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Effect of pulsed electric fields on microbial inactivation and gelling properties of porcine blood plasma

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ABSTRACT

The effect of pulsed electric fields (PEF) on the inactivation of inoculated *Pseudomonas (P.) fragi*, *Escherichia (E.) coli* (K12) and *Staphylococcus (S.) xylosus* as well as on the total aerobic plate count (TPC) of porcine blood plasma was investigated. Furthermore, the impact of PEF on gel strength and solubility was analysed. Inoculated plasma samples were PEF treated at an initial temperature of 30 °C, electric field strength between 9 and 13 kV/cm and a specific energy input in a range of 40 to 182 kJ/kg for total treatment times between 35 μs and 233 μs.
Increased specific energy input led to increased microbial inactivation, but then decrease in soluble protein and gel strength occurred. To avoid undesired protein denaturation, energy input should not exceed 120 kJ/kg. Considering these processing conditions an inactivation of 3.0 ± 0.4 log steps for the total plate count was proven ($p < 0.001$). Inoculated *E. coli* K12 and *P. fragi* could be reduced below the detection limit ($p < 0.0001$) and for at least 3 log steps ($p < 0.0001$), respectively. *S. xylosus* was the most PEF resistant germ and for sufficient inactivation higher energy input was needed which resulted in outlet temperatures above 60 °C ($p < 0.01$).
Industrial relevance: The treatment of blood plasma with pulsed electric fields (PEF) is in principle a suitable method for reducing the total plate count, *Pseudomonas fragi*, *Escherichia coli* and *Staphylococcus xylosus* in porcine blood plasma. Inactivation is limited by the specific energy input which should not exceed 120 kJ/kg at start temperatures of 30 °C to avoid undesired protein denaturation. Improved microbiological quality of PEF-treated blood plasma enables enhanced utilisation possibilities and allows extended storage times.
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1. Introduction

Blood proteins, especially those found in the plasma fraction, have relevant technological properties such as gelation, foaming agents and emulsification (Benjakul, Visessanguan, & Chantarasuwan, 2004; Tsong & Ahmad, 1990). Due to their neutral taste and lack of the dark colour typical of the cellular fraction, plasma and the products derived therefrom are more widely used as value-added ingredients in the food industry (Tsong & Ahmad, 1990).

Nevertheless, blood plasma is a quickly perishable product due to its neutral to slightly alkaline pH and high a_w-value. Spoilage and pathogenic microorganisms from the body surface area and gastrointestinal tract of slaughtered animals can be transferred to the blood and subsequentely recovered in the blood plasma (Carretero & Parés, 2000). Nonacceptable microbiological limits are reached within 7 storage days at 3 °C (Stiebing, 1985). Preservation methods such as freezing and (spray) drying have been developed, but these cause high energy costs, inadequate shelf life, variations in colour and taste and loss of functional properties of plasma proteins (Toldrà, Elias, Parés, Saguer, & Carretero, 2004).

The application of pulsed electric fields (PEF) has been demonstrated to be eligible for inactivating microorganisms and enzymes in liquid food at lower temperatures compared with conventional heat treatment technologies (Amiali, Ngadi, Smith, & Raghavan, 2007; Aronsson, Lindgren, Johansson, & Rönner, 2001; Gerlach et al., 2008; Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2008; Riener, Noci, Cronin, Morgan, & Lyng, 2009; Saldaña, Puértolas, Monfort, Raso, & Álvarez, 2011). Generally, PEF treatment is achieved by delivering high-voltage pulses to a product placed between two conductive electrodes within a treatment chamber (Heinz, Knorr, Lee, & Angersbach, 2002). The application of an external electrical field to biological cells induces an electrical potential across the cell membrane which leads to an electrical breakdown and local structural changes of the cell membrane. PEF application leads to cell membrane permeabilisation but the nature of damage and its relationship with cell death depends on the bacterial species and the pH of the treatment medium. At pH 7 the loss of viability is, depending on the bacterial strain, correlated with the sum of non-permanent and permanent membrane permeabilisation or with a permanent loss of membrane integrity (García, Gómez, Mañas, Raso, &

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Pagán, 2007). As the number of inactivated microorganisms could be higher than cells with irreversibly permeabilised membranes, permeabilisation might be resealed (Aronsson, Rönner, & Borch, 2005; García et al., 2007). The application of PEF at higher temperatures proved to be more effective than PEF at low temperatures (Aronsson & Rönner, 2001; Heinz, Toepfl, & Knorr, 2003; Saldaña, Monfort, Condón, Raso, & Álvarez, 2012; Sepulveda, Góngora-Nieto, San-Martin, & Barbosa-Cánovas, 2005). Thus, PEF technology may be effectively used as an enhanced mild thermal preservation method. Heat is generated volumetrically throughout the treated liquid. Elevated product heating of preheated liquids during PEF application can only be avoided either by limiting the number of applied pulses or by using heat exchangers between treatment chambers or treatment steps (Sepulveda et al., 2005; Toepfl, Heinz, & Knorr, 2007).

So far, there have been only few investigations on the impact of PEF on the durability and safety or on sensory parameters of blood and no investigations concerning blood plasma (Boulaaba, Egen, & Klein, in press; Kiessling & Töpfl, 2012). Hence, the objective of this study was to investigate the effect of PEF on the inactivation of inoculated microorganisms and total aerobic plate count. Gelling properties were analysed to determine the impact of PEF on plasma proteins.

2. Material and methods

2.1. Plasma collection

Porcine blood plasma was retrieved from Sonac Loenen B. V. (Loenen gld, the Netherlands). The company utilised whole porcine blood, hygienically collected in a sticking carousel at commercial slaughterhouses. The anticoagulant trisodium citrate dihydrate was simultaneously added to the blood to achieve a final blood citrate concentration of 0.3% (wt/vol) citrate. Plasma was separated by centrifuging blood using a continuous disc centrifuge (GEA Westfalia separator, Oelde, Germany). Plasma was then packed in 20-litre bag-in-box-systems and transported to the German Institute of Food Technologies e. V., Quakenbrück, Germany (DIL). Immediately after arrival at the DIL, the plasma was stored under cooling conditions (≤3 °C) until PEF treatment was applied on the following day with the pilot plant ELEA® HVP5.

2.2. 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 coaxial continuous treatment chambers. The chambers were composed of two titanium electrodes separated by a gap of 7 mm. The apparatus generated approximately square waveform pulses of a width of 20 μs. Inoculated plasma was, dependent on the electrical conductivity of the native plasma, PEF-processed at electric field strengths between 9 and 13 kV/cm. The same settings were used for each plasma batch. In two plasma batches the electric field strength was, depending on the applied energy input, a range between 9 and 11 kV/cm. The selected frequencies of 50, 100, 150, 200, 250 and 300 Hz corresponded to specific energy densities between 40 and 182 kJ/kg (Table 1). The flow rate was fixed at 50 L/h. The temperature of the blood plasma was adjusted shortly before PEF application. Thus, plasma temperature ranged from 30 ± 2 °C at the PEF chamber inlet to temperatures between 39 ± 2.6 °C and 70 ± 6.5 °C, depending on the specific energy input, at the chamber outlet. A heat exchanger was used to cool the plasma down to 21 °C directly after processing. Samples of processed plasma were placed in a styrofoam box filled with ice prior to microbiological and physical analysis. The pH values and the electrical conductivity (Table 2) of the native blood plasma were determined at 20 °C using a battery-powered pH metre (S 20, Seven Easy™pH, Mettler Toledo, Gießen, Germany) and an electrical conductivity measuring instrument (Type LF, Fa. WTW, Weilheim, Germany).

2.3. Inoculation of indicator microorganisms

To prove the lethal effectiveness of pulsed electric fields (PEF), we used *Escherichia (E.) coli* K12 (DSMZ 498), *Staphylococcus (S.) xylosus* (DSMZ 20266) *and Pseudomonas (P.) fragi* (DSMZ 3456) as indicator microorganisms. These were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Microorganisms were transferred 24 h before each experiment from stock cultures (CRYO-Bank, −70 °C) to Caso-Bouillon CM 0129b (Tryptone Soy Broth from Oxoid, Wesel, Germany). Cells were activated under agitation and enhanced for 24 h at 37 °C (*E. coli*, *S. xylosus*) and for 48 h at 30 °C (*P. fragi*), respectively (Kiessling & Töpfl, 2012). Thus, a bacterial count in the respective suspensions of approximately 10^8 CFU/mL resulted. From these suspensions 10 mL was used for inoculation of 1 L of blood plasma. Native and PEF treated blood plasma was sampled for the total plate count (TPC) before inoculation with indicator microorganisms. For microbiological examinations of the indicator microorganisms samples were taken after inoculation and before PEF treatment to determine the initial cell number (at an average of 10^6–10^7 CFU/mL plasma) and directly after PEF application. The samples were collected in sterile blender bags (Bag light, interscience, Saint Nom la Bretèche, France) and placed on ice immediately after treatment. The total plate count (TPC) was determined using the pour plate technique on plate count agar and incubated at 30 °C for 3 days (detection limit 10 CFU/mL, microbial counts below the detection limit were integrated in the calculation with 5 CFU/mL). The enumeration of *E. coli* K12 was conducted by the spread plate method using the Fluorocult Mc Conkey Agar (Merck, KgaA, Darmstadt, Germany) and incubation at 37 °C for 24 h. *S. xylosus* was enumerated on Baird–Parker–Agar Base CM 275 (Oxoid GmbH, Wesel, Germany, spread plate method) and incubated at 37 °C for 48 h. *P. fragi* were determined using the spread plate method with the Pseudomonas Agar Base CM 559 (Oxoid GmbH, Wesel, Germany). The plates were incubated at 30 °C for 72 h. By using the spread plate method, the lower detection limit was 100 CFU/mL. Microbial counts below the detection limit were integrated with 50 CFU/mL in the calculation. Generally, the survival ratio of microorganisms S was defined as:

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

where N_t represents the number of surviving microorganisms after a session of PEF treatment, and N_0 is the initial number of microorganisms

Table 1
Influence of the selected frequency on treatment intensity, specific energy input and temperature of blood plasma at field strengths between 9 and 13 kV/cm.

Pulse frequency (Hz)	0	50	100	150	200	250	300
Treatment time (μs)	0	39	78	116	155	194	233
Temperature (°C)[a]	–			30 ± 2 °C			
Temperature (°C)[b]	–	39.0 ± 2.6	47.0 ± 4.2	56.2 ± 4.0	59.7 ± 4.5	65.7 ± 6.1	70.0 ± 6.5
Specific energy input (kJ/kg)	–	38 ± 6	68 ± 12	100 ± 14	117 ± 13	139 ± 20	157 ± 21

pH = 7.71 ± 0.19; electrical conductivity = 11.92 ± 1.42.
[a] Before treatment.
[b] Outlet temperature after PEF treatment.

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