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Inactivation of *Penicillum expansum* in sour cherry juice, peach and apricot nectars by pulsed electric fields

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ABSTRACT

Inhibitory effects of pulsed electric fields (PEF) on *Penicillum expansum* inoculated into sour cherry juice, apricot and peach nectars were determined based on germination tube elongation, spore germination rate, and light and scanning electron microscopy (SEM) observations in this study. After inoculation of juice/nectar samples with *P. expansum* spores at the level of 10^5–10^6 cfu/mL, the samples were processed by bench scale PEF pulse generator as a function of differing electric field strengths (0, 13, 17, 20, 23, 27, 30 and 34 kV/cm) and processing times (0, 62, 94, 123, 163, 198 and 218 μs). Results revealed that with an increase in electric field strength and processing time, germination tube elongation and spore germination rate were completely inhibited. Light and SEM observations revealed considerable morphological alterations in fungal conidia such as cytoplasmic coagulation, vacuolations, shrinkage and protoplast leakage. PEF processing of juice/nectars was demonstrated to be effective in inactivating *P. expansum*. To our knowledge, this is the first study confirming the inhibitory effects of PEF on germination tube elongation and spore germination rate of *P. expansum* in fruit juice/nectars.

1. Introduction

Pulsed electric fields (PEF) is a nonthermal food pasteurization method than can be used for microbial inactivation of liquid and semi-liquid food products with minimal or no detrimental changes in their nutrient value, physical characteristics and sensory properties (Zhang et al., 1995; Mertens and Knorr, 1992). Application of electric fields strength can range from 20 to 80 kV/cm (Martin, 1997). Fruit juices including apple juice (Evrendilek et al., 1999; Sen Gupta et al., 2005), cranberry juice (Jin and Zhang, 1999), orange juice (McDonald et al., 2000; Yeom et al., 2000), tomato juice (Min et al., 2003) and grape and pineapple juice (Raso et al., 1998) were processed by PEF to determine changes in microbial inactivation, quality and shelf life extension. Results of these studies revealed that PEF processing alone or in combination with mild heat treatment was a successful option for the pasteurization of the food products processed.

Bacteria inactivated by PEF studies included *Escherichia coli, E. coli* O157:H7, *Salmonella enteritidis*, *Listeria monocytogenes,* lactic acid bacteria, and yeasts such as *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* (Dunn and Pearlman, 1987; Pothakamury et al., 1995; Raso et al., 1998; Reina et al., 1998; Hermawan et al., 2004; Molinari et al., 2004; Elez-Martinez et al., 2005). Inactivation effects of PEF have been mostly conducted on human or food borne bacterial pathogens. PEF-inactivation of molds include inactivation of *Byssochlamys fulva* conidiospores and *Neosartoria fischeri* ascospores suspended in apple, orange, pineapple, cranberry, grape and tomato juices (Raso et al., 1998) and *Byssochlamys nivea* ascospores in malt extract agar (Grahl and Markl, 1996). However, there is a lack of information concerning the use of PEF treatments against fungal plant pathogens.

Therefore, efforts have focused on possible uses of PEF against fungal pathogens such as *Penicillum expansum* in different fruit juice/nectars. Blue mold caused by *P. expansum* is the most important post-harvest decay across the world of stored fruits (Eckert, 1990). The blue mold spores are long-lived and may easily survive in different seasons on contaminated places, where the fungus can grow and produce copious amounts of spores. Contamination can also occur during handling of fruit in water with the fungus in packinghouses. *P. expansum* not only causes fruit decay, but also produces carcinogenic mycotoxin patulin (Morales et al., 2007). Post-harvest treatment of fruits with fungicides has been traditionally the most common method of combating blue mold. Killing spores in dump tanks, on bins, or in flume water with chlorine or sodium *O*-phenylphenate (SOPP) has been effective in reducing the spore load, and thus, the amount of fruit decay (Janisiewicz, 1999).

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Since such chemical applications cannot be used in fruit juices, alternative methods of controls are needed. Therefore, the objective of the study was to evaluate a PEF treatment as a means to control *P. expansum* inoculated in sour cherry juice, apricot and peach nectars by spore germination rate and germination tube elongation. As spores are the primary source of initial mold contamination, spore germination rate is determined. If the contaminated spores are capable of germination, they cause infection on the fruit. Alteration is dependent upon ability of contamination spores to germinate (El Halouat and Debevere, 1997). Thus, germination is measured by germination tube elongation.

2. Materials and methods

2.1. Test microorganism

P. expansum was isolated from infected apple fruit and maintained in potato dextrose agar (PDA) (Merck, Germany). The culture was stored at 4 °C and sub-cultured once a month. Spore suspension was prepared from 2-week-old PDA culture. The spores were removed from the surface of the culture, suspended in 1000 mL sour cherry juice, apricot and/or peach nectar separately. Spore concentration was determined using a heamocytometer and adjusted to 10^5–10^6 spores/mL (Soylu et al., 2006).

2.2. Food samples

Sour cherry, apricot and peach concentrates were obtained from Dimes Gıda Sanayii ve Tic. A.Ş. (Tokat, Turkey) in aseptic pouches. Sour cherry concentrate was diluted to 13.5 °Brix, while apricot and peach concentrates were diluted to 11 and 10 °Brix, respectively. After preparation, the fruit juice/nectars were immediately processed by PEF.

2.3. PEF processing unit

A bench scale continuous PEF system [OSU-4A] (Evrendilek et al., 1999) was used to treat inoculated samples. Six treatment chambers connected in series were integrated into the PEF system (Fig. 1). Each treatment chamber was constructed with a treatment zone of 0.29 cm in diameter and 0.23 cm in gap distance. The electrode material was stainless steel, and the insulator was made of Delrin. The contact between the electrode and insulator was air tight based on a specific design to avoid sample to be trapped. The OSU-4A bench scale PEF generator provided square wave bipolar pulses. There was one positive and one negative square wave pulses apart from one another at 20 μs pulse delaying time. The pre- and post-treatment temperatures (t_2–t_1, t_4–t_3 and t_6–t_5) at inlet and outlet of each pair of the treatment chambers were monitored by K-type dual channel digital thermocouples (Fisher Scientific, Pittsburgh, PA, USA) attached to the outer surface of the thin wall stainless steel tubing. The K-type thermocouple probe used had a time constant of 0.1 s and a response time of 0.5 s.

The treated sample was cooled after each pair of chambers by cooling coils submerged in a water bath at 12 °C (Model RTE-111, NESLAB Instruments Inc., Newington, NH, USA). A pulse generator (Model 9300 series, Quantum Composers, Inc., Bozeman, MT, USA) was used to control pulse duration time, pulse delaying time and pulse repetition rate. Applied voltage and current were monitored by a two-channel digital oscilloscope (Model TDS 320, Tektronix Inc., Beaverton, OR, USA). Specifications of the OSU-4A bench scale PEF units were 12,000 max of output voltage, 60 A max of output current, 10,000 pulse per second (pps) max of repetition rate, 200–1200 Ω of load resistance and 16 J of energy storage in the pulse generator when fully charged. Preliminary tests were performed to determine PEF treatment conditions for sour cherry juice, peach and apricot nectars.

Before and after each trial, tap water was pumped at a flow rate of 40 mL/min for 5 min to remove all the remaining samples for cleaning the system. A 10% (v/v) household bleach solution was pumped through the system until pHydrion Micro Chlorine strip (LaMotte, Chestertown, MD, USA) dipped into the sample outlet port indicated that the chlorine concentration was more than 200 ppm. The system was soaked with this bleach solution for at least 5 min. Sterile de-ionized water was pumped through the system until pHydrion Micro Chlorine strip dipped into the sample outlet port indicated that the chlorine concentration was 0 ppm. The uninoculated sample was pumped through the system for further cleaning from any remaining chlorine residues. Then the entire system was flushed and filled with the uninoculated sample.

2.4. PEF processing parameters

PEF processing parameters were applied as a function of electric field strength and treatment time. For this purpose, 0 (control), 17, 20, 23, 27, 30 and 34 kV/cm electric field strengths were applied with 45 mL/min of flow rate, 3 μs of pulse duration, 500 pulses per second (pps) of frequency and 163 μs of treatment time. Similarly, 0 (control), 62, 94, 123, 163, 198 and 218 μs treatment times were applied with 40 mL/min of flow rate, 3 μs of pulse duration and 17 kV/cm of electric field strength.

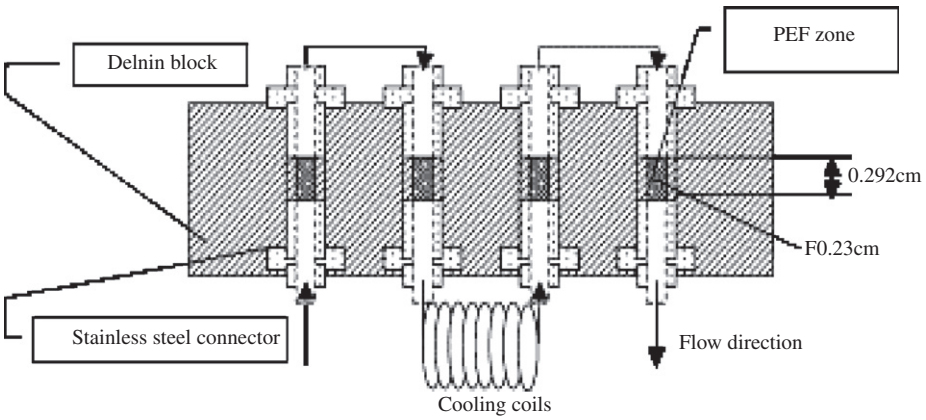

Fig. 1. Diagrammatic drawing of co-field flow treatment chambers for OSU-4 PEF systems.