Italian Journal of Food Science, 2021; 33 (1): 39–45

The antimicrobial activity of two phenolic acids against foodborne *Escherichia coli* and *Listeria monocytogenes* and their effectiveness in a meat system

Oluwatosin Ademola Ijabadeniyi[a]*, Austin Govender[a], Omotola Folake Olagunju[a], Ajibola Bamikole Oyedeji[b]*

[a]Department of Biotechnology and Food Technology, Durban University of Technology, Durban, South Africa; [b]Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, Gauteng, South Africa

***Corresponding Authors:** Oluwatosin Ademola Ijabadeniyi, Department of Biotechnology and Food Technology, Durban University of Technology, S9 Level 1, Steve Biko Campus, Durban 4000, South Africa. Email: oluwatosini@dut.ac.za; Ajibola Bamikole Oyedeji, Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg Doornfontein Campus, P.O. Box: 17011, Gauteng 2028, South Africa. Email: jibanky2@gmail.com

Received: 21 July 2020; Accepted after revision: 14 December 2020; Published: 1 February 2021.
© 2021 Codon Publications

OPEN ACCESS

PAPER

Abstract

Ready-to-eat meats are susceptible to pathogenic contamination during their production, distribution, and sale. This study evaluated the antimicrobial effects of two phenolic acids (caffeic and ferulic acids) against foodborne pathogens in cold-cut meat at low-temperature conditions. The individual and combined antibacterial activities of caffeic and ferulic acids against *Escherichia coli* O157:H7 ATCC 43888 and *Listeria monocytogenes* ATCC 7644 were determined by diffusion disk assay in broth media and cold-cut meat. Broth media and meat samples already inoculated with *E. coli* and *L. monocytogenes* were treated with caffeic acid, ferulic acid, and their combination at the concentrations of 150 ppm and 200 ppm and stored at 4°C. Microbial growths were monitored at 0, 24, 48, and 72 h. Caffeic acid at 200 ppm exhibited a zone of inhibition of 12.33 mm on *E. coli*, and ferulic acid revealed a zone of inhibition of 11.00 mm on *L. monocytogenes*. The combination of caffeic-ferulic acid at a concentration of 200 ppm was most effective against *E. coli*, demonstrating a synergistic effect over 72 h at 4°C in both broth media and meat. For meat samples, the combination of caffeic acid and ferulic acid exhibited a log reduction of 3.63 CFU/g at 150 ppm and 2.51 CFU/g at 200 ppm against *E. coli* O157:H7 at the end of cold storage. Caffeic acid alone exhibited an overall log reduction of 2.48 CFU/g at 150 ppm and 2.75 CFU/g at 200 ppm against *L. monocytogenes*. These results indicate the ability of caffeic and ferulic acids, individually and in combination, to reduce pathogenic contamination and improve safety of cold-cut meats.

Keywords: antibacterial activity; caffeic acid; ferulic acid; cold-cut meat; *E. coli*; *L. monocytogenes*

Introduction

Cold-cut meat products such as ham are one of the most commonly consumed ready-to-eat products. They are often in sliced forms and used as fillings for sandwiches and similar foods. They are manufactured from raw pork and/or beef and characterized by physical and biochemical changes that occur during curing (Marušić *et al.*, 2014). In most modern cold-cut meats, nitrites (either sodium nitrite or potassium nitrite) are employed during curing to prevent bacterial growth and survival and enhance safety and storage stability. However, owing to the toxicity of nitrites, some country regulations specify maximum allowable contents in the final product (Campion *et al.*, 2017). This indulges public health concern because, under certain conditions, nitrites in meat can react with degradation products of amino acids, forming nitrosamines, which are known carcinogens

 ISSN 1120-1770 online, DOI 10.15586/ijfs.v33i1.1933

(Shpazier *et al*., 2018). Furthermore, foodborne pathogenic bacteria may persist and replicate in cold-cut meats due to their low effective doses, antimicrobial resistance, and capability of adapting to stress conditions without observable sensorial changes in the product (Rybarczyk *et al.*, 2017).

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive bacterium that thrives in water, soil, and foods (Chen *et al*., 2017). It is the causative organism for listeriosis and has the potential to contaminate different forms of foods, including low-moisture foods, at different stages within the food chain. It has been implicated in many foodborne disease outbreaks, and some of the most common vehicles involved in these outbreaks include ready-to-eat meals, unpasteurized milk, dairy products, meat, fruits, and vegetables (Pernin *et al*., 2019). Ingestion of foods contaminated with *L. monocytogenes* could result in different symptoms from mild gastroenteritis to severe central nervous system infections. High-risk individuals prone to *L. monocytogenes* infection include pregnant women and immunocompromised people (Hunjak *et al*., 2019). *Escherichia coli* (*E. coli*) is also a Gram-negative, rod-shaped, ubiquitous bacterium, which also lives in the gut of humans and suppresses the growth of harmful bacteria (Saxena *et al*., 2015). One of its pathogenic strains, *E. coli* O157:H7, is listed by the United States Centers for Disease Control (CDC) as a pathogen that has attracted increasing attention due to the number of victims who had to be hospitalized following its ingestion (*De Souza et al*., 2018). The CDC estimated that in the US, there were 10,000 illnesses, thousands of hospitalizations, and hundreds of deaths attributed to the *E. coli* O157:H7 yearly. Most *E. coli* O157:H7 outbreaks are associated with fecal-oral transmission due to poor hygiene and food handling practices. Sources of human infection include poorly cooked meat, apple juice, water, and milk products such as yogurt and unpasteurized milk. These bacteria have been adjudged the most threatening foodborne pathogens because of their severe debilitating effects on humans upon ingestion (Buchanan *et al*., 2017).

Although various measures have been implemented in the past to control and reduce the prevalence of *L. monocytogenes* and *E. coli* O157:H7, reports of foodborne disease outbreaks as a result of their infection subsists. Chemical preservatives are capable of inhibiting or inactivating bacterial growth in foods. However, the use of natural preservatives is currently encouraged due to increasing bacterial resistance to chemical preservatives, food safety regulations, and increased awareness of the adverse effects of chemicals (Pernin *et al*., 2019). Phenolic compounds have attracted much interest due to their antioxidant potential and antimicrobial properties. Phenolic compounds are secondary metabolites produced by plants and can be classified into three different groups based on their chemical structures. These classes include non-flavonoids, flavonoids, and tannins in their simple or complex forms (Pernin *et al*., 2018). In plant-based foods, phenolic compounds are responsible for the color of red fruits, juices, and wines, substrates involved in enzymatic browning and are also considered to contribute to the health benefits associated with dietary consumption of fruits and vegetables (Cheynier, 2012).

In particular, caffeic and ferulic acids possess aromatic rings with a hydroxyl group (-OH) on the fifth position of their rings while their difference is in the presence of methoxy group ($-H_3CO$) for ferulic acid and hydroxyl group (-OH) at the sixth position of aromatic rings (Sanchez-Maldonado *et al*., 2011). They are well distributed in plants, and they possess antioxidant and prooxidant properties (Nystrom *et al*., 2005) substantiated by their functions in the reduction of auto-oxidation by acting as radical scavengers, inhibitors of lipid peroxidation, and low-density lipoproteins (Maurya & Devasagayam, 2010). Their antioxidant and prooxidant abilities in foods have been adduced to factors such as concentration, chemical structure, and the nature of substrates or foods (Maurya & Devasagayam, 2010).

Previous studies have validated the antimicrobial properties of phenolic compounds against bacteria, including pathogenic species. These findings justified that their use could be an alternative means of inhibiting or inactivating the growth of *L. monocytogenes* and *E. coli* (Vaquero *et al*., 2007). When combined, phenolic acids could reflect greater antimicrobial efficacy against foodborne pathogens, compared to individual phenolic acids. Therefore, this study was conducted to investigate the individual and combined antimicrobial effects of caffeic and ferulic acids on the survival of *L. monocytogenes* ATCC 7644 and *E. coli* O157:H7 ATCC 43888 in cold-cut meat products under low-temperature storage conditions.

Materials and Methods

Preparation of inoculum

The bacterial strains *L. monocytogenes* ATCC 7644 and *E. coli* O157:H7 ATCC were obtained from the microbial culture collection of the Department of Biotechnology and Food Technology, Durban University of Technology. Stock cultures of *L. monocytogenes* were grown aerobically for 24 h at 30°C in brain heart infusion (BHI) broth. For *E. coli*, stock cultures were grown at 37°C for 24 h in tryptic soy broth (TSB). A loopful of the stock culture of each microorganism was transferred to 10 mL of BHI and TSB, as applicable, and incubated at 37°C for

24 h. Turbidity of cultures was adjusted to match that of a 0.5 McFarland standard to achieve an approximate 10^8 CFU/mL in each case.

Preparation of phenolic acid extracts

Caffeic acid and ferulic acid (>98% purity) were purchased from Sigma-Aldrich (Johannesburg, South Africa). To elucidate the antimicrobial properties, these phenolic compounds were dissolved in 99.8% ethanol (Merck, Johannesburg, South Africa). However, for broth and meat experiments, the phenolic acids were dissolved in water at 1% w/v (Zhang *et al*., 2016). The solutions were filtered through 0.22-µm membrane filters in each case and stored at 4°C in sterilized glass containers until needed.

Antibacterial activity determination

For agar disk diffusion assay, 0.1 mL of each bacterial culture suspension was transferred into Petri dishes containing Mueller Hinton agar (Merck, Johannesburg, South Africa) and were uniformly spread onto the surface with the help of a sterile plate spreader. Fifty microliters of phenolic acids solutions, at different concentrations (150 and 200 ppm), were pipetted on 6 mm sterile filter paper disks (Whatman No. 1) and air-dried in a laminar flow chamber. Distilled water was used as the negative control, while Ciprofloxacin (Merck, Johannesburg, South Africa) was used as the positive control for its effectiveness against both Gram-positive and Gram-negative bacteria. These dried disks were then transferred into inoculated plates and incubated at 30°C for 24 h for *L.monocytogenes* and 37°C for *E. coli*. Antibacterial activity for each phenolic acid and their combination was determined by measuring the inhibition zone around the disk following a 24-hour incubation period. Each experiment was replicated at least three times.

Broth study

In each case, 1 mL bacterial suspension containing $1x10^8$ CFU/mL *E. coli* and *L. monocytogenes* were transferred into either BHI broth for *L. monocytogenes* or TSB broth for *E. coli* O157:H7. Thereafter, 50 µL of individual solutions of caffeic and ferulic acid of 150 ppm and 200 ppm concentration as well as a combination (1:1) of caffeic and ferulic acid solutions (150 ppm:75 ppm of each and 200 ppm:100 ppm of each) were added to inoculated broths. An inoculum containing a bacterial suspension of $1x10^8$ CFU/mL of each bacterium that was not treated by the phenolic acids served as the control samples. The samples were then stored at 4°C for 72 h. Microbial survival in each broth treatment was determined at 12, 24, 48, and 72 h by pour-plating six serial dilutions on appropriate growth agar. Plates were incubated at 37°C for 24 h before enumeration. Each experiment was repeated at least thrice.

Meat study

Cold-cut meat samples were bought from a trusted retail outlet and sliced into 5 g portions. For sterilization, the samples were dipped into 95% ethanol solution and left to dry in a laminar flow chamber. Afterward, 0.1 mL of each bacterial suspension was spot-inoculated onto the meat samples and spread out onto the surface of the meat using sterile spreader. Inoculated meat samples were then dried for 1 h at room temperature. Thereafter, samples were dipped in phenolic acid solutions containing a concentration of 150 ppm and 200 ppm of each phenolic acid and their combination (1:1) for 60 s. Broth inoculum containing a bacterial suspension of $1x10^8$ CFU/mL for each strain was used as the control. The samples were stored at 4°C for a 72-hour period in sterilized stomacher bags containing phosphate buffer solution (PBS). Microbial survival was also evaluated at 12, 24, 48, and 72 h. Plates were incubated at 37°C for 24 h prior to enumeration. Each experiment was repeated at least three times.

Statistical analysis

All experiments were performed in triplicate. Experimental data were analyzed by ANOVA (Analysis of Variance) ($P<0.05$). The mean values of the experimental growth data were compared using Duncan's multiple range test.

Results and Discussion

Antibacterial activity of phenolic acids using disk diffusion assay

The effects of caffeic acid, ferulic acid, and their combination on the growth of *L. monocytogenes* and *E. coli* O157:H7 are shown in Table 1. They demonstrated various degrees of inhibition against the two pathogenic strains. Ferulic acid was found to be most effective against the *E. coli* O157:H7 strain at 150 ppm concentration, with a zone of inhibition of 10 mm, compared to caffeic acid and their combination at the same concentration. There was no difference ($P<0.05$) in the inhibitory actions of caffeic acid and the combination of caffeic and ferulic acids at this same concentration (150 ppm). With an increase in the ferulic acid concentration to 200 ppm, the zone of

inhibition was found to increase further. For *L. monocytogenes*, caffeic acid was the most effective antibacterial agent at the two concentrations tested, giving zones of inhibition of 7.66 and 11 mm at 150 and 200 ppm, respectively. The structure-function relationships between the antimicrobials and the respective microorganisms could justify the contrasting activities of the phenolic acids against Gram-positive and Gram-negative bacteria (Sánchez-Maldonado *et al.,* 2011). Distilled water did not inhibit the growth of organisms as no inhibition zones was detected during screening. Ciprofloxacin, used as the positive control, demonstrated greater inhibitory potential for *L. monocytogenes,* possibly due to differences in the cell wall structures of Gram-positive and Gram-negative bacteria. The combination of caffeic acid and ferulic acid exhibited a joint effect at both concentrations, suggesting an affinity relationship between the two antimicrobials. However, they tend to possess a greater antimicrobial activity individually than in combination.

Inhibitory effect of phenolic acids against *E. coli* O157:H7 and *L. monocytogenes* in broth

The growth response of *E. coli* O157:H7 and *L. monocytogenes* in broth media treated with phenolic acids at 150 or 200 ppm and stored at 4°C for a 72 h period is presented in Table 2. The high levels of *L. monocytogenes* and *E. coli* inocula in broth and meat samples in this study are necessary to clearly understand the efficiency of the different concentrations of phenolic acids and the extent of their activity in highly contaminated food materials. Inoculum levels excess of 10^7–10^9 have been used by previous authors (De Souza *et al.,* 2018; Kwon *et al.,* 2019; Rodriguez-Vaquero *et al.,* 2007). In the control medium, the number of viable cells of *E. coli* O157:H7 increased from $7.70x10^8$ to $8.01x10^8$ CFU/mL at the end of the incubation period (72 h). For *L. monocytogenes*, the number of viable cells in the control medium increased from $7.87x10^8$ after 12 h to $8.21x10^8$ at the end of the incubation period. At a concentration of 150 ppm, ferulic acid was the most effective antimicrobial agent against *E. coli* O157:H7, showing a reduction of 2.98 log CFU/mL, while caffeic acid was the most effective against *L. monocytogenes* with a log reduction of 2.17 log CFU/mL following the incubation period. The combination of caffeic acid and ferulic acid in a 1:1 ratio demonstrated a synergistic effect whereby the viability of microorganisms diminished by 2.42 and 2.14 log cycles, respectively. At a concentration of 200 ppm, the order of effectiveness remained the same for both Gram-positive and Gram-negative strains; however, a higher inhibition rate was observed (Table 2) – a reduction of 3.49 log CFU/mL against *E. coli* O157:H7 by ferulic acid and 2.35 log CFU/mL against *L. monocytogenes* by caffeic acid. The synergistic effect of the combination of caffeic acid and ferulic acid enhanced the reduction of viability of microorganisms by 2.42 and 2.25 log CFU/mL (at 150 ppm) as well as 3.14 and 2.62 log CFU/mL for *E. coli* and *L. monocytogenes*, respectively. In a previous study, the combination of caffeic acid and gallic acid, at 100 ppm and 200 ppm solution produced 1 log CFU/mL and 2 log CFU/mL reduction of *L. monocytogenes*, respectively, in meat (Rodriguez-Vaquero *et al.,* 2011). In broth media, phenolic acids were less effective against *L. monocytogenes.* A possible explanation could be that the microorganism can proliferate under refrigeration conditions, allowing it to be more adaptable to the stress imposed by phenolic acids.

Inhibitory effect of phenolic acids against *E. coli* O157:H7 and *L. monocytogenes* in cold-cut meats

Table 3 illustrates the growth response of *E. coli* O157:H7 and *L. monocytogenes* in cold-cut meats treated with phenolic acids and stored at 4°C for over 72 h. The number of viable cells in the control samples increased from $7.49x10^8$ to $7.75x10^8$ CFU/mL for *E. coli* O157:H7 and

Table 1. Antibacterial activity of phenolic compounds against *E. coli* O157:H7 and *L. monocytogenes* determined by disk diffusion assay.

Treatments	Concentration	Zone of inhibition (mm) Organism	
		***E. coli* O157:H7 (ATCC 43888)**	***L. monocytogenes* (ATCC 7644)**
Caffeic acid	150 ppm	7.00 ± 1.73^b	7.66 ± 2.51^b
	200 ppm	9.33 ± 1.15^a	11.00 ± 3.00^a
Ferulic acid	150 ppm	10.00 ± 2.00^b	6.00 ± 1.72^b
	200 ppm	12.33 ± 2.51^a	8.33 ± 2.52^a
Combination (1:1)	150 ppm	7.00 ± 1.73^b	6.67 ± 1.15^b
	200 ppm	8.67 ± 1.16^a	7.66 ± 2.51^a
Control	Positive	26.67 ± 2.88^a	36.67 ± 2.89^a
	Negative	ND	ND

Superscripts indicate significant differences ($P < 0.05$) across the columns only for each phenolic acid and concentration.

Table 2 The effect of phenolic compounds (at 150 ppm and 200 ppm) against *E. coli* O157:H7 and *L. monocytogenes* at 4°C for over 72 h in a broth medium.

Phenolic acids (150 ppm)	Population (log CFU/mL) over storage period (h) of			
	12	24	48	72
***E. coli* O157:H7 (ATCC 43888)**				
Control	7.70 ± 0.04[a]	7.80 ± 0.04[a]	7.92 ± 0.06[a]	8.01 ± 0.04[a]
Caffeic acid	6.08 ± 0.07[b]	5.73 ± 0.05[b]	5.71 ± 0.05[b]	5.63 ± 0.06[b]
Ferulic acid	5.89 ± 0.03[c]	5.57 ± 0.03[b]	5.55 ± 0.05[b]	5.03 ± 0.05[b]
Combination (1:1)	6.01 ± 0.03[b]	5.70 ± 0.13[b]	5.61 ± 0.03[b]	5.59 ± 0.07[b]
***L. monocytogenes* (ATCC 7644)**				
Control	7.87 ± 0.03[a]	8.08 ± 0.05[a]	8.17 ± 0.04[a]	8.21 ± 0.03[a]
Caffeic acid	6.24 ± 0.02[b]	6.13 ± 0.02[b]	6.08 ± 0.03[b]	6.04 ± 0.05[b]
Ferulic acid	6.36 ± 0.03[b]	6.18 ± 0.02[b]	6.14 ± 0.03[b]	6.11 ± 0.03[b]
Combination (1:1)	6.33 ± 0.03[b]	6.18 ± 0.03[b]	6.12 ± 0.02[b]	6.07 ± 0.04[b]
Phenolic acids (200 ppm)				
***E. coli* O157:H7 (ATCC 43888)**				
Control	7.70 ± 0.04[a]	7.80 ± 0.04[a]	7.92 ± 0.06[a]	8.01 ± 0.04[a]
Caffeic acid	6.30 ± 0.02[b]	6.27 ± 0.02[b]	5.34 ± 0.01[b]	5.29 ± 0.02[b]
Ferulic acid	6.29 ± 0.03[b]	6.21 ± 0.02[b]	4.85 ± 0.04[c]	4.52 ± 0.06[c]
Combination (1:1)	6.33 ± 0.05[b]	6.26 ± 0.03[b]	4.97 ± 0.04[c]	4.87 ± 0.04[c]
***L. monocytogenes* (ATCC 7644)**				
Control	7.87 ± 0.03[a]	8.08 ± 0.05[a]	8.17 ± 0.04[a]	8.21 ± 0.03[a]
Caffeic acid	6.19 ± 0.02[b]	6.01 ± 0.03[b]	5.92 ± 0.06[b]	5.86 ± 0.08[b]
Ferulic acid	6.24 ± 0.03[b]	6.09 ± 0.03[b]	6.03 ± 0.05[b]	6.01 ± 0.02[b]
Combination (1:1)	6.22 ± 0.02[b]	6.05 ± 0.03[b]	6.01 ± 0.04[b]	5.96 ± 0.03[b]

Superscripts indicate significant differences (P<0.05) across the columns only for each phenolic acid used.

$7.51x10^8$ to $7.96x10^8$ CFU/mL for *L. monocytogenes*. At a concentration of 150 ppm, the combination of caffeic acid and ferulic acid was found to be most effective against *E. coli* O157:H7 in comparison to caffeic acid, and ferulic acid was applied individually, with a log reduction of 3.38 CFU/mL at 72 h. As observed in broth media, caffeic acid revealed the greatest effects against *L. monocytogenes* in cold-cut meats with a log reduction of 2.44 CFU/mL at the maximum incubation period. At a concentration of 200 ppm, a similar pattern of effectiveness was documented for both Gram-positive and Gram-negative strains, with a higher inhibition rate (Table 3). The survival rate was lower for both strains, indicating that the synergistic effect of the combination of caffeic acid and ferulic acid was more effective in the meat compared to the broth. This could be attributed to the presence of macromolecules (proteins, lipids) and micromolecules (vitamins) in the food matrix (meat), which provided enhanced affinity for phenolic compounds. Arima *et al.* (2002) reported that the combinations of quercetin and quercitrin, quercetin and morin, and quercetin and rutin portrayed synergistic effects resulting in improved efficacy than individual flavonoids against *Bacillus cereus* and *Salmonella enteritidis*.

The inhibitory effect of phenolic compound mixtures was greater at 4°C incubation temperature than at 20°C for meat (Rodriguez-Vaquero *et al.*, 2011) and fish (Rodríguez-Vaquero *et al.*, 2013), reducing the viability of *L. monocytogenes* at two concentrations (100 and 200 mg/L). This is because their mode of action depends on their migration into bacterial membranes, which reduce fluidity at lower temperatures (Ultee *et al.*, 2000). Beuchat *et al.* (1994) substantiated the improved antibacterial effect of phenolic compounds at low storage conditions. The shelf lives of foods could be further preserved by hurdle technology, such as in the case of combining refrigeration temperatures between 0°C and 4°C with modified atmosphere packaging to preserve foods (Leistner & Gorris, 1995).

Conclusions

Caffeic acid, ferulic acid, and their combination have potentials for the effective reduction or inhibition of foodborne pathogens; thus, providing a good alternative to chemical additives. Zones of inhibition of *E. coli* and *L. monocytogenes* were widened with an increase in the

Table 3 The effect of phenolic compounds (at 150 ppm) against *E. coli* O157:H7 and *L. monocytogenes* at 4°C for over 72 h in meat.

Phenolic acids (150 ppm)	Population (log CFU/g) over storage period (h) of			
	12	24	48	72
***E. coli* O157:H7 (ATCC 43888)**				
Control	7.49 ± 0.06^{a}	7.70 ± 0.05^{a}	7.76 ± 0.06^{a}	7.75 ± 0.11^{a}
Caffeic acid	5.67 ± 0.04^{b}	5.51 ± 0.04^{b}	5.22 ± 0.05^{b}	4.98 ± 0.05^{b}
Ferulic acid	5.49 ± 0.02^{c}	5.31 ± 0.04^{c}	5.06 ± 0.08^{b}	4.51 ± 0.11^{b}
Combination (1:1)	5.42 ± 0.03^{c}	5.21 ± 0.04^{c}	4.91 ± 0.02^{b}	4.37 ± 0.04^{c}
***L. monocytogenes* (ATCC 7644)**				
Control	7.51 ± 0.05^{a}	7.71 ± 0.05^{a}	7.87 ± 0.04^{a}	7.96 ± 0.02^{a}
Caffeic acid	6.29 ± 0.02^{c}	6.09 ± 0.03^{b}	5.76 ± 0.03^{b}	5.52 ± 0.04^{b}
Ferulic acid	6.52 ± 0.02^{b}	6.39 ± 0.02^{b}	6.12 ± 0.03^{b}	5.97 ± 0.06^{b}
Combination (1:1)	6.41 ± 0.03^{c}	6.27 ± 0.08^{b}	5.99 ± 0.03^{b}	5.68 ± 0.04^{b}
Phenolic acids (200 ppm)				
***E. coli* O157:H7 (ATCC 43888)**				
Control	7.49 ± 0.06^{a}	7.70 ± 0.05^{a}	7.76 ± 0.06^{a}	7.75 ± 0.11^{a}
Caffeic acid	5.34 ± 0.08^{b}	5.21 ± 0.05^{b}	4.94 ± 0.07^{b}	4.67 ± 0.08^{b}
Ferulic acid	5.24 ± 0.05^{b}	5.10 ± 0.02^{b}	4.76 ± 0.08^{b}	4.23 ± 0.04^{c}
Combination (1:1)	5.19 ± 0.02^{b}	5.07 ± 0.06^{b}	4.66 ± 0.08^{b}	4.09 ± 0.02^{c}
***L. monocytogenes* (ATCC 7644)**				
Control	7.51 ± 0.05^{a}	7.71 ± 0.05^{a}	7.87 ± 0.04^{a}	7.96 ± 0.02^{a}
Caffeic acid	6.02 ± 0.04^{b}	5.63 ± 0.02^{b}	5.52 ± 0.05^{b}	5.25 ± 0.02^{c}
Ferulic acid	6.19 ± 0.02^{b}	5.77 ± 0.04^{b}	5.74 ± 0.04^{b}	5.63 ± 0.04^{b}
Combination (1:1)	6.12 ± 0.03^{b}	5.77 ± 0.04^{b}	5.68 ± 0.03^{b}	5.49 ± 0.02^{c}

Superscripts indicate significant differences (P<0.05) across the columns only for each phenolic acid used.

concentration of phenolic acids, with ferulic acid having the greatest inhibition effect on *E. coli* at 200 ppm. For the two concentrations tested, phenolic acids were more efficacious against *E. coli* in broth and meat as the storage hours increased, compared to *L. monocytogenes.* However, the greater effects were obtained at 200 ppm for the two phenolic acids and their combination for both microorganisms. These results show the effectiveness of phenolic acids tested, at the stipulated concentrations, against the pathogenic organisms investigated and, as such, may allow the formulation of new antimicrobial products for potential use as food preservatives. Further studies involving sensory evaluation may be necessary to determine consumer acceptability of caffeic and ferulic acid-treated meats.

Acknowledgments

This work is based on research supported in part by the National Research Foundation of South Africa, SA (NRF)/Russia (RFBR) Joint Science and Technology Research Collaboration (Grant Number: 118910).

References

Arima, H., Ashida, H. and Danno, G.I., 2002. Rutin-enhanced antibacterial activities of flavonoids against *Bacillus cereus* and *Salmonella enteritidis*. Bioscience, Biotechnology, and Biochemistry 66(5):1009–1014. https://doi.org/10.1271/bbb.66.1009

Beuchat, L.R., Brackett, R.E. and Doyle, M.P., 1994. Lethality of carrot juice to *Listeria monocytogenes* as affected by pH, sodium chloride and temperature. Journal of Food Protection 57(6): 470–474. https://doi.org/10.4315/0362-028X-57.6.470

Buchanan, R.L., Gorris, L.G., Hayman, M.M., Jackson, T.C., and Whiting, R.C., 2017. A review of *Listeria monocytogenes*: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. Food Control 75:1–13. https://doi.org/10.1016/j.foodcont.2016.12.016

Campion, A., Morrissey, R., Field, D., Cotter, P.D., Hill, C., and Ross, R.P., 2017. Use of enhanced nisin derivatives in combination with food-grade oils or citric acid to control *Cronobacter sakazakii* and *Escherichia coli* O157: H7. Food Microbiology 65: 254–263. https://doi.org/10.1016/j.fm.2017.01.020

Chen, J.Q., Regan, P., Laksanalamai, P., Healey, S., and Hu, Z., 2017. Prevalence and methodologies for detection, characterization and subtyping of *Listeria monocytogenes* and

L. ivanovii in foods and environmental sources. Food Science and Human Wellness 6(3):97–120. https://doi.org/10.1016/j.fshw.2017.06.002

Cheynier, V., 2012. Phenolic compounds: from plants to foods. Phytochemistry Reviews 11:153–177. https://doi.org/10.1007/s11101-012-9242-8

De Souza, J., Ahmed, R., Strange, P., Barbut, S. and Balamurugan, S., 2018. Effect of caliber size and fat level on the inactivation of *E. coli* O157: H7 in dry fermented sausages. International Journal of Food Microbiology 266:167–172. https://doi.org/10.1016/j.ijfoodmicro.2017.11.024

Hunjak, B., Andric, N., Beslic, I. and Fabris, A., 2019. *Listeria monocytogenes* – clinical significance in pregnant women and newborns. International Journal of Infectious Diseases 79:88. https://doi.org/10.1016/j.ijid.2018.11.221

Kwon, S.A., Song, W.J. and Kang, D.H., 2019. Combination effect of saturated or superheated steam and lactic acid on the inactivation of *Escherichia coli* O157: H7, *Salmonella typhimurium* and *Listeria monocytogenes* on cantaloupe surfaces. Food Microbiology 82:342–348. https://doi.org/10.1016/j.fm.2019.03.012

Leistner, L. and Gorris, L.G., 1995. Food preservation by hurdle technology. Trends in Food Science and Technology 6(2):41–46. https://doi.org/10.1016/S0924-2244(00)88941-4

Marušić, N., Vidaček, S., Janči, T., Petrak, T. and Medić, H., 2014. Determination of volatile compounds and quality parameters of traditional Istrian dry-cured ham. Meat Science 96(4):1409–1416. https://doi.org/10.1016/j.meatsci.2013.12.003

Maurya, D.K. and Devasagayam, T.P.A., 2010. Antioxidant and prooxidant nature of hydroxycinnamic acid derivatives ferulic and caffeic acids. Food and Chemical Toxicology 48(12):3369–3373. https://doi.org/10.1016/j.fct.2010.09.006

Nyström, L., Mäkinen, M., Lampi, A.M. and Piironen, V., 2005. Antioxidant activity of steryl ferulate extracts from rye and wheat bran. Journal of Agricultural and Food Chemistry 53(7):2503–2510. https://doi.org/10.1021/jf048051t

Pernin, A., Dubois-Brissonnet, F., Roux, S., Masson, M., Bosc, V. and Maillard, M.N., 2018. Phenolic compounds can delay the oxidation of polyunsaturated fatty acids and the growth of *Listeria monocytogenes*: structure-activity relationships. Journal of the Science of Food and Agriculture 98(14):5401–5408. https://doi.org/10.1002/jsfa.9082

Pernin, A., Guillier, L. and Dubois-Brissonnet, F., 2019. Inhibitory activity of phenolic acids against *Listeria monocytogenes*: deciphering the mechanisms of action using three different models. Food Microbiology 80:18–24. https://doi.org/10.1016/j.fm.2018.12.010

Rodriguez-Vaquero, M.R., Alberto, M.R. and de Nadra, M.M., 2007. Antibacterial effect of phenolic compounds from different wines. Food Control 18(2):93–101. https://doi.org/10.1016/j.foodcont.2005.08.010

Rodriguez-Vaquero, M.J., Aredes Fernández, P.A. and Manca de Nadra, M.C., 2011. Effect of phenolic compound mixtures on the viability of *Listeria monocytogenes* in meat model. Food Technology and Biotechnology 49(1):83–88.

Rodríguez-Vaquero, M.J., Aredes Aredes-Fernández, P. and Manca de Nadra, M.C., 2013. Phenolic compounds from wine as natural preservatives of fish meat. Food Technology and Biotechnology 51(3):376–382.

Romaniuk, J.A. and Cegelski, L., 2015. Bacterial cell wall composition and the influence of antibiotics by cell-wall and whole-cell NMR. Philosophical Transactions of the Royal Society B: Biological Sciences 370(1679):20150024. https://doi.org/10.1098/rstb.2015.0024

Rybarczyk, J., Kieckens, E., Vanrompay, D. and Cox, E., 2017. In vitro and in vivo studies on the antimicrobial effect of lactoferrin against *Escherichia coli* O157: H7. Veterinary Microbiology 202:23–28. https://doi.org/10.1016/j.vetmic.2016.05.010

Sánchez-Maldonado, A.F., Schieber, A. and Gänzle, M.G., 2011. Structure–function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria. Journal of Applied Microbiology 111(5):1176–1184. https://doi.org/10.1111/j.1365-2672.2011.05141.x

Saxena, T., Kaushik, P. and Mohan, M.K., 2015. Prevalence of *E. coli* O157: H7 in water sources: an overview on associated diseases, outbreaks and detection methods. Diagnostic Microbiology and Infectious Disease 82(3):249–264. https://doi.org/10.1016/j.diagmicrobio.2015.03.015

Shpaizer, A., Nussinovich, A., Kanner, J. and Tirosh, O., 2018. S-nitroso-N-acetylcysteine generates less carcinogenic N-nitrosamines in meat products than nitrite. Journal of Agricultural and Food Chemistry 66(43):11459–11467. https://doi.org/10.1021/acs.jafc.8b04549

Ultee, A., Slump, R.A., Steging, G. and Smid, E.J., 2000. Antimicrobial activity of carvacrol toward *Bacillus cereus* on rice. Journal of Food Protection 63(5):620–624. https://doi.org/10.4315/0362-028X-63.5.620

Zhang, H., Wu, J. and Guo, X., 2016. Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality. Food Science and Human Wellness 5(1):39–48. https://doi.org/10.1016/j.fshw.2015.11.003