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Effectiveness of combined Pulsed Electric Field (PEF) and Manothermosonication (MTS) for the control of *Listeria innocua* in a smoothie type beverage

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ABSTRACT

The combination of novel, non-thermal technologies for preservation purposes is a recent trend in food processing research. The objectives of the current study were (i) to optimise PEF or MTS treatment conditions which would achieve a maximum reduction of up to 3 log cycles of *Listeria innocua* in a milk based smoothie, when these technologies were applied individually, and (ii) to investigate possible additive or synergistic effects of the combined technologies. Microbiological analysis was performed by inoculating the smoothie with *L. innocua* and enumerating populations pre- and post-processing. All technologies applied within combinations significantly reduced *L. innocua* in the smoothie, when compared to untreated controls ($p \leq 0.0001$). The sequence in which the MTS and PEF were applied was found to have a significant impact on the level of microbial reduction achieved ($p \leq 0.05$). The sequence of MTS followed by PEF was the most effective in inactivating *L. innocua* achieving a mean reduction of 5.6 log cfu/ml, thereby exceeding the 5 log cycles minimum requirement specified by the United States Food and Drug Administration (US FDA). Significantly ($p \leq 0.05$) lower reductions of 4.2 log cfu/ml were achieved when the PEF + MTS sequence combination was applied. The combination of MTS + PEF achieved inactivation comparable to thermally treated samples ($p > 0.05$). This study has shown the combination MTS + PEF is a promising hurdle preservation approach to control undesirable microorganisms in milk based smoothie beverages.

1. Introduction

Fresh fruit based beverages such as smoothies have become increasingly popular in recent years and are considered to be high quality, healthy and nutritious products with a fresh-like flavour and appearance (Leistner & Gorris, 1995). However, their shelf life can be short due to their ability to support microbial growth as a result of the minimal level of processing associated with these products. Such microbial activity can cause economic losses for the food producer by causing undesirable organoleptic changes and also may be potentially harmful to the health of consumers. Smoothies have gained in popularity in recent times among consumers and can offer a means of fulfilling the daily intake of fresh fruit as recommended by nutritionists (Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2008). This as led to increasing research focus on non-thermal processing technologies for preservation purposes, since conventional thermal processing, the most extensively used method of preservation, can adversely affect the quality characteristics of food (Lado & Yousef, 2002). It has been suggested that non-thermal technologies such as PEF and MTS have great potential as preservation treatments for liquid products such as milk and fruit juices (Guerrero-Beltrán, Sepulveda, Góngora-Nieto, Swanson & Barbosa-Cánovas, 2010).

Microbial inactivation by PEF is based on electroporation of cell membranes, causing reversible or irreversible pore formation depending on the electric field intensity. Microbe inactivation is assumed to start when the voltage drop across the membrane exceeds 1 V, which is created by electric fields of the order of 20 kV/cm. This results in loss of the semipermeability properties of the cell membranes, altering homeostasis and causing cell death (Weaver & Chizmadzhev, 1996).

Ultrasound is another non-thermal technology which has attracted interest by researchers and industry in recent years, particularly when applied in conjunction with heat (thermosonication TS) or heat and pressure (manothermosonication MTS) (Piyasena, Mohareb, & McKellar, 2003). When ultrasound, at high power and low frequency (20–100 kHz) propagates in liquid media, resulting in longitudinal waves, cavitation is induced, which

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consists of the formation, growth and sudden implosion of bubbles. The implosion of bubbles generates localised spots with very high pressures (104–105 kPa) and temperature (5500 °C) that can disrupt cellular structures (Demirdoven & Baysal, 2009).

The objective of the current study was to assess the inactivation of *Listeria innocua* in a milk based smoothie using the individual technologies PEF or MTS as well as the hurdle combinations: comprising PEF + MTS and the reverse sequence MTS + PEF. *L. innocua* was chosen as a surrogate for *Listeria monocytogenes* as this pathogen represents a major public health concern and has been associated with illness outbreaks originating in ready to eat foods (Jemmi, & Stephan, 2006).

2. Materials and methods

2.1. Smoothie preparation

A blend of fresh juices consisting of 35% orange, 20% pineapple, 25% mango, 20% apple juice (v/v) was obtained from a local juice manufacturer (Keeling Fresh Juices, Dublin, Ireland) and stored at −20 °C. The frozen blend of juices was thawed overnight and then filtered through a stainless steel kitchen mesh to remove larger pulp particles. Low fat milk (Avonmore, Dublin, Ireland) was added at 20% v/v. The smoothie was homogenised using a Silverson mixer (L4RT, Chesham, Bucks, U.K.) at 8000 rpm for total time of 8 min and then autoclaved at 110 °C for 10 min.

2.2. Bacterial strains, culture conditions and enumeration

Experiments were performed using *L. innocua* NCTC 11288. Prior to usage, the cultures were maintained in glycerol at −20 °C. *L. innocua* was cultured in 300 ml of Tryptone Soya Broth (TSB) (Oxoid, Basingstoke, Hampshire, UK) for 24 h at 37 °C. Following incubation cultures were centrifuged (Sigma, Model No. 4K15, DJB Labcare Ltd, Buckinghamshire, England) at 8000 rpm (Rotor No. 12256, producing a relative centrifugal force 10,375 × *g*) for 10 min at 4 °C and the resulting pellets were added to 1000 ml of juice to give an initial concentration of approximately 10^7 cfu/ml. Viable counts of cells before and after processing were estimated by preparing 10-fold dilutions and plating out on Tryptone Soya Agar (TSA) (Oxoid) for total bacterial counts and *Listeria* Selective Agar (LSA) with selective supplement (Oxoid) for *L. innocua.* Duplicate plates were incubated at 37 °C, for 48 h and the inactivation of vegetative cells was evaluated by calculating the difference between the viable counts in treated and untreated samples.

2.3. Processing

2.3.1. Pulsed electric fields

The smoothie was processed in a pilot scale PEF system (ELCRACK HVP 5, DIL, German Institute of Food Technologies, Quackenbruck, Germany) with bipolar square-wave pulses through an electrode gap of 3 mm. The system maximum voltage was 25 kV, the maximum frequency was 1 kHz and the pulse width was adjustable between 4 and 32 μs. The system consisted of two pairs of co-linear treatment chambers with each pair followed by a refrigerated cooling module (−5 °C). The system was connected to a digital oscilloscope (Model No. TDS 2012, Tektronix, Beaverton, OR, USA). Temperatures were monitored by two thermocouples (Model No.925, Testo AG, Lenzkirch, Germany) with a pipe wrap-type probe attached to the surface of the stainless-steel tubes at the inlet and outlet points of unit. Recorded temperatures did not exceed 35 °C. The smoothie was pumped through the system using a peristaltic pump (Model No. L/S 77200-60, Masterflex, Cole-Parmer Instruments, Illinois, USA) at a flow rate of 160 ml/min. Volumes (50 ml) of sample were collected in sterilized containers and kept in ice until analysis.

2.3.2. Manothermosonication

Ultrasound treatments were conducted using an ultrasonic processor (Hiescher UIP1000hd, Teltow, Germany), with a frequency of 20 kHz, maximum amplitude of 31 μm and intensity 40 W/cm^2. The sonotrode (Model No. BS2d40) was fitted with a booster (B2-1.8) and placed in a stainless steel flow cell (Model No. FC100L1 K-1S). The pressure applied was 200 kPa gauge. The preheating temperature was set at 35 °C. A circulating water bath (Model No. LTD20 G, Grant, Instruments Ltd., Cambridge, UK) maintained at −15 °C was used to recirculate coolant through the jacket surrounding the ultrasonic processing chamber to maintain a target outlet temperature of 52 °C. After treatment the smoothie samples were further refrigerated by passing through a coil submerged in iced water. The temperature was monitored at the inlet and outlet points at 1 s intervals using a T type thermocouple and data logger (Squirrel SQ 2020, Grant Instruments Ltd., Cambridge, UK).

2.3.3. Thermal processing

For thermal treatment (used as a control) the inoculated smoothie was passed through a tubular heat exchanger (Model No. FT74 T, Armfield, Ringwood, UK) at a flow rate of 94 ml/min. The temperature of the holding tube was set at 72 °C with a residence time of 26 s. The time and temperature profiles were monitored using the logging system supplied with the unit.

2.4. Experimental treatments and design

2.4.1. Application of individual non-thermal hurdles

The smoothie was exposed to MTS or PEF using the equipment described in Section 2.3, under the conditions shown in Table 1, in order to establish the capability of the individual hurdles to inactivate *L. innocua* under various treatment conditions. The objective was to obtain an inactivation of the order of 3 log cycles when the hurdles were applied individually. A 3 × 2 factorial design was used with 3 energy levels for each one of the two factors. Residence time and amplitude were used as factors for MTS with pressure and temperature set as constants at 200 kPa and 35 °C respectively. Pulse amplitude and field strength were variable factors for PEF while using a constant residence time and pulse frequency as illustrated in Table 1. All the treatments were carried out in triplicate.

2.4.2. Application of paired non-thermal hurdles

Following the preliminary experiments, conditions that produced between 2 and 3 logs of inactivation for MTS and PEF as stand-alone technologies were selected to study their combined effect (synergistic, additive or antagonistic) when applied in combination. The smoothie was treated by a combination of PEF (34 kV/cm, 32 μs) and MTS (100%, 160 ml/min, 200 kPa) and by the reverse sequence in continuous systems. Thermally pasteurised smoothie was used as a control. All treatments were carried out in triplicate.

Table 1
Pulsed Electric Field (PEF) and Manotheromosonication (MTS) processing conditions applied to inactivate of *L. innocua* in the smoothie.

Factors		Levels		
PEF	Electric field (kV/cm)	18	25	34
	Pulse width (μs)	12	24	32
MTS	Amplitude (%)	50	75	100
	Residence Time (min)	2.1	1.0	0.7