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Effect of the food matrix on pressure resistance of Shiga-toxin producing *Escherichia coli*

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ABSTRACT

The pressure resistance of Shiga-toxin producing *Escherichia coli* (STEC) depends on food matrix. This study compared the resistance of two five-strain *E. coli* cocktails, as well as the pressure resistant strain *E. coli* AW1.7, to hydrostatic pressure application in bruschetta, tzatziki, yoghurt and ground beef at 600 MPa, 20 °C for 3 min and during post-pressure survival at 4 °C. Pressure reduced STEC in plant and dairy products by more than 5 logs (cfu/ml) but not in ground beef. The pH affected the resistance of STEC to pressure as well as the post-pressure survival. *E. coli* with food constituents including calcium, magnesium, glutamate, caffeic acid and acetic acid were treated at 600 MPa, 20 °C. All compounds exhibited a protective effect on *E. coli*. The antimicrobial compounds ethanol and phenylethanol enhanced the inactivation by pressure. Calcium and magnesium also performed protective effects on *E. coli* during storage. Glutamate, glutamine or glutathione did not significantly influence the post-pressure survival over 12 days. Preliminary investigation on cell membrane was further performed through the use of fluorescence probe 1-N-phenylnaphthylamine. Pressure effectively permeabilised cell membrane, whereas calcium showed no effects on membrane permeabilisation.

1. Introduction

The application of hydrostatic pressure for food preservation experiences worldwide commercial growth (Balasubramaniam et al., 2015; Georget et al., 2015). Pressure ranging from 400 to 600 MPa eliminates pressure-sensitive pathogens and spoilage organisms (Patterson et al., 1995; Balasubramaniam et al., 2015; Georget et al., 2015); however, some foodborne pathogens including *Staphylococcus aureus* and Shiga-toxin producing *Escherichia coli* (STEC) are highly resistant to pressure (Hauben et al., 1997; Tassou et al., 2008; Liu et al., 2015; Gänzle and Liu, 2015). STEC cause severe foodborne disease; they are primarily associated with ruminants but plant foods including fruit juice and produce are also recognized as vectors for their transmission (Frenzen et al., 2005; Karch et al., 2005). Pressure treatments aiming to eliminate pathogens in fresh meat or plant products thus target STEC. The pressure resistance of *E. coli* is variable (Hauben et al., 1997; Liu et al., 2015). The lethality of 600 MPa towards 100 strains of STEC differed by more than 5 log(cfu/mL) and approximately 30% of strains of STEC were highly pressure resistant (Liu et al., 2015). The food matrix, process temperature, and pH also influence the pressure resistance of *E. coli* (Gänzle and Liu, 2015). The pressure resistance of several strains of *E. coli* was assessed in different food products; however, the comparison of literature data is confounded by the use of different process parameters in different studies (Garcia-Graells et al., 1998; Lavinas et al., 2008; Liu et al., 2012, 2015; Reineke et al., 2015).

As pressure processing alone does not sufficiently inactivate STEC, the use of additional antimicrobial hurdles is necessary. The targeted design of improved pressure processes necessitates an improved understanding of the role of matrix constituents on pressure resistance. Multiple pressure-sensitive targets have been described in *E. coli*. Pressure permeabilises the outer membrane of Gram-negative bacteria (Gänzle and Vogel, 2001; Ritz et al., 2000). Pressure also induces a phase transition in the cytoplasmic membrane (Casadei et al., 2002), resulting in the dissipation of the proton motive force (Wouters et al., 1998; Winter, 2002; Kilimann

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et al., 2005), and the elimination of acid resistance (Garcia-Graells et al., 1998). Ribosomes, protein folding, and the disposal of misfolded proteins also are pressure-sensitive targets in *E. coli* (Niven et al., 1999; Aertsen et al., 2004; Govers et al., 2014). Moreover, pressure induces oxidative stress in *E. coli* which enhances pressure-mediated inactivation (Aertsen et al., 2005). In keeping with pressure-induced oxidative stress as "suicide mechanism" in *E. coli*, thiol reactive antimicrobials exhibited a strong synergistic bactericidal activity with pressure (Feyaerts et al., 2015).

The use of hurdle technology in food included combinations of pressure with high (40–60 °C) temperature (Liu et al., 2012; Reineke et al., 2015). However, even moderately elevated temperatures in the range of 40–60 °C may alter food quality when combined with high pressure (Omana et al., 2011). The pressure treatment at low pH also eliminates *E. coli* after pressure treatment (Alpas et al., 2000; Garcia-Graells et al., 1998) but not all food products can be acidified. The synergistic activity of antimicrobial compounds, including thiol-reactive antimicrobials and bacteriocins, was demonstrated in model systems but rarely in food. This study therefore aimed to compare the pressure resistance of *E. coli* in foods and to assess the matrix effect on pressure resistance. Experiments were performed with a cocktail of 5 pathogenic *E. coli* and a cocktail of non-pathogenic strains (Garcia-Hernandez et al., 2015). Moreover, model studies were carried out in buffer systems with the heat- and pressure resistant *E. coli* AW1.7 (Dlusskaya et al., 2011; Liu et al., 2012).

2. Materials and methods

2.1. Bacterial strains and culture conditions

This study employed two cocktails each containing five strains of *E. coli* (Garcia-Hernandez et al., 2015). One strain cocktail was composed of four strains of STEC (05-6544, 03-2832, 03-6430 and C0283) and the enteropathogenic *E. coli* O145:NM PARC 449. These strains were selected to represent the most pressure resistant strains of more than 100 strains of STEC (Liu et al., 2015). *E. coli* PARC 449 harbors the locus of enterocyte effacement but not the gene coding for the shiga-like toxin (Liu et al., 2015; Mercer et al., 2015). The second strain cocktail was composed of the non-pathogenic *E. coli* AW1.7, AW1.3, GM16.6, DM18.3 and MG1655. *E. coli* strains were streaked from the frozen (−80 °C) stock cultures onto Luria–Bertani (LB) agar (Difco, Sparks, MD, USA) and incubated for 24 h at 37 °C. Strains were subcultured in LB broth and incubated at 37 °C and 200 rpm for 16–18 h. Equal volumes of each of the five single cultures were mixed to form the respective strain cocktails.

2.2. Preparation of samples for pressure treatment

Bruschetta (pH 4.1) and tzatziki (pH 4.0) were obtained from Food Processing and Development Centre located in Leduc of Alberta, Canada. The formulation of the products is shown in Table 1. Plain low-fat yoghurt (pH 4.0, Astro, Canada) and ground beef (20% fat) were purchased from a local supermarket. Products were used as obtained, or after adjusting the pH to 5.5 or 7.5. Cell counts of each batch of each food product were quantified by surface plating on LB agar; all cell counts were less than 2.6 log(cfu/g). Strain cocktails or the pressure resistant strain *E. coli* AW1.7 (1.5 ml) were inoculated into the food products (10 ml or g) to an initial population of around 10^7-10^8 cfu/ml. The inoculated food products were homogenized for 2 min. Subsamples of 250 μL or μg were packed into 3-cm R3603 tygon tubes (Akron, PA, USA) and heat-sealed after exclusion of air. Prior to pressure treatment, tubes were placed into a 2-ml Cryovial (Wheaton, Millville, NJ) filled with 10% bleach.

Table 1
Product composition of bruschetta and tzatziki.

Bruschetta (pH 4.1)	%	Tzatziki (pH 4.0)	%
Tomato	94.821	Cucumber	24.093
Balsamic Vinegar (6% acidic acid)	1.546	Sour Cream (14%)	34.36
Olive Oil	1.288	Plain Yogurt	34.36
Garlic (diced in oil)	1.031	Olive Oil	4.014
Basil Paste	0.644	Lemon Juice	1.608
Salt	0.386	Garlic (pre-chopped)	0.964
Black Pepper (80 mesh)	0.077	Salt	0.45
Xanthan Gum	0.155	Pepper	0.063
Crushed Chilis	0.052	Xanthan Gum	0.088

2.3. Pressure treatments of food samples

Pressure treatments were carried out as described previously (Liu et al., 2012). Samples were treated in a Multivessel Apparatus U111 (Unipress Equipment, Warsaw, Poland) at 600 MPa and 20 °C for 3 min. After the pressure treatment, the cell counts were determined by serial 10-fold dilution and surface plating on LB agar. Lactic acid bacteria in untreated or pressure treated yoghurt were enumerated by surface plating on modified de Man Rogosa Sharpe medium. Samples were stored at 4 °C over 16 days and cell counts were determined during storage. Cell counts of uninoculated and untreated as well as uninoculated and pressure-treated samples were used as controls. During enumeration of the colonies, the colony morphology was noted to determine whether it matched the colony morphology of the *E. coli* inoculum. All experiments were performed in triplicate.

2.4. Effect of food constituents on pressure resistance of E. coli

The effect of the following food constituents on the pressure resistance of *E. coli* was evaluated: calcium, magnesium, glutamate, acetic acid and caffeic acid. Experiments were carried out in 100 mmol/L MES (Fisher, Ottawa, Canada) buffer at pH 5.5. The food constituents were used at the following concentration: 10 mmol/L calcium chloride (Sigma, new Jersey, USA), 10 mmol/L magnesium sulfate heptahydrate (Sigma, new Jersey, USA), 10 mmol/L L-glutamic acid monosodium salt hydrate (Sigma, new Jersey, USA), 1 g/L caffeic acid (Sigma, St. Louis, USA) and 0.1% acetic acid in MES buffer. MES buffer or MES buffer supplemented with the respective compounds was mixed with an overnight culture of *E. coli* AW1.7 in a volumetric ratio of 9:1 (vol:vol). Samples were prepared for pressure treatment as described above and treated at 600 MPa and 20 °C for 0–16 min. Cell counts of untreated and pressure-treated samples were determined by surface plating on LB agar. Experiments were performed in triplicate.

2.5. Determination of effects of ethanol and phenylethanol on pressure resistance

The effect of ethanol and phenylethanol on pressure resistance was evaluated in acetate:MES:MOPS buffer (Sigma–Aldrich, St. Louis, MS, USA). The use of three buffering components with different pKa allows changing the buffer pH without changing the buffering component. The pH of the buffer was adjusted to 5.5. Ethanol and 2-phenylethanol (Sigma) were added to the buffer to a final concentration of 2% and 20 mM, respectively. Addition of *E. coli* AW1.7, and preparation and treatment of cultures was performed as described above. Cell counts of untreated and pressure-treated samples were determined on LB and Violet Red Bile agar (Difco) plates to enumerate the surviving with or without injury. Experiments were performed in triplicate.