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Use of phenolic compounds for sensitizing *Listeria monocytogenes* to high-pressure processing

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Abstract

Three *Listeria monocytogenes* strains (Scott A, OSY-8578, and OSY-328) that differ considerably in barotolerance were grown to stationary phase and suspended individually in phosphate buffer (pH 7.0). Twelve phenolic compounds, including commercially used food additives, were screened for the ability to sensitize *L. monocytogenes* to high-pressure processing (HPP). Each *L. monocytogenes* strain was exposed to each of the 12 phenolic compounds (100 ppm each) for 60 min; this was followed by a pressure treatment at 400 MPa for 5 min. Six phenolic compounds increased the efficacy of HPP against *L. monocytogenes* but *tert*-butylhydroquinone (TBHQ) was the most effective. The additives alone at 100 ppm were not lethal for *L. monocytogenes*. Subsequently, the three *L. monocytogenes* strains were exposed to TBHQ before or after pressure treatments at 400 or 500 MPa for 5 min. When TBHQ was added after the pressure treatment, the combined treatment was more lethal than was pressure alone. However, the lethality attributable to TBHQ was greater when the additive was applied before rather than after pressure treatment. The inactivation kinetics of the *L. monocytogenes* strains at 300, 500, and 700 MPa, in the presence or absence of TBHQ, was investigated. All survivor plots showed non-linear inactivation kinetics, but tailing behavior was most pronounced when HPP was used alone. Combinations of TBHQ and HPP eliminated tailing behavior when survivors were monitored by direct plating or an enrichment procedure. Pressure and phenolic additives are apparently a potent bactericidal combination against *L. monocytogenes*.

Keywords: *Listeria monocytogenes*; High-pressure processing; Phenolic additives; *Tert*-butylhydroquinone; Inactivation kinetics; Tailing

1. Introduction

High-pressure processing (HPP) is a non-thermal alternative food preservation technology. Food products are treated with pressures of up to 900 MPa for a period of 2 to 10 min to achieve microbial inactivation with minimum quality loss (Cheftel, 1995; Matser et al., 2004). However, HPP often results in microbial inactivation patterns that do not follow first-order kinetics and a small fraction of the population may remain viable after prolonged processing. This tailing phenomenon was observed during pressure treatment of *Listeria monocytogenes* in vacuum-packaged frankfurters (Lucore et al., 2000) and in microbiological media (Tay et al., 2003).

L. monocytogenes is one of the most barotolerant non-sporeforming pathogens, but there is a wide variation in pressure resistance between strains (Alpas et al., 1999; Tay et al., 2003). Therefore, designing an HPP treatment that is effective for the most barotolerant strains is needed to ensure the efficacy of this technology in a given food. High-pressure processing may be combined with other preservation methods to increase its efficacy and commercial feasibility. Currently used and potential food additives, such as bacteriocins (Yuste et al., 2002), potassium sorbate (Mackey et al., 1995), and carvacrol (Karatzas et al., 2001), have been tested in combination with HPP.

L. monocytogenes were occasionally detected, by enrichment procedures, in inoculated, pressure-treated foods that

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contained some of the previously studied additives (unpublished data). Additionally, these additives cannot be used in many foods due to sensory and quality considerations. Combining phenolic compounds, which are fairly hydrophobic, with high pressure may be useful in controlling pathogens, particularly in fat-rich foods such as salad dressings and sausages. Phenolic food additives have been used to retard oxidative deterioration in foods, but some of these compounds also show antimicrobial activity. The antimicrobial activity of phenolic antioxidants against *L. monocytogenes* has been investigated in a model milk system (Payne et al., 1989) and in tryptose broth (Yousef et al., 1991). These studies indicated that *tert*-butylhydroquinone was a better bacteriostatic agent against *L. monocytogenes* Scott A than the other phenolics that were tested. Carvacrol and cinnamic acid are potentially effective phenolic preservatives in foods including fresh fruits, rice, and soft cheese (Ultee et al., 2000; Smith-Palmer et al., 2001; Roller and Seedhar, 2002). However, phenolic compounds at concentrations that are high enough to exert an antimicrobial activity may impact food flavor and this limits their applications in foods. A number of solutions have been suggested to overcome this problem, including the use of these compounds in combination with other antimicrobial factors (Smith-Palmer et al., 2001). Combination of phenolic food-grade ingredients with HPP could well be useful for eradicating pressure-resistant *L. monocytogenes* in food. Therefore, the goal of this study is to identify phenolic compounds, preferably currently used food additives, that enhance the efficacy of high pressure against *L. monocytogenes*, particularly the barotolerant strains.

2. Materials and methods

2.1. Culture preparation

The three strains of *L. monocytogenes* (Scott A, OSY-8578, and OSY-328) used in the study were obtained from the culture collection of the Food Safety Laboratory at The Ohio State University. Stock cultures were stored at −80 °C in tryptose broth (TB; Difco, Becton Dickinson and Co., Sparks, MD, USA) containing 40% (*v*/*v*) glycerol. Cultures were transferred in TB two times prior to use. All experiments were carried out using stationary phase cells. Bacteria were grown in TB at 37 °C for 18 h, pelleted at 8000 ×*g* for 15 min using a centrifuge (Sorvall RC-5B; Dupont, Wilmington, DE, USA), washed, and resuspended in sterile 0.2 M sodium phosphate buffer solution (PBS; pH 7.0) to obtain cell suspensions with approximately 10^9 CFU/ml.

2.2. High-pressure processing

Pressure treatments were performed using a hydrostatic food processor (Quintus QFP6; Flow Pressure Systems, Kent, WA, USA) as described by Tay et al. (2003). Before the treatment, the temperatures of the sample and the pressure-transmitting fluid were adjusted to compensate for adiabatic heating (3 to 4 °C/100 MPa). The water jacket temperature was also maintained to meet the final temperature during pressurization. Samples were treated with pressures in the range 300 to 700 MPa.

2.3. Treatments

2.3.1. Screening of phenolic compounds

Twelve phenolic compounds were screened for their synergy with pressure against *L. monocytogenes*; these were butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), carvacrol (Carv), catechin (Cat), hydroquinone (HQ), *iso*-eugenol (*i*-Eug), phenol (Phe), propyl gallate (PG), rosemary extract (Rose-Ext), *tert*-butylhydroquinone (TBHQ), thymol (Thy), and trihydroxybutyrophenone (THBP). These compounds were purchased from Sigma Chemical Co. (St. Louis, MO, USA) with the exception of rosemary extract (RFI-1280 OSR) which was obtained from RFI Ingredients (Blauvelt, NY, USA). A stock solution containing 1000 ppm of each additive was prepared in dimethyl sulfoxide (DMSO, 99.9% spectrophotometric grade; Sigma). Solutions were sterilized by filtration through a 0.2 μm PTFE membrane filter (Millipore Corp., Bedford, MA, USA).

The phenolic compounds were tested as two groups, with TBHQ included in both groups. Group I was BHA, BHT, Carv, PG, and Phe and group II was *i*-Eug, HQ, THBP, Cat, Thy, and Rose-Ext. Three *L. monocytogenes* strains were tested for group I phenolics and only *L. monocytogenes* OSY-8578 was tested for group II compounds. Each strain of *L. monocytogenes* was treated as follows: (i) control (culture in phosphate buffer with no treatments), (ii) phenolic compound only, (iii) HPP only, and (iv) phenolic–HPP combination. The pathogen was also treated with DMSO with or without HPP. Cell suspensions (1.8 ml) were transferred into sterile polyethylene bags. For each phenolic-containing treatment, 0.2 ml phenolic stock solution was added to each bag to obtain a final concentration of 100 ppm. For treatments without phenolics, 0.2 ml of PBS was added to the cell suspension (1.8 ml). The sample bags were sealed using a vacuum sealer (Vacmaster, Kansas City, MO, USA) and held for ~60 min at 4 °C prior to the pressure treatment. Sample bags were pressurized at 400 MPa for 5 min, at 18 to 20 °C. Pressure-treated and untreated bags were opened aseptically. The contents were serially diluted in 0.1% peptone water (Difco) and appropriate dilutions were spread-plated on tryptose agar (TA; Difco) plates, which were incubated at 35 °C for 48 h for enumeration of survivors. Each experiment was repeated four times.

2.3.2. Phenolic-pressure treatment sequence

Tert-butylhydroquinone was used for testing the effect of treatment sequence on inactivation of *L. monocytogenes*. Washed cells, TBHQ solution, and sample bags for HP treatment were prepared as previously described. The additive was dispensed into the bag containing the cell suspension before or after pressurization. Pressure treatments were performed at 400 and 500 MPa for 5 min and the temperature at the holding pressure was 18 to 20 °C. The contents of treated and untreated bags were plated and survivors were enumerated as before. Each experiment was performed three times.