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ARTICLE

Inactivation Efficiency of *Escherichia coli* and *Listeria monocytogenes* in Ground Pork by Combination of Natural Food Ingredients and High Pressure Processing

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Abstract

The objective of this study was to examine the effects of a combined treatment regarding antimicrobial food ingredients and high pressure processing (HP) on the inactivation efficiency of *Escherichia coli* and *Listeria monocytogenes* inoculated into ground pork. Ethanol extracted from garlic, leeks, onions, and ginger powder was prepared. Half of the prepared powder was irradiated at 5 kGy to see the effect of pasteurization before addition. The prepared food ingredients were added into radiation-sterilized ground pork (1%, w/w), and inoculated with *E. coli* and *L. monocytogenes*. The samples were vacuum-packed and applied with HP at 0.1 (control), 300, 450, and 600 MPa. Microbial log reduction increased with the increase of pressure up to 600 MPa. With minor exceptions, overall efficiency of HP treatment with regards to inactivation of pathogens increased. Inoculated microorganisms showed approximately 7-8 Log reductions by 600 MPa, except for *L. monocytogenes* treated with garlic (5.7 Log reductions). The *E. coli* reduction in ground pork mixed with ethanol extracted garlic showed the highest efficiency (1.86) compared to leeks (1.25-1.31), onions (1.17-1.44), and ginger (1.50-1.82) when treated at an HP of 450 MPa. There was no evidence for the advantage of pasteurization concerning the food ingredients before addition of antimicrobial food ingredients and HP. Results demonstrate that the combination of antimicrobial food ingredients and HP treatment may help improve the efficiency of sterilization in meat systems.

Key words: food ingredients, pork, high pressure, combination

Introduction

Technological advances developed by the food industry have enabled the control of microbial hazards and reduction of foodborne diseases. Usually food preservation method used has been thermal processing including pasteurization and sterilization. However, theses processing affect sensory, textural, and nutritional qualities. Consumer demand is increasing for high quality, fresh tasting foods free from additives, microbiological safety, and extended shelf life (Leistner, 2000). Within this context the combination of hurdles to inhibit pathogen growth acquired high importance. Hurdle technology advocates the deliberate combination of existing and novel preservation techniques in order to establish a series of hurdles that no microorganisms present should be able to overcome (Leistner and Gorris, 1995).

High pressure processing (HP) is a non-thermal food preservation technology for inactivating post-processing contaminants, especially for foods whose nutritional, sensory and functional characteristics are thermo-sensitive (Marcos *et al*., 2008). HP kills and/or sub-lethally injures the cells by destroying the functionality of the cell wall and the cytoplasmic membrane, dissociating the proteins and the ribosomal subunit structures, and inactivating some enzymes (Hoover *et al*., 1989). Recently, HP is successfully applied on a commercial scale for pasteurization and available in world food market of a whole range of food products such as oyster and ham (Marcos *et al*., 2008; Prapaiwong *et al*., 2009). HP has also developed in combination with other technologies such as bacteriocin

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or other natural materials (Rastogi *et al*., 2007).

Several researchers found that microbial inactivation can be achieved efficiently by combining high pressure with heat or antimicrobial agents than by applying only HP (Marcos *et al*., 2008). Caillet *et al*. (2006) reported that the combination of natural materials with irradiation, another well-known non-thermal processing, increased the radiation sensitivity of bacteria. Yun *et al*. (2011) reported that food ingredients usually added into Korean processed meat products also increased the radiation sensitivity and increased inactivation efficiency. Such hurdle effect have many advantages in food processing including reduction of energy costs and the production of safer and more palatable products.

Therefore, the objective of this study was to examine the effect of combined treatment with natural antimicrobial food ingredients, ethanol extracted garlic, leek, onion, and ginger, and HP on inactivation efficiency of *E. coli* and *L. monocytogenes* inoculated into ground pork.

Materials and Methods

Meat preparation and irradiation

Ground pork was purchased from a local market in Daejeon, Korea and sealed into polyethylene pouch (2 mL O_2/m^2/24 h at 0°C, Sunkyung Co. Ltd., Korea). For the inoculation test of pathogens, samples were irradiated (35 kGy) in a cobalt-60 gamma irradiator (AECL, IR-79, MDS Nordion International Co. Ltd., Canada) at Advanced Radiation Technology Institute, Korea. The source strength was approximately 11.1 PBq with a dose rate of 10 kGy/h at 10±0.5°C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR Analyzer.

Test pathogens preparation

Escherichia coli (KCTC 1682) and *Listeria monocytogenes* (KCTC 3569) were obtained from a Korean Collection for Type Culture (KCTC, Korea). The strains were cultivated at 37°C for 18 h in a tryptic soy broth (Difco, Laboratories, SA), and 10 mL cultures of each strain were transferred aseptically to a 50 mL centrifuge tube and were vortexed for 10 s. Each strain was centrifuged (1,950 g for 10 min at 4°C) in a refrigerated centrifuge (VS-5500, Vision Scientific Co., Korea). The pellet was washed twice with sterile saline (0.85%), and suspended in saline to a final concentration of approximately 10^9 CFU/mL of the stock inoculum.

Sample preparation

Fresh garlic (*Allium sativum* L.), leek (*Allium tuberosum* R.), onion (*Allium cepa* L.), and ginger (*Zingiber officinale*) were purchased from a local market in Daejeon, Korea. Each sample (500 g) was extracted three times with 70% ethanol for 12 h in enclosed flask with constant shaking (100 rpm) at room temperature. After filtration with a Whatman No. 2 filter paper, the residue was re-extracted with additional 200 mL of 70% ethanol for an additional for 12 h and then filtered. Ethanol was evaporated form the combined filtrates using a rotary evaporator (EYELA N-1000, Japan) at 50°C. Then, it was freeze dried. Dried extracts were placed in sealed bottles and stored at -20°C before use. The dried powders were dissolved in water (10% w/w) prior to use.

To see the effect of contamination of the ethnol-extracted antimicrobial food ingredients, the half of the prepared food ingredients were irradiated at 5 kGy as the same method previously described to reduce the original number of microorganisms present in the additives. The other half was used without irradiation.

Inoculation of pathogens and high pressure treatment

The prepared natural compounds, both irradiated at 5 kGy or none, were mixed with radiation-sterilized pork (50 g) at a concentration of 1% (w/w, final concentration). The control sample without any additive was also prepared. The sample was sub-divided into portions each have 5 g in sterile polyethylene pouch bag (8×10 cm), and inoculated with *L. monocytogenes* and *E. coli*. The test culture suspension (50 μL) was uniformly and aseptically inoculated in different areas on the samples and mixed to achieve uniform dispersal at the desired concentration throughout the sample for 5 min in enclosed polyethylene bag. The bags were sealed and the inoculated samples were stored at 4°C for approximately 12 h prior to irradiation.

The samples were transported to the Korea Food Research Institute (Korea) in a cooled container and were subjected to treatment. Samples were placed in a pressure vessel submerged in hydrostatic fluid medium (Quintus food processor 6; ABB Autoclave Systems, Inc., USA) and pressurized at 300, 450, and 600 MPa for 5 min with the initial temperature of the pressure vessel at 15±3°C. Control samples were maintained under atmospheric pressure at 4°C while the other samples were treated.

Microbiological analysis

Samples of HP treated were blended with sterile saline using a stomacher (BagMixer® 400, Interscience Ind., France) for 2 min. Then, a series of decimal dilution was prepared with sterile saline and each diluent (0.1 mL) was spread in tryptic soy agar (Difco). The plates were incubated at 37°C for 48 h and microbial counts were expressed as Log CFU/g. HP efficiency ratio was determined ratio of reduced viable cell for HP treatment/control at 450 MPa.

Statistical analysis

Experiment was conducted as 3 independent trials with 2 observations for treatment combinations per each trial. Statistical analysis was performed by one-way Analysis of Variance (ANOVA), and when significant differences were detected, the differences among the mean values were identified by Student-Newman-Keul's multiple range test using SAS version 9.1 software (2002) with the confidence level at $p<0.05$. Mean values and standard error of the means are reported.

Results and Discussion

The initial number of total aerobic bacteria of ethanol extracted garlic, leek, and ginger were in the range of 1-3 Log CFU/g (data not shown). There was no viable cell detected in onion. When the ethanol-extracted food ingredients were irradiated at 5 kGy for pasteurization, the number of total aerobic bacteria was not above the detection limit of $<10^2$ CFU/g for all the samples.

The initial number of *E. coli* in the control, which is the sample without any additives, was 7.18 Log CFU/g and was reduced to undetected level when HP of 600 MPa was treated (Table 1). The number of *E. coli* was different in the sample by addition of food ingredients. The sample mixed with ethanol extracted garlic and leek showed a little lower initial number when compared with onion and ginger. After 300 MPa of HP, the numbers were slightly reduced but further increase of pressure to 450 MPa decreased the number of *E. coli* rapidly. Similarly, the number of *E. coli*, *Salmonella* Typhimurium, *and L. monocytogenes* decreased only 1 Log cycles by 300 MPa but 4-8 Log reduction was achieved by 450 MPa in chicken breast fillet (Kruk *et al*., 2011). The result suggests that HP treatment with 300 MPa is not enough to control these pathogens. There were no viable cells detected after HP of 600 MPa regardless of treatments.

The initial load of the samples with irradiated food ingredients for 5 kGy was higher than those with non-irradiated ones. A slightly but significantly higher reduction ($p<0.05$) was achieved when 300 MPa was treated when compared with the sample with non-irradiated additives. However, in general, there was no difference in pathogen reduction found at 450 and 600 MPa.

Table 1. The combination effect of ethanol-extracted food ingredients and high pressure on *Escherichia coli* in ground pork (Log CFU/g)

Irradiation dose (kGy)[1)]	Food ingredient	High pressure (MPa)				SEM[2)]
		0.1	300	450	600	
0	Control	7.18	6.41	3.46	ND[3)]	
	Garlic	6.90[a]	6.59[b]	ND[c]	ND[c]	0.044
	Leek	6.67[a]	6.29[a]	1.79[b]	ND[b]	0.562
	Onion	7.35[a]	6.07[a]	1.98[b]	ND[c]	0.496
	Ginger	7.75[a]	6.94[a]	2.15[b]	ND[c]	0.054
5	Control	7.70	5.57	3.37	ND	
	Garlic	8.01[a]	5.97[b]	ND[c]	ND[c]	0.013
	Leek	8.10[a]	5.87[b]	2.71[c]	ND[d]	0.109
	Onion	7.88[a]	6.07[b]	2.84[c]	ND[d]	0.047
	Ginger	7.86[a]	5.97[b]	ND[c]	ND[c]	0.035

[1)]Irradiation was applied for pasteurization of ethanol-extracted food ingredient.
[2)]Standard errors of the mean (n=12)
[3)]Viable cell was not detected with a detection limit at $<10^1$ CFU/g.
[a-d]Values with different letters within the same row differ significantly ($p<0.05$).

In the result from the sample treated with 450 MPa of HP, at least 1 Log or higher pathogen reduction was observed by comparison of the samples between control and samples added with ethanol extracted food ingredients. It indicates the increase of efficiency in inactivation of pathogen by HP using combination with natural antimicrobial food ingredients. Among the food ingredients used, ethanol extracted garlic was the best in increase of efficiency of HP inactivation of *E. coli*. Yun *et al*. (2011) studied the effect of several food ingredients on radiation inactivation and reported that the most efficient ingredient against *E. coli* was ethanol extracted leek, followed by freeze-dried ginger and leek. The relative radiation sensitivity of ethanol extracted leek, freeze-dried ginger and leek, calculated as D_{10} of control/D_{10} of sample mixed with food ingredients, were 3.89, 3.66, and 3.63, respectively.

Natural food ingredients have been known to possess antibacterial and antifungal activities, and contain the powerful sulfur and other numerous phenolic compounds (Benkeblia, 2004). The antimicrobial activities of phenolic compounds may involve multiple modes of action.

Shan *et al*. (2007) suggested that phenolic compounds can degrade the cell wall, disrupt the cytoplasmic membrane, cause leakage of cellular components, change fatty acid and phospholipid constituents, influence the synthesis of DNA and RNA, and destroy protein translocation. Phenolic compounds also involve a sensitization of the phospholipid bilayer of the cell membrane, causing an increase in permeability and leakage of vital intercellular constituents (Kim *et al*., 1995) or impairment of bacterial enzymes (Wendakoon and Sakaguchi, 1995). Ponce *et al*. (1998) reported that high pressure produces and addition of nisin on gram-negative cells such as *E. coli* caused sub lethal injury in the outer membrane. Microbial cellular membrane is affected by high pressure, resulting in osmotic changes, lysis, alterations of nuclear materials, and other modifications which can result in cell death (Mackey *et al*., 1994).

Relatively higher reduction (1.5-2.5 Log reductions vs 0.5-2.0 Log reductions) of *L. monocytogenes* was achieved after 300 MPa of HP treatment when compared with *E. coli* (Table 2). However, the result was reversed in the sample with 450 MPa of HP, which showed that *L. monocytogenes* was slightly more resistant against HP. At 600 MPa, the viable cells were not detected except for the ground pork with non-irradiated garlic.

Previously, Black *et al*. (2005) studied individual and combined effect of HP and nisin on bacteria contaminated in milk. The authors reported that the combination of HP and nisin enhanced the levels of inactivation for both gram-negative and gram-positive bacteria. Usually, gram-negative bacteria are more sensitive to HP than gram-positive bacteria, probably due to the thicker cell wall of the former (Carlez *et al*., 1994). Low water activity protects cells against pressure, but microorganisms injured by HP are generally more sensitive to low water activity (Cheftel and Culioli, 1997). The combined treatment of enterocins and lactate-diacetate, with HP treatment eliminated microorganisms inoculated on cooked ham (Marcos *et al*., 2008). Evrendilek and Balasubramaniam (2011) also reported that HP processing combined with mint essential oil appeared to be a promising technique for preserving microbiologically-safe yogurt with no significant impacts to product quality. The decrease in intracellular ATP concentration in microorganisms such as *E. coli* and *L. monocytogenes* by the addition of essential oil is associated with the ability of essential oil to disrupt bacterial cell wall membranes and cause lysis (Lacroix *et al*., 2009; Rhayour *et al*., 2003).

The ratios of the reduction of pathogens in sample with food ingredients and control at 300 and 450 MPa were calculated (Table 3). With minor exceptions, overall efficiency of HP treatment in inactivation of pathogens was increased. The *E. coli* reduction in ground pork with ethanol extracted garlic showed the highest (1.86) when HP-treated for 450 MPa. There was no evidence found for the advantage of pasteurization of ethanol extracted food ingredients. The present study demonstrates that the combination of antimicrobial food ingredient and HP treatment may improve the efficiency of sterilization in meat system.

Table 2. The combination effect of ethanol-extracted food ingredients and high pressure on *Listeria monocytogenes* in ground pork (Log CFU/g)

Irradiation dose (kGy)[1)]	Treatment	High pressure (MPa)				SEM[2)]
		0.1	300	450	600	
0	Control	7.40	5.30	2.90	ND[3)]	
	Garlic	7.15[a]	4.95[b]	2.94[c]	1.49[d]	0.406
	Leek	7.25[a]	3.83[b]	2.59[c]	ND[d]	0.282
	Onion	7.37[a]	4.95[b]	3.00[c]	ND[d]	0.072
	Ginger	7.33[a]	4.80[b]	ND[c]	ND[c]	0.031
5	Control	7.40	5.30	2.90	ND	
	Garlic	7.43[a]	5.65[b]	ND[c]	ND[c]	0.022
	Leek	7.41[a]	5.32[b]	3.55[c]	ND[d]	0.029
	Onion	7.46[a]	5.81[b]	ND[c]	ND[d]	0.038
	Ginger	7.89[a]	5.48[b]	3.20	ND[d]	0.024

[1)]Irradiation was applied for pasteurization of ethanol-extracted food ingredient.
[2)]Standard errors of the mean (n=12)
[3)]Viable cell was not detected with a detection limit at $<10^1$ CFU/g.
[a-d]Values with different letters within the same row differ significantly ($p<0.05$).

Table 3. Pathogen inactivation efficiency of high pressure after combination of ethanol-extracted food ingredient

Pathogen	Food ingredients	Inactivation efficiency of high pressure			
		300 MPa[1)]		450 MPa[2)]	
		0 kGy	5 kGy	0 kGy	5 kGy
Escherichia coli	Garlic	0.40	0.96	1.86	1.86
	Leek	0.49	1.05	1.31	1.25
	Onion	1.66	0.85	1.44	1.17
	Ginger	1.05	0.89	1.50	1.82
Listeria monocytogenes	Garlic	1.05	0.85	0.94	1.65
	Leek	1.63	1.00	1.04	0.86
	Onion	1.15	0.79	0.97	1.67
	Ginger	1.20	1.15	1.63	1.04

[1)]Calculated by the ratio of (Difference of viable cell in sample with food ingredient between 300 MPa and 0.1 MPa)/(Difference of viable cell in control sample between 300 MPa and 0.1 MPa)
[2)]Calculated by the ratio of (Difference of viable cell in sample with food ingredient between 450 MPa and 0.1 MPa)/(Difference of viable cell in control sample between 450 MPa and 0.1 MPa)

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