In our investigations, gram-positive coagulase positive staphylococci fell below the detection limit subsequent to PEF application. However, it must be kept in mind that only four batches contained this microorganism with rather low bacterial counts. Although detection of sulphite-reducing anaerobic bacteria was no longer possible after PEF application, statistical significance of PEF treatment could not be proved. This was mainly due to the low number of contaminated batches and low initial bacterial counts slightly above the detection limit.

As described in various studies, temperature intensification increases the lethal effect of PEF on microorganisms (Moody et al., 2014; Dunn and Pearlman, 1987; Cregenzan-Alberti et al., 2015). Hence, we used a combination of PEF and moderate heat (30 ± 0.5 °C inlet temperature) with short processing times. At the measured outlet temperature of 58 °C, microorganisms may be inactivated by heat alone if the heating time is long enough (Boulaaba et al. 2014a; Aronsson et al., 2001). However, 5 sec is the maximum length of thermal impact in the ELEA® HVP5 and the actual processing time in the treatment chamber was 31.82 ms. A plate heat exchanger cooled the plasma down to 21 °C directly after processing and plasma samples were packed on crushed ice and in refrigerated rooms. In a previous study we analyzed the effect of heat generated during PEF application on microbial inactivation in blood plasma and could show that effective inactivation due to thermal stress was only achieved from 60 °C upwards and a holding time of at least 35 s (Boulaaba et al. 2014a). Likewise, Aronsson, Lindgren (2001) stated that thermal effect has a limited direct influence on the inactivation of microorganisms during PEF processing since exposure time in the PEF equipment is very short. The use of plate heat exchangers is important to cool down large amounts of fluids subsequent to PEF application. As a consequence, the thermal stress on the valuable blood proteins will be reduced. Furthermore, in avoidance of potential microbial regrowth, it is important to cool down the plasma to ≤3 °C according to the regulations for offal (Regulation EC No. 853/2004 (Anonym, 2004)). Concerning industrial scale it would be necessary to fulfill the requirements with further cooling processes.

Microbial shelf life of porcine blood plasma stored at 3 °C

For this study part, porcine blood plasma of five plasma batches was PEF processed with the treatment conditions described above (2.3) and stored at ≤3 °C for 14 days to analyze microbial shelf life. A temperature of ≤3 °C for offal is regulated by law (Regulation EC No 853/2004 (Anonym, 2004)). Samples were periodically taken after selected storage times for microbial growth (days 0, 4, 8 and 14 after PEF treatment). PEF led to significantly reduced counts of TPC (p<0.05) and *Pseudomonas* spp. (p<0.0001). On storage days 8 (p<0.05) and 14 (p<0.01), PEF treated plasma showed a significantly lower growth of TPC (Figure 3). For *Pseudomonas* spp. this could be proved for the entire storage

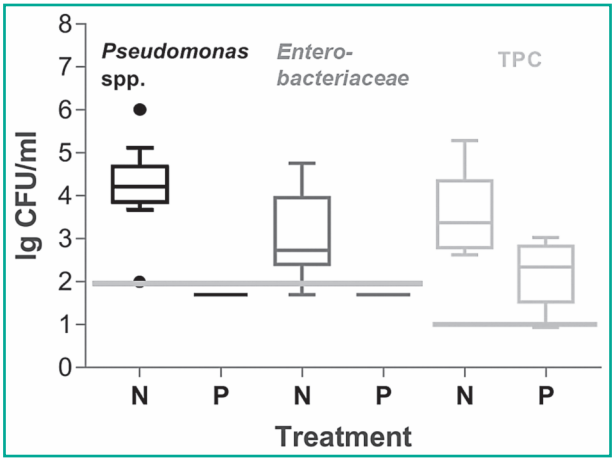

FIGURE 2: *TPC (lg CFU/mL), Pseudomonas spp. (lg CFU/mL) and Enterobacteriaceae (lg CFU/mL) in porcine blood plasma before and after PEF treatment (N = untreated, native; P = PEF-treated; TPC = Total aerobic plate count; grey borderline = detection limit; n = 10 plasma batches; Tukey whiskers).*

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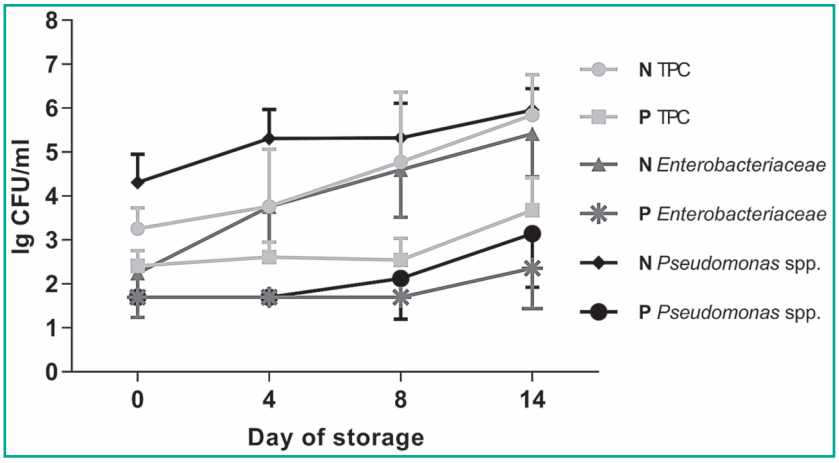

FIGURE 3: *Total aerobic plate count (lg CFU/mL), Pseudomonas spp. (lg CFU/mL) and Enterobacteriaceae (lg CFU/mL) in porcine blood plasma before and after PEF treatment (day 0) and during storage at 3 °C for 14 days (N = untreated, native; P = PEF treated n = 5 plasma batches)*

period (p<0.0001 for days 4 and 8, p<0.01 for day 14). Two plasma batches contained *Enterobacteriaceae* (2.6 ± 1.2 CFU/mL) with rather low bacterial counts just above the detection limit. Hence, no significant inactivation by PEF application was observed. However, on storage days 4 (p<0.01), 8 (p<0.001) and 14 (p<0.001) significantly reduced growth in PEF treated samples was demonstrated. For *Enterobacteriaceae,* differences between native and PEF treated samples were dependent on storage days (p<0.01).

In the case of TPC and *Enterobacteriaceae,* even a smaller reduction of 1 or 2 $\log_{10}$ steps by PEF treatment led to significant reduced bacterial growth during storage. PEF processing changes the aggregative structure of the proteins (Kiessling and Töpfl, 2012; Floury et al., 2006) which in turn could result in a poorer capture of water molecules by the proteins. Consequently, compared with untreated plasma, PEF processing could cause unfavorable conditions for microbial growth. Moreover, exept being dead or alive, bacterial cells could be sublethally injured after beeing PEF treated. In the study of Wang, Zeng (2015) a large part of *Saccharomyces cerevisiae* cells was sublethally injured when they were exposed to PEF with lower electric field strength (e. g. approx. 10 kV/cm) or a shorter treatment time (<1.2 ms). In the present study, we applied with 12 kV/cm rather low electric field strenght combined with short treatment times. Sublethally injured bacterial cells aditionally would be further weakened by the cold storage period ≤3 °C.

With the absence of any legal microbial criteria for blood plasma, Stoeppler and Promberger (1991) set 1.0 x 10^5 CFU/mL as *m* (represents an acceptable level and values above it are marginally acceptable or unacceptable in the terms of the sampling plan) and 5 x 10^5 CFU/mL as *M* (values above *M* are unacceptable in the terms of the sampling plan and detection of one or more samples exceeding this level would be cause for rejection of the lot) for TPC in blood plasma. According to our study concerning shelf life of whole blood (Boulaaba et al. 2014b) we followed stricter criteria and used 5 x 10^4 CFU/mL for TPC, 5 x 10^3 CFU/mL for *Pseudomonas* spp., 5 x 10^2 CFU/mL for *Enterobacteriaceae,* 5 x 102 CFU/mL for coagulase-positive staphylococci and 1 x 10^2 CFU/mL for sulphite-reducing anaerobic bacteria as the maximum acceptable limits in our investigations.

When kept at 3 °C, the unacceptable microbiological limit of TPC >4.7 $\log_{10}$ CFU/mL in PEF plasma was not exceeded within the storage period of 14 days (table 2). In native blood plasma 4 of 5 and 5 of 5 batches reached the limit on storage days 8 and 14, respectively. For *Enterobacteriaceae* (limit 2.7 $\log_{10}$ CFU/mL) and *Pseudomonas* spp. (limit 3.7 $\log_{10}$ CFU/mL), the limits were reached in PEF treated plasma from 2 of 5 batches on the final storage day. In native plasma all batches presented >3.8 $\log_{10}$ CFU/mL *Pseudomonas* spp. as of storage day 4 and >2.7 $\log_{10}$ CFU/mL *Enterobacteriaceae* as of storage day 8.

Bacillus spp. could not be detected in any of the samples. Sulphite-reducing anaerobic bacteria could only be detected in one untreated sample (2.06 $\log_{10}$ CFU/mL) and coagulase-positive staphylococci were found in two native plasma samples (3.05 $\log_{10}$ ± 1.22 CFU/mL). After PEF treatment and during the 14 day storage period, CFU were below the detection limit (<10 CFU/mL and <100 CFU/mL, respectively). Remarkably, from storage day 4, counts of sulphite-reducing anaerobic bacteria and coagulase-positive staphylococci in native blood plasma were below the detection limit as well. In this context, it must be taken into account that the number of positive batches was very limited (one and two batches, respectively) and the microbial load was rather low. Besides, plasma was stored at a refrigerating temperature of ≤3 °C. This causes a selective pressure towards the development of psychrotrophic bacteria (Dàvila; 2006). Within psychrotrophs, *Pseudomonas* is the dominating group (Eriksson and Bockelmann, 1975) which could be confirmed in the present study for the medium blood plasma. Hence, it is possible that the selective pressure not only facilitated growth of *Pseudomonas* spp., but also retarded growth of sulphite-reducing anaerobic bacteria and coagulase-positive staphylococci.

Physico-chemical parameters in native and PEF treated blood plasma

In the native blood plasma, the average of protein was 6.2 ± 0.26 % and the proportion of ash was 1.1 ± 0.1 %. These

TABLE 2: *Number of porcine plasma batches (n) kept at 3 °C reaching the unacceptable microbiological limits on the defined storage days.*

	[1]CFU/mL	N	PEF	day 0	day 4	day 8	day 14
TPC	> 4.7	5	N[2]	0	0	4	5
			T[3]	0	0	0	0
Enterobacteriaceae	>2.7	5	N	0	4	5	5
			T	0	0	0	2
Pseudomonas spp.	> 3.7	5	N	4	5	5	5
			T	0	0	1	2
Sulphite-reducing anaerobic bacteria	> 2	5	N	1	0	0	0
			T	0	0	0	0
Coagulase-positive staphylococci	> 2.7	5	N	2	1	0	0
			T	0	0	0	0

n = number of blood batches kept at 3 °C reaching the unacceptable microbiological limits on the defined storage days. N = total number of blood batches. [1] unacceptable microbiological limit; [2] native blood plasma; [3] PEF treated blood plasma with the following treatment conditions: 12 kV/cm, 168 Hz, 113 kJ/kg, treatment time 132 µs, pulse width 20 µs.

TABLE 3: *Means of the aw values, pH values, electrical conductivity, protein, ash and hemoglobin in the native blood plasma and after PEF treatment (n = 10 plasma batches).*

	Native (± sd)	PEF[1)] (± sd)	*p* value
a_w value	0.99 ± 0.001[A]	0.99 ± 0.001[A]	ns
pH value	7.73 ± 0.60[A]	7.80 ± 0.57[A]	ns
Electrical conductivity	15.51 ± 0.82[A]	15.36 ± 0.80[A]	ns
Protein	6.02 ± 0.26	5.97 ± 0.34	ns
Ash	1.1 ± 0.1	1.1 ± 0.1	ns
Hemoglobin	0.69 ± 0.83[A]	1.83 ± 0,99[B]	0.002

sd = standard deviation; ns = no significance; [1)] PEF treated with the following PEF treatment conditions: 12 kV/cm, 168 Hz, 113 kJ/kg, treatment time 132 µs, pulse width 20 µs. Means with different superscripts in capital letters (A and B) in each row differ significantly.

results are in agreement with previous studies [5, 36], the authors just describe slightly higher values for protein, 6.61 % and 7.19 %, respectively. As expected, PEF application had no measurable impact on these examination parameters or on a_w- and pH values (table 3).

The native blood plasma was shown to contain between 0.07 and 2.39 g/dl hemoglobin with a median of 0.25 g/dl. Lower average values of 0.22 g/dl were found by Nowak and von Mueffling, 2006). The variation of hemoglobin content in the present study reflects the differences in quality of the plasma batches. Overall, only two native plasma batches contained high hemoglobin values above 2 g/dl. Besides, plasma batches used for the storage experiment showed with 0.18 ± 0.08 g/dl low hemoglobin levels, hence we assume that the influence of hemoglobin content on microbial growth was marginal. Contrary to expectations, hemoglobin values increased subsequent to PEF application. As hemoglobin content is virtually impossible to increase intrinsically owing to PEF application, we assume that the noticed slight cloudiness of PEF treated samples led to inaccuracy of measurement. This cloudiness may be explained by precipitation of plasma proteins since aggregation and denaturation of plasma proteins already starts at 55 °C (Hermansson, 1982).

Initial electrical conductivity of native blood plasma was relatively uniform and amounted to 15.51 ± 0.82 mS/cm. In a previous study, we obtained similar values from different blood plasma batches (Boulaaba et al. 2014a). However, compared with beer, orange-milk-beverage, orange-, red grape, apple juice (Evrendilek et al., 2004; Sampedro et al., 2007; Toepfl et al., 2007) and whole porcine blood (Kiessling and Töpfl, 2012; Boulaaba et al. 2014b), blood plasma offers a rather elevated electrical conductivity. In whole porcine blood PEF treatment leads to hemolysis of the red blood cells. Consequently, the release of ions increases the electrical conductivity (Boulaaba et al. 2014b). In contrast and as expected, PEF application had no effect on the electrical conductivity of blood plasma (15.63 ± 0.8 mS/cm).

Conclusions

With the chosen processing conditions of the pilot plant PEF system ELEA® HVP5, the effectiveness of PEF application on naturally occurring microorganisms in porcine blood plasma could be shown. After PEF application bacterial counts for *Enterobacteriaceae, Pseudomonas* spp., sulphite-reducing anaerobic bacteria and coagulase-positive staphylococci dropped below the detection limit. In addition, TPC was significantly reduced by 1.44 $\log_{10}$ CFU/mL.

PEF application and low temperature storage at ≤3 °C resulted in a strongly retarded growth of microorganisms in the PEF treated plasma samples, extending its shelf-life to 14 days based on strictest criteria for microbiological acceptability. Thus, PEF treatment may be considered as an appropriate method for reducing microorganisms in porcine blood plasma and for extending shelf life. In overall terms, PEF application is considered to be beneficial compared to preservation methods such as freezing and (spray) drying which are known to cause high energy costs, inadequate shelf life and loss of functional properties of plasma proteins.

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Conflict of interest

The authors declare that there is no conflict of interest.

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