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Influence of treatment time and pulse frequency on *Salmonella* Enteritidis, *Escherichia coli* and *Listeria monocytogenes* populations inoculated in melon and watermelon juices treated by pulsed electric fields

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Abstract

Consumption of unpasteurized melon and watermelon juices has caused several disease outbreaks by pathogenic microorganisms worldwide. Pulsed electric field (PEF) has been recognized as a technology that may inactivate those bacteria present in fluid food products at low temperatures. Hence, PEF treatment at 35 kV/cm, 4 µs pulse duration in bipolar mode and square shape were applied on *Salmonella* Enteritidis, *E. coli* and *L. monocytogenes* populations inoculated in melon and watermelon juices without exceeding 40 °C outlet temperatures. Different levels of treatment time and pulse frequency were applied to evaluate their effects on these microorganisms. Treatment time was more influential than pulse frequency ($P \leq 0.05$) on the PEF microbial reduction levels for both melon and watermelon juices. Populations of *S.* Enteritidis, *E. coli* and *L. monocytogenes* were experimentally reduced and validated in a single process up to 3.71±0.17, 3.7±0.3 and 3.56±0.26 $\log_{10}$ units, respectively, in melon juice when 1440 µs and 217 Hz were used; whereas reductions up to 3.56±0.12, 3.6±0.4 and 3.41±0.13 $\log_{10}$ units of those microorganisms, respectively, were reached in watermelon juice treated for 1727 µs at 188 Hz. Although PEF treatment reduced the populations of the three microorganisms, *L. monocytogenes* was more resistant to PEF than *S.* Enteritidis and *E. coli* in both juices when treated at the same processing conditions.

Keywords: PEF; Treatment time; Pulse frequency; *Salmonella* Enteritidis; *E. coli*; *L. monocytogenes*; Melon; Watermelon; Juice

1. Introduction

Melon and watermelon products are regarded as potentially hazardous foods by the Food and Drug Administration (FDA) (FDA, 2001) because they may favor the growth of pathogenic microorganisms due to their low acidity (pH 5.2 to 6.7) and high water activity (0.97 to 0.99). Outbreaks of *Salmonella* spp. and *E. coli* O157:H7 have been linked with the consumption of fresh-cut as well as juice of melon and watermelon (CDC, 1991; Mohle-Boetani et al., 1999; Powell and Leudtke, 2000; CDC, 2001; Meng et al., 2001; FDA, 2001; CDC, 2002). The majority of outbreaks are linked to the presence of these pathogens on the fruit rind, presumably contaminated in the field by improperly composted fertilizer, irrigation with infected water or through infected workers (FDA, 2001). Hence, these pathogenic microorganisms can be transferred to the edible tissues and juices when melons and watermelons are cut during preparation (Ukuku and Sapers, 2001; Sharma et al., 2005). Incidence, survival and growth of *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* on watermelon and melon slices and juices have been reported by several researchers (Fernandez Escartin et al., 1989; Golden et al.,1993; Del Rosario and Beuchat, 1995; Penteado and Leitao, 2004; Eswaranandam et al., 2004). Nowadays, fresh juices from those fruits are sold without pasteurization and, thus, they could be potential sources of pathogenic microorganisms such as *Salmonella, E. coli* and *Listeria* which at low doses (1–100 cells ml^{-1}) may produce illness (D'Aoust et al., 2001; Meng et al., 2001; Swaminathan, 2001; Bell and Kyriakides, 2002a,b,c).

These pathogenic microorganisms can be easily eliminated through heat, but sensorial and nutritional attributes are

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Nomenclature

Q	Is the electric energy density input (J/cm^3)
V	Is the peak voltage (V)
I	Is the intensity of current (A)
Tt	Is the treatment time (s)
v	Is the total volume of all treatment chambers (cm^3)
Y	Is the maximum microbial reduction obtained after PEF treatment ($\log_{10}$ CFU/ml)
F	Is the pulse frequency (Hz)
K	Is a constant of the Eq. (2)
A	Is a regression coefficient of treatment time (μs^{-1})
B	Is a regression coefficient of pulse frequency (Hz^{-1})
C	Is a regression coefficient between treatment time interactions (μs^{-2})
D	Is a regression coefficient between pulse frequency interactions (Hz^{-2})
E	Is a regression coefficient between treatment time and pulse frequency interaction (μs^{-1}·Hz^{-1})

extensively damaged (Jeyamkondan et al., 1999; Espachs-Barroso et al., 2003; Elez-Martínez and Martín-Belloso, 2005). Nevertheless, significant efforts are leading to the development of novel non-thermal processes. One of them is the use of pulsed electric fields (PEF) as an alternative preservation process for fluid foods. The aim of this technology is to inactivate spoilage and pathogenic microorganisms and to decrease the activity of enzymes in order to extend the shelf life and safety of foods without undesirable heat and chemical effects (Cserhalmi, 2006). Moreover, the organoleptic and nutritional properties seem to be maintained after PEF treatment (Hodgins et al., 2002; Cserhalmi et al., 2006; Elez-Martínez et al., 2006a,b). The effectiveness of PEF treatment for inactivating or reducing of some strains and serovars of *Salmonella* spp. and *E. coli* in some fruit juices has been studied (Evrendilek et al., 1999; Iu et al., 2001; Liang et al., 2002; Evrendilek and Zhang, 2005; Zhong et al., 2005; Mosqueda-Melgar et al., 2006). Evrendilek and Zhang (2005) have reported that bipolar pulses were more effective than monopolar pulses for reducing *E. coli* O157:H7 in apple juice. Iu et al. (2001) and Liang et al. (2002) obtained a higher inactivation of *Salmonella* Thyphimurium and *E. coli* O157:H7 populations in orange juice and apple cider, respectively, when higher number of pulses and electric field strength were applied. On the other hand, Evrendilek et al. (1999), Zhong et al. (2005) and Mosqueda-Melgar et al. (2006) reached higher microbial inactivation of *E. coli* O157:H7, *E. coli* and *Salmonella* Enteritidis in several fruit juices when treatment time was increased. However, studies on *L. monocytogenes* inactivation in fruit juices by PEF treatment were not found in the literature, although its incidence, survival and growth in fresh-cut as well as pulp of melon and watermelon has been reported (Penteado and Leitao, 2004; Eswaranandam et al., 2004). Thus, the inactivation of *L. monocytogenes* by PEF in these fluid foods represents a new challenge to the fruit and derivatives industry.

The aims of this study were to evaluate the effect of the treatment time and pulse frequency, as variable parameters of PEF treatment, on *S.* Enteritidis, *E. coli* and *L. monocytogenes* populations inoculated in melon and watermelon juices, as well as to obtain optimized values of these processing factors for the standardization of the PEF treatment.

2. Materials and methods

2.1. Juice preparation

Melon (*Cucumis melo* var. "Piel de sapo") and watermelon (*Citrullus lanatus* var. "Seedless") fruits at commercial ripeness were selected in a supermarket of Lleida, Spain. The fruits were washed, peeled and cut into pieces. Then juices were made through an Ufesa blender (Model BP 4512; Vitoria, Spain) and centrifuged at 12,500 rpm for 15 min at 4 °C in an Avanti™ J-25 Centrifuge (Beckman Instrument, Inc.; USA). The supernatant juice was filtered, bottled and autoclaved in a Presoclave 75 (J.P. Selecta, S.A; Barcelona, Spain) at 121 °C for 15 min. Finally, the samples were stored at refrigeration temperature (5 °C) until inoculation and PEF treatment.

2.2. Physicochemical analysis of the juices

Electric conductivity (Testo 240 conductivimeter; Testo GmBh & Co; Lenzkirch, Germany), pH (Crison 2001 pH-meter; Crison Instruments S.A; Barcelona, Spain) and soluble solid content (Atago RX-1000 refractometer; Atago Company Ltd., Japan) were measured. pH and soluble solid were carried out according to the B.O.E (1988) (Table 1).

2.3. Microbial culture preparation

Pure cultures of *S.* Enteritidis 1.82 (National Collection of Type Culture (NCTC) 9001, PHLS Central Public Health Laboratory; London, UK) and *E. coli* 1.107 (Laboratoire de

Table 1
Analytical parameters of melon and watermelon juices

Parameters (unit)	Values[a]	
	Melon juice	Watermelon juice
Electrical conductivity (mS/cm)	5.23 ± 0.03	3.66 ± 0.05
Soluble solids(%)	11.1 ± 0.0	6.5 ± 0.0
pH	5.82 ± 0.04	5.46 ± 0.11

[a]Results are the mean ± standard deviation of three measurements.