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Application of caffeine, 1,3,7-trimethylxanthine, to control *Escherichia coli* O157:H7

S.A. Ibrahim *, M.M. Salameh, S. Phetsomphou, H. Yang, C.W. Seo

Food Safety and Microbiology Laboratory, 161 Carver Hall, North Carolina Agricultural and Technical State University, Greensboro, NC 27411-1064, United States

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Abstract

The objective of this research was to determine the effectiveness of caffeine on inactivation of *Escherichia coli* O157:H7 in brain heart infusion (BHI) broth. Overnight samples of five *E. coli* O157:H7 strains of (E0019, F4546, H1730, 944 and Cider) were used in this study. These strains were individually inoculated at an initial inoculum level of 2 log CFU/ml into BHI broth containing caffeine at different concentrations (0.00%, 0.25%, 0.50%, 0.75%, 1.00%, 1.25%, 1.50%, 1.75%, and 2.00%). Samples were then incubated at 37 °C for 24 h. Bacterial growth was monitored at different time intervals by measuring turbidity at 610 nm using a spectrophotometer. Results revealed that the addition of caffeine inhibited the growth of *E. coli* O157:H7. Significant growth inhibition was observed with concentration levels of 0.50% and higher. These results indicate that caffeine has potential as an antimicrobial agent for the treatment of *E. coli* O157:H7 infection and should be investigated further as a food additive to increase biosafety of consumable food products.

Keywords: Caffeine; Antimicrobial; *Escherichia coli*; Pathogen

1. Introduction

Escherichia coli O157:H7, a leading cause of bacterial foodborne disease outbreaks, is responsible for approximately 73,500 cases of foodborne illnesses per year (CDC, 1995). *E. coli* O157:H7 is a Gram-negative rod that causes serious illness, such as hemorrhagic colitis and hemolytic uremic syndrome. The bacterium was first identified as a dangerous foodborne pathogen in 1982 when several people in Michigan and Oregon became ill with severe abdominal pain and bloody diarrhea (Riley et al., 1983). This outbreak was associated with the consumption of undercooked ground beef from a fast food restaurant chain. In 1993, over 700 people, in four states, were infected with *E. coli* O157:H7 and four deaths were attributed to contaminated hamburgers served at a food restaurant (CDC, 1983). More recently, foods have been implicated in outbreaks, including fresh produces, acid or acidified food products, such as apple cider, salad dressings and mayonnaise (Besser et al., 1993; CDC, 1995; Mayerhauser, 2001).

Food preservation processes are used by the food industry to ensure that consumers receive safe wholesome food products. Among the most safe and effective approaches in food preservation is the use of preservative agents that have antimicrobial activity. These antimicrobial agents can be classified into natural and artificial chemical compounds. Artificial compounds include chemicals such as organic acids, whereas natural compounds are derived from plants and biopreservatives from fermentation of lactic acid bacteria. Currently, consumers are paying closer attention not only to the risk of foodborne pathogens but also to artificial chemical preservatives often used to control these foodborne pathogens (Abee, Lrockel, & Hill, 1995). This concern has generated great interest in the use of natural ingredients as antimicrobial compounds as a way of avoiding consumption of perceived ''unhealthy''

* Corresponding author. Tel.: +1 336 334 7328; fax: +1 336 334 7239.
E-mail address: ibrah001@ncat.edu (S.A. Ibrahim).

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ingredients present in artificial chemical compounds used in food products.

The antimicrobial effects of many plant extracts have been well studied. For example, essential oils from plants such as basil, cumin, and oregano have been shown to inhibit different foodborne pathogens (Fridman, Henika, Levin, & Mandrell, 2004). Arrowroot tea extract have been shown to have antimicrobial activity against *E. coli* O157:H7 (Kim & Fung, 2004). The antimicrobial action found in plants is believed to be present as a defence mechanism. Consequently, plants that manifest relatively high levels of antimicrobial action may be sources of compounds that may inhibit the growth of foodborne pathogens.

Caffeine-based products are among the most widely consumed foods in the world, e.g., tea, coffee and cocoa. Structurally related to uric acid, caffeine (1,3,7-trimethyl xanthine) is one of three methylated xanthine alkaloid derivatives that are present in many plant species throughout the world. A few of studies on the antimicrobial activities are related to the Maillard reaction products present in coffee (Daglia, Cuzzoni, & Dacarro, 1994a; Daglia, Cuzzoni, & Dacarro, 1994b; Daglia, Papetti, Dacarro, & Gazzani, 1998). However, there is no study on using caffeine as a food preservative/antimicrobial, especially against foodborne pathogens, such as *E. coli* O157:H7. The purpose of this study was to determine the antimicrobial ability of different concentrations of caffeine against five different strains of *E. coli* O157:H7.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Five samples of *E. coli* O157:H7 strains (Table 1) were supplied by Dr. S.S. Sumner, Department of Food Science and Technology at Virginia Tech. Strains were kept in −80 °C stock cultures in tryptic soy broth with 20% glycerol. Strains were activated by transferring 50 μl of the stock into 5 ml of brain heart infusion (BHI) and incubating at 37 °C for 18 h. All working cultures were kept at 4 °C on tryptic soy agar (TSA) slants. Strains were maintained everyday by transferring 100 μl of overnight culture into fresh 9 ml BHI broth and incubating at 37 °C for 24 h. Overnight cultures were then centrifuged at 6000*g* and 4 °C for 15 min. Cell pellets were washed twice with sterile peptone water. Bacterial cells were suspended in 9 ml of sterile peptone water and the cell suspension was serially diluted in sterile peptone water to give a cell number of 10^3 CFU/ml and used immediately for sample inoculation.

Table 1
Bacterial strains and food-associated outbreaks

Strain	Isolate	Food source of outbreak
E. coli O157:H7	944	Salami
E. coli O157:H7	Cider	Cider
E. coli O157:H7	E0019	Beef
E. coli O157:H7	H1730	Lettuce
E. coli O157:H7	F4546	Alfalfa sprout

2.2. Sample preparation and treatment

Ten ml batches of BHI broth containing caffeine (Sigma Chemicals, St. Louis, MO, USA) at concentrations of 0.0%, 0.25%, 0.50%, 0.75%, 1.00%, 1.25%, 1.50%, 1.75% and 2.00% (w/v) were prepared and autoclaved at 121 °C for 15 min. Samples were inoculated with 100 μl of bacterial cells and incubated at 37 °C for 24 h. Bacterial growth was monitored by measuring the turbidity at different time interval during the incubation period using a spectronic 21 Milton Roy Spectrophotometer (Thermo Electron Scientific Co., Madison, WI).

2.3. Bacterial enumeration

At the end of the incubation period, 1 ml of the *E. coli* strains was removed, and 0.1 ml of the appropriate dilution was surface plated on duplicate plates of BHI agar. The plates were incubated at 37 °C for 48 h and bacterial colonies were counted.

2.4. Experimental design

The experimental design for this study was a complete $5 \times 9 \times 8$ factorial design of *E. coli* strains (944, cider, E0019, F4546, and H1730), caffeine concentrations (0.0%, 0.25%, 0.50%, 0.75%, 1.00%, 1.25%, 1.50%, 1.75% and 2.00%) and incubation times (0, 2, 4, 6, 8, 10, 12, and 24 h). For each sample, bacterial growth was monitored by measuring the turbidity at different time intervals during the incubation period.

2.5. Statistical analysis

The experiments tests were conducted three times, independently, for each bacterial strain to determine whether caffeine influenced microbial growth. The microbiological data were analyzed by the general linear model (GLM) procedure of SAS/STAT software (SAS Institute Inc., Cary, NC).

3. Results and discussion

Figs. 1–5 show the survival and growth of the five strains of *E. coli* O157:H7 in the presence of different concentrations of caffeine in laboratory medium (BHI) during the incubation at 37 °C for 24 h. When *E. coli* strains were grown in BHI broth without caffeine (controls), the strains continued to grow during the incubation period and reached the stationary phase within 8–10 h. The turbidity readings reached absorbance at 610 nm of 1.00–1.10. When caffeine was added to BHI at 0.25%, significant growth inhibition ($P < 0.05$) was observed in all tested strains.

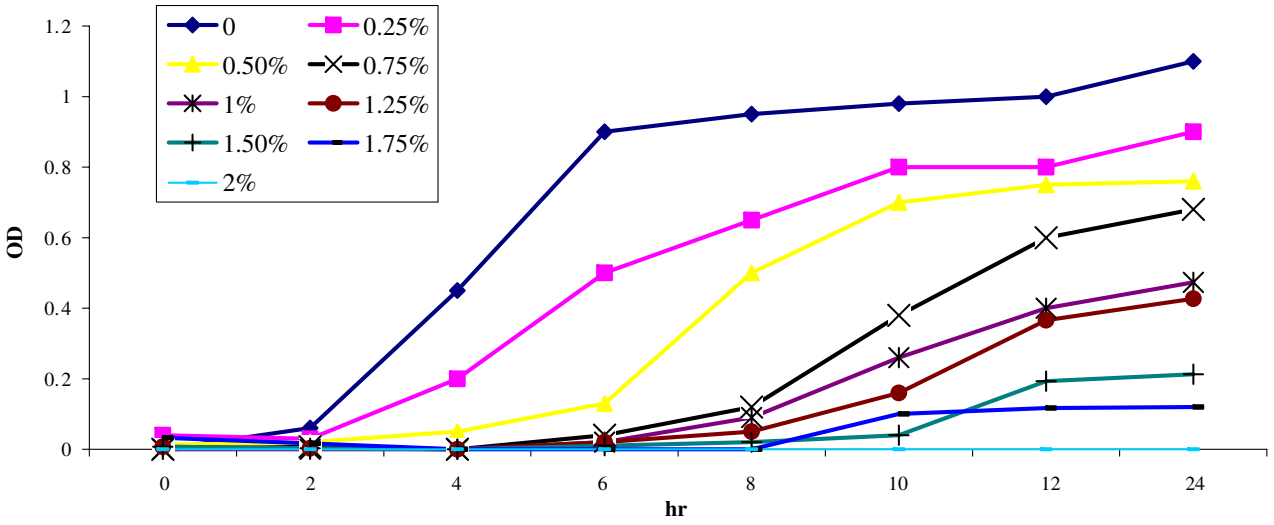

Fig. 1. Survival and growth of *E. coli* O157:H7 (strain 944) in the presence of caffeine at different concentrations.

The growth inhibition ranged between 27% and 50% within 4 h of incubation at 37 °C; however, after a 24 h period, the growth of *E. coli* in laboratory media with 0.25% caffeine, continued but was less than the control samples ($P < 0.001$). The growth inhibition was at least 90% when caffeine was added at 0.50% or more in all tested strains, indicating that caffeine had significant growth inhibition ($P < 0.0001$). This also indicates that a 0.50% caffeine level is effective in controlling the growth of *E. coli* in laboratory media. With the use of 0.75% caffeine, there was little if any bacterial growth for the first 8 h; thereafter, growth commenced, reaching a maximum after 24 h, but was significantly lower than the control ($P < 0.0001$). Higher concentrations of caffeine continued to cause further reduction of *E. coli* growth. The pattern of growth inhibition with caffeine at different concentrations (0–2%) was similar among all strains of *E. coli* O157:H7 used in this study. When caffeine concentrations increased in broth, growth inhibition was significantly increased among all tested strains ($P < 0.0001$) (Table 2).

Table 3 shows the effects of different concentrations of caffeine on the survival and growth of five strains of *E. coli* O157:H7 grown in BHI broth during the incubation at 37 °C for 24 h. The initial bacterial populations in all samples were less than 10^2 CFU/ml. When *E. coli* strains were grown in broth without caffeine, bacterial population continued to grow and reached an average of 9.06 CFU/ml among the tested strains. When *E. coli* was grown in BHI

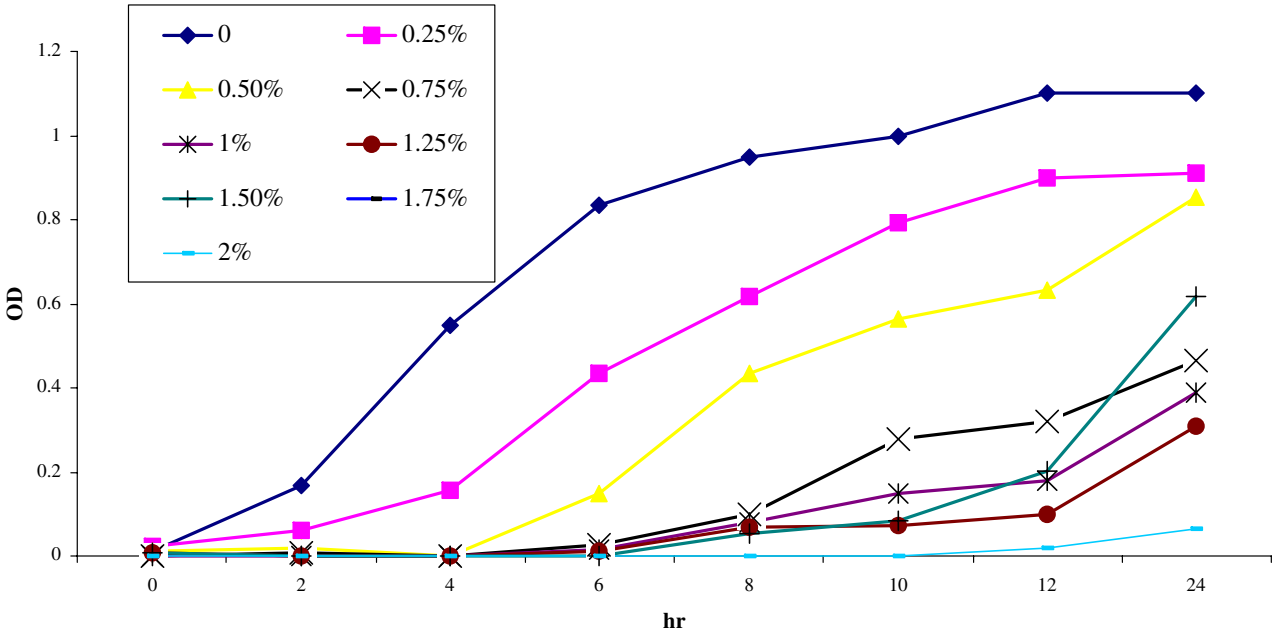

Fig. 2. Survival and growth of *E. coli* O157:H7 (cider) in the presence of caffeine at different concentrations.

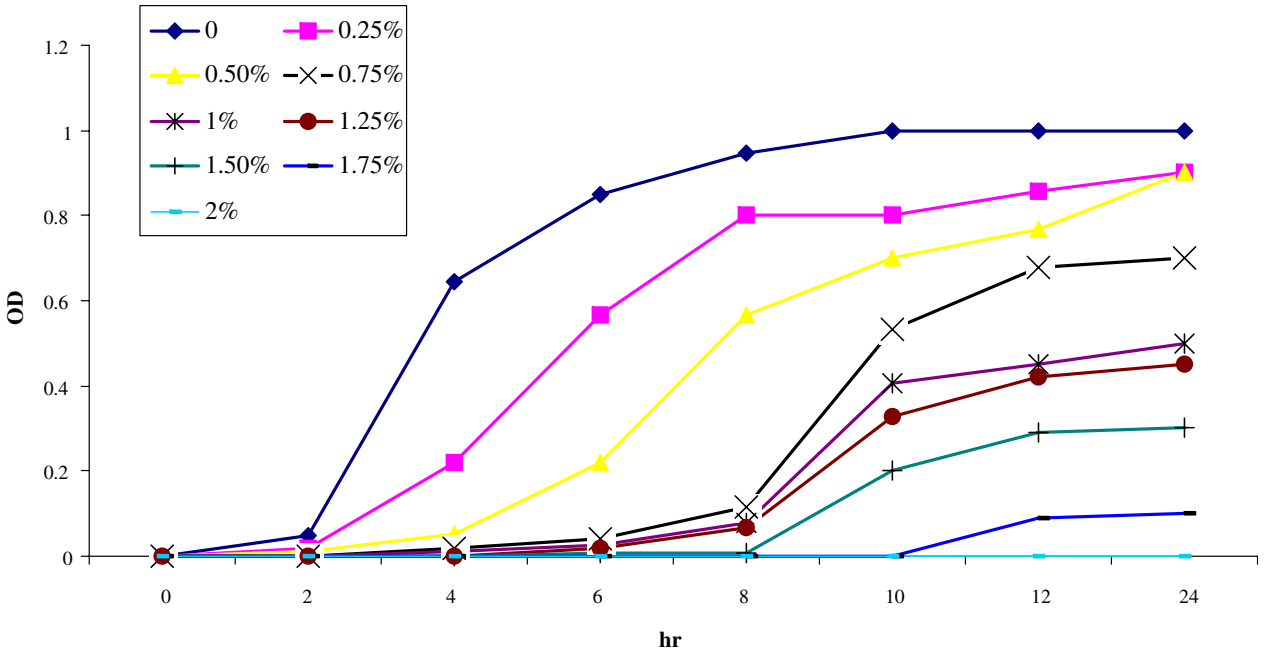

Fig. 3. Survival and growth of *E. coli* O157:H7 (strain E0019) in the presence of caffeine at different concentrations.

broth containing 0.25% caffeine, the bacterial growth significantly decreased by 0.37 log CFU/ml ($P < 0.001$) when compared to the control. Increasing concentrations of caffeine were shown to cause further growth inhibition ($P < 0.001$) for all *E. coli* strains tested in this study. With the use of 0.75% caffeine, bacterial population was reduced by 1.4 log CFU/ml. The addition of 1.00% caffeine caused a reduction of bacterial cells by an average of 2.00 log CFU/ml. More than a 3 log CFU/ml reduction of *E. coli* population was observed with the 1.50% caffeine level. Our results also showed that 2% was needed to achieve more than 4 log CFU/ml among the tested strains (<0.001).

This study represents the first published work on the antimicrobial activity of caffeine against the foodborne pathogen, *E. coli* O157:H7. The objective of this study was to determine the effect of different concentrations of caffeine on the survival and growth *E. coli* O157:H7.

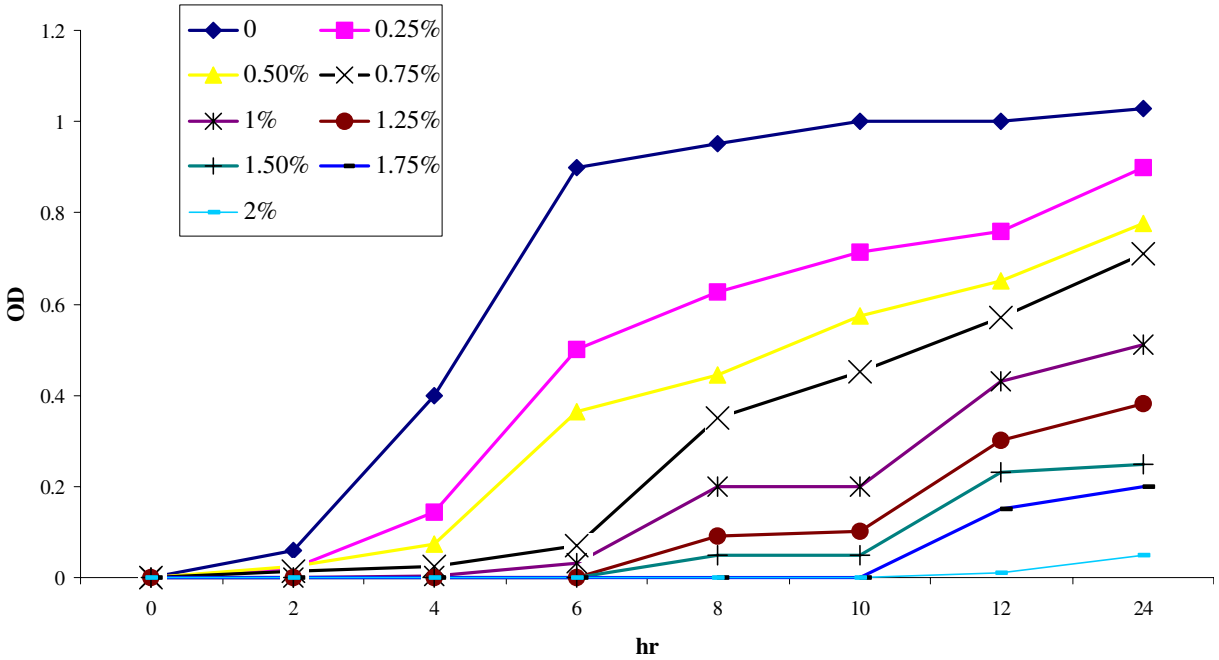

Fig. 4. Survival and growth of *E. coli* O157:H7 (strain F4546) in the presence of caffeine at different concentrations.

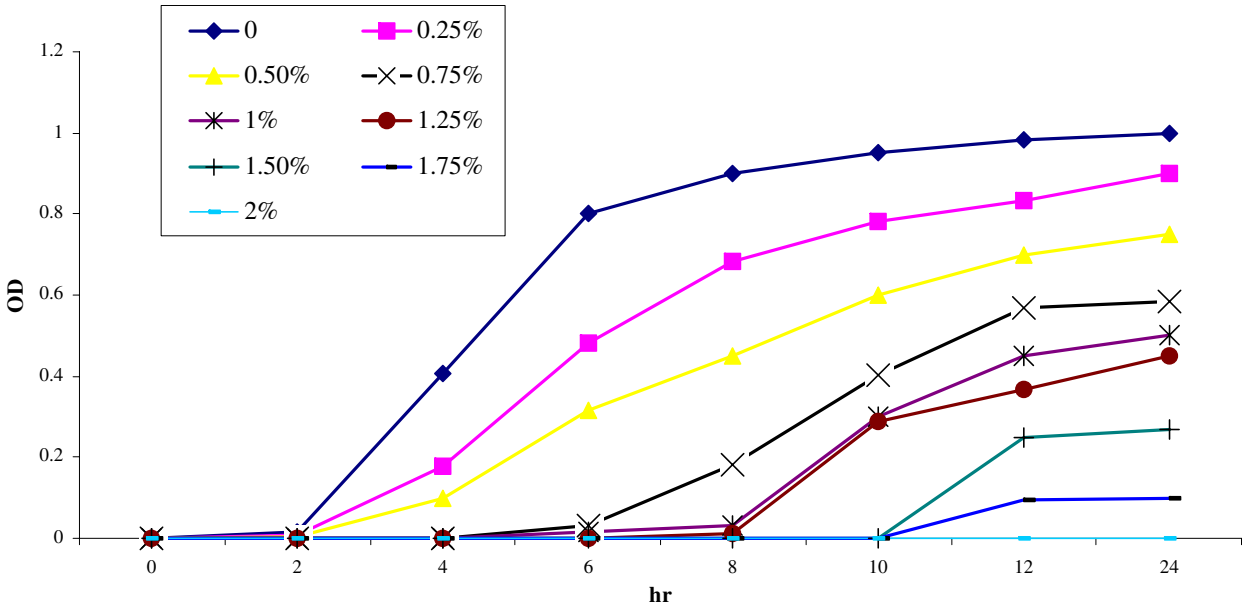

Fig. 5. Survival and growth of *E. coli* O157:H7 (strain H1730) in the presence of caffeine at different concentrations.

The concentration levels used in this study were based on preliminary data (Ibrahim, 2004) and the concentrations commonly found in food and food products, between 0.01% and 1.00%. Our results have demonstrated that caffeine has a strong antimicrobial activity against *E. coli* O157:H7. Significant growth reduction was recorded in all tested strains, indicating that , *E. coli* O157:H7 could not grow but survive in the presence of caffeine (see Table 3).

The mechanism of antimicrobial activity of caffeine against *E. coli* O157:H7 test strains was not investigated in this study. It is likely that caffeine could have damaged the bacterial DNA. Grigg (1972) and Middelhoven and Lommen (1984) used caffeine to inhibit DNA repair in bacteria as a method to introduce mutants. Caffeine at 0.1% concentration also inhibits protein synthesis in bacteria.

Most experts agree that 300 mg of caffeine (about the amount contained in 3 cups of coffee) is a moderate intake. People who have certain health problems need to check with their doctor as they consider their caffeine intake (Wardlaw, 1999). Due to the taste of caffeine, its application as a food ingredient or preservative should be combined with all other food components. For example, the addition of the caffeine should not overcome the main flavour or taste of food products. For special food products, such as soda, there is a need for further detailed study of the doses of caffeine required to control pathogenic bacteria.

Table 2
Statistical analysis data for the effect of different concentrations of caffeine on the survival and growth of five strains of *E. coli* O157:H7

The GLM procedure

Source	DF	Sum of squares	Mean square	*F* value	Pr > *F*
Dependent variable: OD					
Model	359	104.5582966	0.2912487	69.38	<0.0001
Error	646	2.7119333	0.0041980		
Corrected total	1005	107.2702299			

R-square	Coefficient variance	Root MSE	OD mean
0.974719	26.90407	0.064792	0.240827

Source	DF	Type IV SS	Mean square	*F* value	Pr > *F*
Bacteria	4	0.24843326	0.06210832	14.79	<0.0001
Caffeine	8	45.39701633	5.67462704	1351.73	<0.0001
Time	7	34.30513499	4.90073357	1167.39	<0.0001
Bacteria * caffeine	32	0.52104063	0.01628252	3.88	<0.0001
Bacteria * time	28	0.52855124	0.01887683	4.50	<0.0001
Caffeine * time	56	19.39212436	0.34628793	82.49	<0.0001
Bacteri * caffeine * time	224	1.22595391	0.00547301	1.30	0.0065

Table 3
Populations of (CFU/ml) of *E. coli* O157:H7 strains grown in BHI broth containing different concentrations of caffeine at 37 °C for 24 h

Strain	Caffeine concentrations (% w/vol)								
	0.00	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00
944	9.05[a]	8.45[b]	7.55[c]	7.05[d]	6.55[e]	6.00[f]	5.50[g]	5.00[h]	4.50[i]
Cider	9.06[a]	8.80[b]	8.25[c]	7.95[d]	6.70[e]	6.60[e]	6.15[f]	5.85[g]	4.85[h]
E0019	8.75[a]	8.70[a]	7.95[b]	7.55[c]	7.35[e]	7.00[f]	5.95[g]	5.70[h]	5.00[i]
F4546	9.22[a]	8.65[b]	8.32[c]	7.99[d]	7.62[e]	6.70[f]	6.05[g]	5.77[h]	4.90[i]
H7113	9.20[a]	8.80[b]	8.55[c]	7.75[d]	6.35[e]	6.12[f]	5.45[g]	5.05[h]	4.20[i]
Average	9.06	8.69	8.12	7.66	6.92	6.49	5.83	5.48	4.69

Data represent means of at least three measurements.
Means with different letters in the same row are significantly different ($P < 0.001$).
Initial population $\sim < 10^2$ CFU/ml.

4. Conclusion

Results have demonstrated that caffeine can significantly decrease the survival of *E. coli* strains grown in laboratory media. Caffeine is naturally present in different food products, such as coffee, tea, cola and chocolate. Therefore, caffeine could be used in foods as an natural ingredient or preservative to control the growth of several foodborne pathogens. Our results have important implications for the food industry. On the basis of this study, caffeine could be used, in combination with other natural ingredients, to control foodborne pathogens. Such combinations would be very powerful tools to ensure the safety of many food products. Additional research is needed to determine the survival of *E. coli* in specific food products because many factors can affect the pathogen's survival. Some possibilities include adding caffeine to several juices during the production stage and monitoring the survival of several foodborne pathogens and natural flora. A study is in progress to determine the efficiency of caffeine, in combination with organic acids, to inhibit listeria in ready-to-eat foods. Results obtained from this research could improve the safety of food products and establish an effective natural system to guard against foodborne pathogens. Future work will be needed to better understand the mechanism of antimicrobial activity and long-term effect of caffeine on *E. coli* O157:H7.

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