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Inactivation of *Listeria monocytogenes* and *Listeria innocua* in yogurt drink applying combination of high pressure processing and mint essential oils

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ABSTRACT

Influences of high pressure processing (HPP) on some physical properties and on inactivation of *Listeria monocytogenes* and *Listeria innocua* in a yogurt drink (ayran) were quantified with or without addition of mint essential oil. Pressure treatment alone or combined with mint essential oil did not cause significant changes in pH, water activity, color and serum protein separation ($p > 0.05$). HPP of ayran samples at 600 MPa for treatment time of 300 s reduced *L. monocytogenes* and *L. innocua* by more than 5-log units ($p \leq 0.05$) at ambient temperature. Addition of mint essential oil further enhanced inactivation of both bacteria by more than 1 log cfu mL^{-1}. Combination of mint essential oil with HPP provided a reduction in pressure treatment severity by 100–300 MPa or by 210 s to achieve the same amount of inactivation relative to HPP alone. Both Weibull distribution and log-logistic models were fitted to survival data. High pressure-processing combined with mint essential oil appeared to be a promising technique for preserving microbiologically-safe ayran with no significant impacts to product quality.

1. Introduction

Non-thermal food preservation technologies, and especially, high pressure processing (HPP) have drawn considerable attention among scientists, food industry, and consumers owing to their involvement of minimal food processing with fewer preservatives. The commercial use of HPP has been increasingly and successfully applied to various foods such as dairy, meat, seafood, poultry products, vegetables and vegetable products, fruit products, and acidified products (Ahmed, Ramaswamy, & Hiremath, 2005; Aymerich, Jofre, Garriga, & Hugas, 2005; Fernández et al., 2007; Laboissière et al., 2007; Trujillo, Capellas, Saldo, Gervilla, & Guamis, 2002).

Ayran as a popular traditional yogurt drink in Turkey, is prepared by mixing yoghurt, water and salt. Salt is added at a maximum level of 1 g 100 g^{-1} to impart flavor (TSE, 1982). With the increasing demand for ayran, it is now industrially produced from homogenized and pasteurized milk (90 or 95 °C for 5 or 10 min). A few studies existed about microbial efficacy of HPP treatment on yogurt drinks (Walker, Farkas, Loveridge, & Meunier-Goddik, 2006). Pressure treatment was used to eliminate typical yogurt micro biota to reduce acidification process, increase the products' shelf life (Trujillo et al., 2002) and inactivate lactic acid bacteria in fruit yogurt samples (Walker et al., 2006).

Listeria monocytogenes is a foodborne pathogen that causes outbreaks in different food products including dairy products, vegetables, meat and fish products (McLauchlin, Mitchell, Smerdon, & Jewell, 2004). Multiple listeriosis outbreaks were linked to contaminated cheese and other dairy products (Ho, Lappi, & Wiedmann, 2007). However, to our knowledge, there exists no prior study that investigated survival of *L. monocytogenes* in yogurt-based drinks using pressure treatments.

Antibacterial and antifungal activities of plant essential oils were reported against various foodborne pathogens and spoilage organisms (Franzios et al., 1997). The antimicrobial effects of plant essential oils against microorganisms can be influenced by their composition, processing method, and type of microorganisms (Adam, Sivripoulou, Kokkini, Lanaras, & Arsenakis, 1998). When plant essential oils are combined with pressure treatment, it may help to reduce process severity. A few studies evaluated efficacy of pressure treatment alone or combined with plant essential oils in dairy products. Therefore, the objective of the study was to evaluate efficacy of pressure treatment both alone and in combination with mint essential oil on inactivation of *L. monocytogenes* and *L. innocua* inoculated in ayran samples. Color, pH, water activity, and serum protein separation were also measured.

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2. Materials and method

2.1. Preparation of bacterial cultures

L. monocytogenes (ATCC 19115) and *L. innocua* (ATCC 33090) cultures were obtained from the Department of Microbiology, The Ohio State University (Columbus, OH, USA) in trypticase soy agar (TSA) (Becton Dickinson, Maryland, USA) slants. The cultures were transferred into trypticase soy broth (Becton Dickinson) and incubated at 30 ± 2 °C overnight.

2.2. Preparation and extraction of mint essential oil

Leaves and aerial parts of mint (*Mentha piperita*) were purchased from a local store in Antakya (Hatay, Turkey). Leaves were separated from their stem parts, and air-dried until use. Air-dried plant parts were steam-distilled for 2 h using a Clevenger-type apparatus (Ildam, Ankara, Turkey) as per European Pharmacopoeia (1975, p.68) guidelines. The essential oils were stored at 4 °C in dark colored air-tight glass vials covered with aluminum foil.

2.3. Preparation of ayran samples for pressure treatment

Full–fat yogurt (12.10 g 100 g^{-1} total solid, 3.25 g 100 g^{-1} total lipid, 4.66 g 100 g^{-1} total sugar, 3.47 g 100 g-^{1} protein and 0.72 g 100 g^{-1} ash) was purchased from local stores (Columbus, OH, USA). Yogurt and water were mixed at the ratio of 1:1 and stomached for 30 s. Salt was added with water at the ratio of 1 g 100 g^{-1}. Ayran samples were inoculated with either *L. monocytogenes* or *L. innocua* cultures at the level of *ca.* 10^5–10^6 CFU mL^{-1}. The inoculated samples (100 mL) were aseptically packaged into sterile pouches (CN-530 film, Cryovac, Sealed Air Corp., Duncan, SC). After the removal of air bubbles, the pouches were heat sealed. The sample pouches were then placed in a high barrier film bag (P640B film, Cryovac, Sealed Air Corp., Duncan, SC, USA) and heat-sealed. The sample pouches were then placed inside a larger high barrier film bag and vacuum packed at −97 kPa using an MC-30 sealer (Sipromac Inc., St. Germain Grantham, Quebec, Canada). An additional set of experiments was also carried out to evaluate efficacy of combining mint essential oils and pressure treatment. The mint essential oil was added at the concentrations of 0.05 and 0.1 mL 100 mL^{-1} to the inoculated ayran samples. These samples were also vacuum packaged as described above and subsequently pressure treated. All the samples were pressure treated within 20 min of sample preparation.

2.4. High pressure processing

A Quintus model QFP-6 high pressure food processor (Flow Autoclave Systems Inc., Columbus, OH) with a 2 L pressure chamber was used for the study. A digital data monitoring system attached to the pressure unit was used for collecting data. Food grade glycol i.e. Houghto Safe 620TY (HS) (Houghton International Inc., Valley Forge, PA) mixed with water (1:1) was used as the pressure-transmitting fluid. A K-type thermocouple mounted within the test area of the high-pressure processor monitored the temperature of the pressure-transmitting fluid. Another K-type thermocouple monitored the temperature of the water jacket surrounding the pressure vessel. Depending on the pressure application, treatment temperature increased during HPP. Therefore, sample temperature was adjusted before HPP taking into account heat of compression, and initial sample temperature was changed from 18 to 23 °C in order to reach 25 ± 2 °C (Rasanayagam et al., 2003). Inoculated ayran samples packaged in sterile pouches were treated over a range of pressures (0.1–600 MPa) at 25 °C for up to 300 s (5 min) pressure holding time. All the treatments were repeated for three times. Pressure-treated samples were kept at refrigeration temperature (*ca.* 4 °C) until further analysis. Untreated pre-packaged samples served as a control group. The samples were taken for analyses maximum 2 h after HPP.

2.5. Analysis of composition of mint essential oils

The gas chromatography (GC)-mass spectrophotometer (MS) analyses of the essential oils was conducted using Hewlett Packard GC (model 6890) and Hewlett Packard MS (model 5972) equipped with a mass selective detector (MSD). The GC was equipped with an HP-5MS column (30 m length × 0.25 mm i.d. × 0.25 μm d_f) and HP18593B automatic injection system. Thirty μL of mint essential oil was transferred into 1 mL of diethyl ether (Sigma, Hamburg, Germany) and injected to GC/MS sampling port. The chromatogram was produced by holding the oven temperature at 50 °C for 5 min initially, and then increasing the temperature to 90 °C at a rate of 2 °C min^{-1} followed by increasing to 210 °C at a rate of 5 °C min^{-1} where it was held for over 40 min. MSD conditions were as follows: capillary direct interface temperature: 280 °C; ionization energy: 70 eV; mass range: 33–330 amu; EM voltage: Atune+200; and scan rate: 5 scan s^{-1}. Helium was used as the carrier gas at a flow rate of 1.5 mL min^{-1}. Identification of components in essential oils was carried out with Willey 275 MS data library (Dadalioglu & Evrendilek, 2004).

2.6. Microbial analysis

The control and pressure treated samples were started to be analyzed for reductions in *L. monocytogenes* and *L. innocua* within 20–30 min after pressure treatment, the pouches were aseptically opened, the samples were diluted with 0.1 g 100 mL^{-1} peptone water and surface plated onto TSA, Oxford agar and PALCAM agar (Becton Dickinson) separately. Plates were incubated at 30 ± 2 °C for 24–48 h. Results were expressed as log CFU mL^{-1}.

2.7. Chemical analyses of the ayran samples

Water activity, pH, color (L, a and b), and serum protein separation in ayran samples were measured before and after the

Table 1
Essential oil constituents of mint determined by gas chromatography/mass spectrophotometer analyses.

Constituent	Retention time (min)	Area (%)
α-Pinene	6.19	1.29
β-Pinene	7.95	1.42
3-Octanol	9.21	0.26
1-Octen-3-ol	11.04	0.95
Limonene	11.58	1.73
Menthone	12.25	8.04
Sabinene	13.74	0.93
Pulagone	14.89	44.84
Isomenthone	15.01	12.89
Neomenthole	15.20	1.23
Piperitone	18.19	7.42
Z-Piperitone oxide	20.98	2.11
Piperitenone	23.13	9.12
Piperitenone oxide	26.23	4.76
Thymol	28.16	0.16
Carvacrol	28.26	0.46
β-Caryophyllene	30.95	0.47
Germacrene-D	32.61	1.29
Δ-Cadinene	33.83	0.63