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Research Article

Ethyl Acetoacetate and β-Phenylethylamine Inhibit Spoilage Bacteria in Ground Beef

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Abstract

Ethyl acetoacetate (EAA) and β-phenylethylamine (PEA) were previously identified as inhibitors of bacterial growth and reducer of biofilm amounts for numerous bacterial pathogens. With this study, the effect of EAA and PEA on spoilage bacteria was determined in ground beef that was treated with either EAA or PEA and incubated at an abusive temperature of 10°C for up to five days. At a concentration of 0.5% w/w, EAA caused a 2.5 log reduction in total bacterial counts after three days of incubation at 10°C and similar reductions for a selection of specific spoilage organisms, including *Lactobacilli* and *Pseudomonads*. For *Brochothrix thermosphacta* and *Enterobacteriaceae*, reductions of approximately 1 log were observed after three days. PEA reduced total bacterial counts by 1.4 log. To ensure that the removal of the natural background flora of the meat would not increase the number of pathogens, *Escherichia coli* O157:H7 and *Salmonella enterica* serovar *typhimurium* were externally added to the ground beef. EAA reduced *Escherichia coli* O157:H7 by 1.06 log, whereas bacterial counts of *Salmonella enterica* were unaffected. PEA did not increase the bacterial counts of externally added *E. coli* or *S. enterica*. In conclusion, EAA and PEA were effective at inhibiting spoilage bacteria (*e.g. B. thermosphacta*) on ground beef and did not increase the numbers of the two tested bacterial pathogens, *E. coli and S. enterica*.

Keywords: *Meat Spoilage Microorganisms; Meat Safety; Beef Meat Anti-Microbials*

Abbreviations

EAA: Ethyl Acetoacetate; PEA: β-Phenylethylamine

Introduction

Meat harbors two different groups of microbiota; spoilage organisms whose metabolic activities affect the sensory attributes of the meat [20] and sometimes pathogens that can cause consumer illness [2]. The bacteria that contribute to spoilage during aerobic cold storage are predominantly *Pseudomonads*, while the bacterial counts of *Brochothrix thermosphacta* and *Enterobacteriaceae* are typically lower [10,11,25]. Among the pathogens, several species of *Salmonella* and shiga-toxin producing *Escherichia coli* are included in the Foodborne Diseases Active Surveillance Network (FoodNet) of the Centers for Disease Control and Prevention. *Salmonella* spec. and *E. coli* were also the two predominant bacterial pathogens that caused food borne outbreaks associated with the consumption of red meat and meat products, as summarized in a recent systematic review [15]. The predominant serovars were *Salmonella enterica*

serovar *typhimurium* among the *Salmonella* serovars and *E. coli* O157:H7 among the shiga toxin producing *E. coli* [15]. Intriguingly, *Lactobacilli* can act as spoilage organisms and anti-microbial for spoilage and pathogenic bacteria [17]. As one specific example, increases in *Lactobacillus sakei* caused a decrease in *Escherichia coli* O157:H7 [29].

Interventions to reduce bacterial growth on beef are diverse and include chemical, physical, and biological treatments, all of which are geared towards providing 'farm to table food safety' by managing reservoirs of pathogens throughout the food processing chain [9]. Among the chemical interventions, a variety of organic acids that reduce pathogens on beef and other meat are in use [4,31]. At the consumer end, the Food Safety and Inspection Service (FSIS) from the United States Department of Agriculture (USDA) recommends a storage temperature of 4.4°C for ground beef, to be consumed within 2 days. However, people don't always adhere to these recommendations. A recent study from the food retail business determined that 17.1% of the investigated delis operated at least one refrigerator above the recommended temperature [3]. Likewise, an analysis of domestic refrigerator temperatures among European countries demonstrated that temperatures followed a normal distribution, with southern European countries exhibiting an N of (7.0, 2.7)°C and northern European countries showing an N of (6.1, 2.8)°C [21]. An even higher temperature of 10°C is considered 'abusive' [16,22]. With this study, we wanted to develop an intervention technique that helps businesses and consumers who keep their meat at higher temperatures than recommended by the USDA. The primary goal was to reduce spoilage bacteria, externally added bacterial pathogens were tested as a secondary goal.

We determined the reduction of live bacterial counts for spoilage bacteria on ground beef stored at an abusive temperature of 10°C by the addition of ethyl acetoacetate (EAA) or β-phenylethylamine (PEA). PEA and acetoacetate (AAA) were identified as anti-microbials in a screen of *E. coli* O157:H7 on 196 carbon and nitrogen sources [12]. EAA is the ethylester of AAA and was described as an anti-microbial with a tested efficacy against *Yersinia enterocolitica*, *Serratia marcescens* and *Cronobacter sakazakii* [7]. PEA also reduced biofilm by several pathogenic bacteria, when used as a flush in silicone tubings [23]. Both, EAA and PEA reduced meat spoilage bacteria by several log, EAA also reduced externally added *E. coli* by approximately 1 log.

Materials and Methods

Meat processing

Ground beef was obtained from the NDSU Meat Lab (www.ag.ndsu.edu/ansc/facilities/shepperd-arena) from two independent slaughter events. At each event, meat from two angus cattle that had been killed by the captive bolt method and aged for two weeks at a temperature between 0 and 2.2°C was ground and transferred on ice to our research lab. Precautionary steps that the slaughter facility undertakes to prevent microbial contamination of muscle meat include rinses of the hot carcass with hot water and 2.5% lactic acid (Birko Corp., Henderson, CO). The workflow for the experiment including the biological and technical replicates are summarized in figure 1.

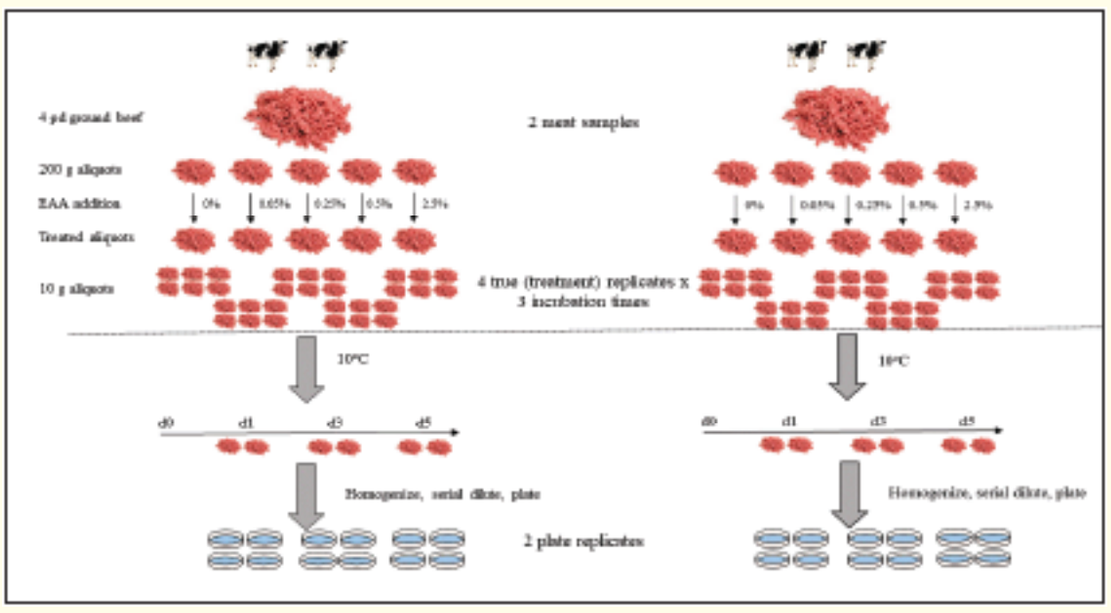


Figure 1: *Wsorkflow with the cows on top*

Each of the two meat samples was weighed into five aliquots of 200g that were supplemented with 0g, 0.05%, 0.25%, 0.5%, or 2.5% g of liquid EAA (Alfa Aesar, Ward Hill MA) or 0g, 0.25%, 0.5%, 2.5%, or 5% of crystalline PEA-HCl (TCI America, Portland, OR). Aliquots of 10g were produced from each 200g aliquot, which allowed for two replicates of each of the two meat samples and determination of bacterial counts at three different time points during the incubation period. These samples are designated true replicates throughout this manuscript. Samples were stored in Ziploc bags at -20°C.

Determination of the effect of EAA or PEA on meat spoilage bacteria

Meat samples were removed from the freezer and incubated at 10°C for five days; bacterial counts were determined on days 1, 3 and 5. The content of each bag was transferred into a stomacher bag (VWR, Radnor PA) and Maximum Recovery Diluent (MRD, Becton Dickinson, Franklin Lakes, NJ) was added to a total of 50g. Meat was homogenized in a Seward Stomacher 400 Circulator (Cole Parmer, Vernon Hills, IL). The total and selective bacterial counts of each homogenate were determined by plating serial dilutions onto appropriate agar plates. Each serial dilution was plated onto two separate agar plates to allow for two plate replicates. The compositions of the selective agar plates are summarized in table 1. Incubation temperatures were room temperature for plate count agar plates (PCA, total live counts), *Pseudomonas* agar plates (PSA, *Pseudomonads*) and Streptomycin sulphate, thallous acetate, actidione agar plates (STAA, *B. thermosphacta*). All purpose Tween agar plates (APT, *Lactobacilli*) were incubated anaerobically at 30°C, violet red bile glucose agar plates (VRGB, Enterobacteriaceae) at 37°C (aerobically). Colonies were counted after 1 to 2 days of incubation.

Name	Abbrev.	Purpose	Composition	Brand
Maximum recovery diluent	MRD	Diluent	1 g/l peptone, 8.5 g/l NaCl, pH 7.0	
Plate count agar	PCA	Total bacterial counts	5 g/l tryptone, 2.5 g/l yeast extract, 1 g/l glucose, 15 g/l agar, pH 7.0	Difco BD
Pseudomonas agar	PSA	Detection of pseudomonads	16 g/l gelatin peptone, 10 g/l casein hydrolysate, 10 g/l K_2SO_4, 1.4 g/l $MgCl_2$, 0.5 mg/ml cetrimide, 0.5 mg/ml fucidin, 2.5 mg/ml cephalosporin, 11 g/l agar, pH 7.1	Oxoid
All purpose tween agar	APT	Detection of lactobacilli	7.5 g/l yeast extract, 12.5 g/l pancreatic digest of casein, 10 g/l dextrose, 5 g/l sodium citrate, 0.001 g/l thiamine HCl, 5 g/l NaCl, 5 g/l K_2HPO_4, 0.14 g/l $MnSO_4 \cdot H_2O$, 0.8 g/l $MgSO_4 \cdot 7H_2O$, 0.04 g/l $FeSO_4$, 0.2 g/l polysorbate, 15 g/l agar, pH 6.7	Difco BD
Streptomycin sulphate, thallous acetate, actidione agar	STAA	Detection of *B. thermospacta*	20 g/l peptone, 2 g/l yeast extract, 1 g/l K_2HPO_4, 1 g/l $MgSO_4 \cdot 7H_2O$, 500 streptomycin sulphate, 50 mg/ml thallous acetate, 50 mg/ml cycloheximide, 13 g/l agar, pH 7.0	Oxoid BD
Violet red bile glucose agar	VRGB	Detection of *Enterobacter*	7 g/l peptone, 3 g/l yeast extract, 1.5 g/l bile salts No. 3, 5 g/l NaCl, 0.03 g/l neutral red, 0.002 g/l crystal violet, 10 g/l glucose, 12 g/l agar, pH 7.4	Oxoid
Luria Bertani agar	LB	Bacterial growth	10 g/l tryptone, 5 g/l yeast extract, 5 g/l NaCl, 15 g/l	Difco BD
Brain heart infusion	BHI	Bacterial growth	7.7 g/l calf brain infusion solids, 9.8 g/l beef heart infusion solids, 10 g/l proteose peptone, 5 g/l NaCl, 2 g/l glucose, 2.5 g/l Na_2HPO_4, pH 7.4	Difco BD

Sorbitol McConkey agar	SMAC	Detection of *E. coli*	15.5 g/l peptone, 3 g/l proteose peptone, 10 g/l D-sorbitol, 1.5 g/l bile salts, 5 g/l NaCl, 0.03 g/l neutral red, 0.001 g/l crystal violet, 15 g/l agar, pH 7.1	Difco BD
Shigella Salmonella agar	SSA	Detection of *S. enterica*	5 g/l beef extract, 2.5 g/l pancreatic digest of casein, 2.5 g/l peptic digest of animal tissue, 10 g/l lactose, 8.5 g/l bile salts mixture, 8.5 g/l sodium citrate, 8.5 g/l sodium thiosulphate, 1 g/l ferric citrate, 0.025 g/l neutral red, 15 g/l agar, 0.33 mg/l brilliant green, pH 7.0	Difco BD

Table 1: *Composition of the bacterial growth media*

Determination of the effect of EAA or PEA on *E. coli* O157:H7 and *S. enterica*

The *E. coli* O157:H7 strain used for this experiment was ATCC 43894 [13], previously made resistant to nalidixic acid [26]. The *S. enterica* strain was a clinical isolate *of S. enterica typhimurium*, designated FSL R6-0207 [28]. *S. enterica* was adapted to 50 µg/ml of nalidixic acid as described [27]. Bacterial inocula were prepared in liquid Brain Heart Infusion broth (BHI), supplemented with 50 µg/ml of nalidixic acid. Cultures were incubated at 37°C overnight, 2 ml from each overnight culture was added to 18 ml of fresh broth and incubated at 37°C for 2h. Cultures were diluted with MRD to a bacterial count of 2.6 x 10^3 CFU/ml for *E. coli* and 5.7 x 10^4 to 1.4 x 10^5 CFU/ml for *S. enterica*. 10g meat portions that were treated with 0% or 0.5% EAA/PEA were removed from the freezer, thawed at 10°C, inoculated with 1 ml of the respective inoculum and mixed within the Ziploc bag. Control meat pieces that were not inoculated with bacteria received 1 ml of MRD instead. Meat samples were incubated at 10°C for up to 5 days and treated as described above. *E. coli* O157:H7 were enumerated on Sorbitol MacConkey agar (SMAC), *S. enterica* on *Salmonella Shigella* Agar (SSA), both supplemented with 50 µg/ml nalidixic acid (Table 1). SMAC and SSA plates were incubated at 37°C, colonies were counted after 1 to 2 days.

Data analysis

Each experiment was performed in a total of 8 replicates (4 biological x 2 technical replicates, see figure 1). Data sets were pre-processed by determining the averages for the 2 plate replicates. Data were analyzed for each EAA/PEA concentration and selective media and expressed as CFU/g of meat and $\log_{10}$ CFU/g of meat. The lower limit of detection was 49 CFU, which is the equivalent of 1.69 $\log_{10}$. This number was used for all experiments that yielded zero colonies from the undiluted homogenate. To determine log reductions, the $\log_{10}$ CFU/g of meat at a given concentration of EAA/PEA was subtracted from that obtained from the untreated control. Average and standard deviations were calculated across the four true replicates.

Statistical analysis for the data from the spoilage bacteria was started with a two-way ANOVA that compared the means of the $\log_{10}$ CFU/g of meat data across concentrations, days, and biological replicates. For comparisons that yielded statistically significant differences between the means (*p*-value below 0.05), Fisher's Least Significant Difference (LSD) test and a pairwise Student's *t*-test were performed as *post hoc* test to determine which of the groups were different from the others.

Statistical analysis for the *E. coli* and *S. enterica* data was done with Student's *t*-test to determine the statistical significance of the difference between bacterial counts obtained from the EAA or PEA treated meat sample and the untreated one. A p-value < 0.05 indicated statistical significance of the difference.

Results and Discussion

EAA reduced live counts of spoilage bacteria

To determine the effect of EAA on the total bacterial counts and selected beef spoilage bacteria, bacteria were enumerated in 10g aliquots of ground beef, either left untreated or treated with concentrations of EAA between 0.05 and 2.5% after a maximal storage time of five days at 10°C. For all media plates (total plate counts and selective plate counts), the analysis of variance (ANOVA) provided evidence that there were statistically significant differences between the $\log_{10}$ CFU/g of meat data from the five different concentrations of EAA and the three different days of harvest (Table 2). Groupings data from the *post hoc* test are also included in figure 2. For all media plates, the 0.5% (yellow bars) and 2.5% samples (dark blue bars) yielded data that were significantly different from those of the untreated meat pieces (marked B and C).

Concentration (p-value)[1]	Day (p-value)[2]	Biol. Repl. (p-value)[3]	Different from 0 %[4]	Different from one another[5]
PCA, total bacterial count				
< 0.0001	<0.0001	< 0.0001	0.5	0.5 from 0.05
			2.5	2.5 from all others
PSA, *Pseudomonas*				
<0.0001	<0.0001	< 0.0001	0.5	0.5 from 0.05
			2.5	2.5 from all others
LPT, *Lactobacilli*				
< 0.0001	<0.0001	< 0.0001	0.5	0.5 from 0.05
			2.5	2.5 from all others
STAA, *B. thermosphacta*				
0.0010	0.0004	NA	0.5	0.5 from 0.05 and 0.25
			2.5	5 from 0.05 and 0.25
VRGB, *Enterobacteriaceae*				
0.0009	< 0.0001	NA	0.5	0.5 from 0.05
			2.5	5 from all others

Table 2: *Statistical analysis of the data from the EAA treatments (spoilage bacteria).*

[1]*This is the p-value from the ANOVA, where the means of the $\log_{10}$ CFU/g values were compared across the five different concentrations.*

[2]*This is the p-value from the ANOVA, where the means of the $\log_{10}$ CFU/g values were compared across the three different days.*

[3]*This is the p-value from the ANOVA, where the means of the $\log_{10}$ CFU/g values were compared across the two different biological replicates.*

[4]*Concentrations are listed that resulted in a grouping that was different from the untreated (0% PEA) control meat pieces, as determined by Fisher's LSD test and Student's t-test.*

[5]*Concentrations are listed that resulted in a grouping that differed from any other concentration (except 0% PEA).*

After 1 day of incubation at an abusive temperature of 10°C, the total bacterial count from the PCA plates decreased with increasing concentrations of EAA to a maximum log reduction of 5.1 at 2.5% of EAA after 5 days of incubation (Figure 2A). Under the more practical condition of 0.5% and 3 days of incubation, a 2.5 log reduction was observed. Among the specific spoilage bacteria, the counts for *Pseudomonads* (Figure 2B) and *Lactobacilli* (Figure 2C) were considerably higher than those for *B. thermosphacta* (Figure 2D) and *Enterobacteriaceae* (Figure 2E). This is in agreement with current literature [18,19]. Reductions in live bacterial counts were similar for *Pseudomonads* and *Lactobacilli* as for total bacterial counts. For *B. thermosphacta* and *Enterobacteriaceae*, reduction at 0.5% and three days were still around 1 log. Note that day 1 data for *B. thermosphacta* and *Enterobacteriaceae* were omitted from figure 2D and 2E because the counts were below the lower limit of detection of 49 colony forming units (CFU).

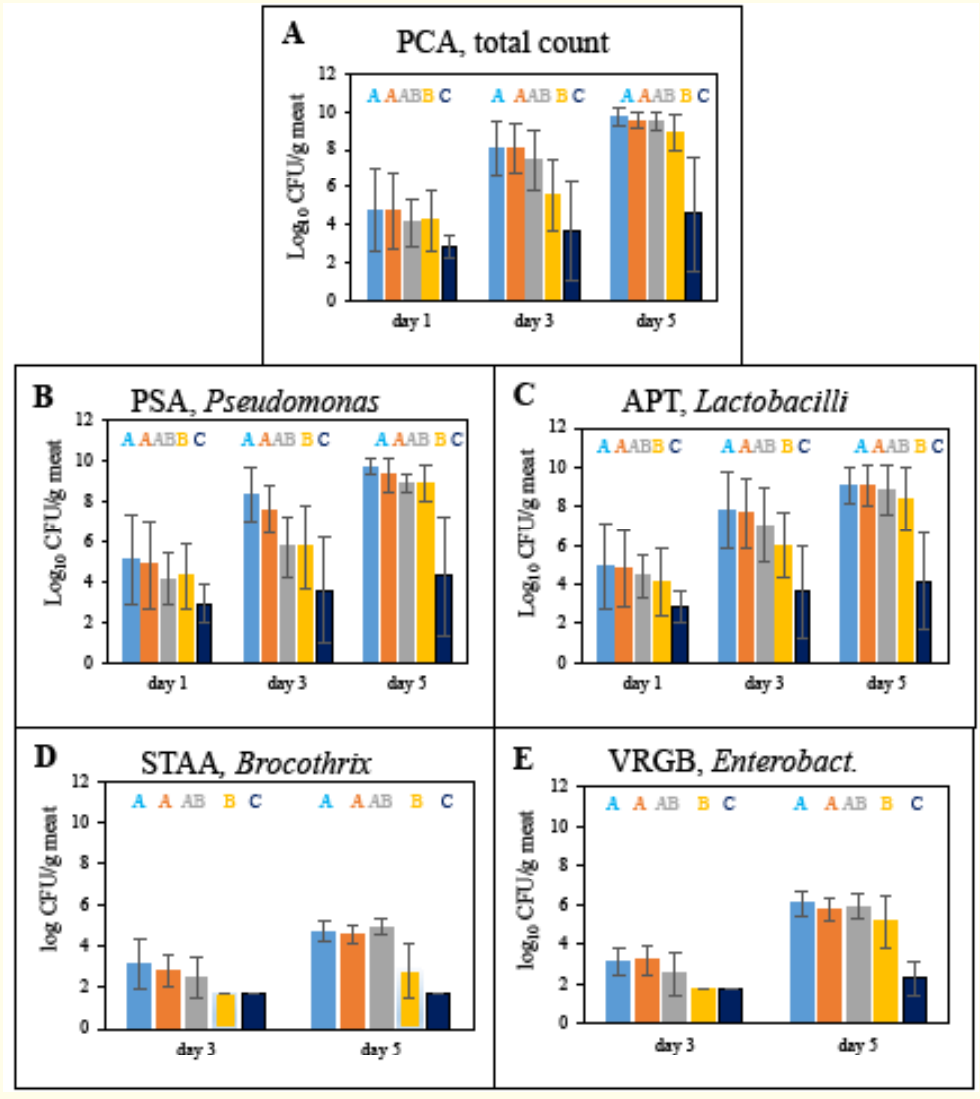


Figure 2: *Bacterial counts of naturally occurring spoilage bacteria in response to EAA. Panel A contains the log10 CFU/g of meat data from the PCA plates, Panel B the data for pseudomonads from the PSA plates, Panel C the data for lactobacilli from the APT plates, Panel D the B. thermosphacta data from the STAA plates, and Panel E the Enterobacteriaceae data from the VRGB plates. Light blue, untreated control; orange, 0.05% of EAA; grey, 0.25% EAA; yellow, 0.5% EAA; dark blue, 2.5% EAA. The characters on top of the bars are the groupings from the post hoc test that was done as part of the statistical analysis of the data.*

Since the amount of spoilage bacteria can impact the number of pathogens [17,29], we then tested whether the EAA treatment would increase the number of two bacterial pathogens, *E. coli* and *S. enterica*. Since the lowest concentration at which a statistically significant difference was seen between the treated and the untreated meat pieces was 0.5% for all selective media plates and spoilage bacteria, we selected this concentration for the pathogen experiment. CFU/g meat data from this experiment are presented in figure 3, day 1 data were omitted because they bacterial counts were below the detection limit. Log reductions for *E. coli* O157:H7 were 1.06 at day 3 and 1.6 at day 5. For *S. enterica*, there was no reduction in bacterial counts by the EAA treatment, but more importantly the pathogen was not increased. The *t*-test yielded *p*-values of 0.01 and 0.018 for *E. coli* at days 3 and 5, respectively. For *S. enterica*, the corresponding *p*-values were 0.18 and 0.33.

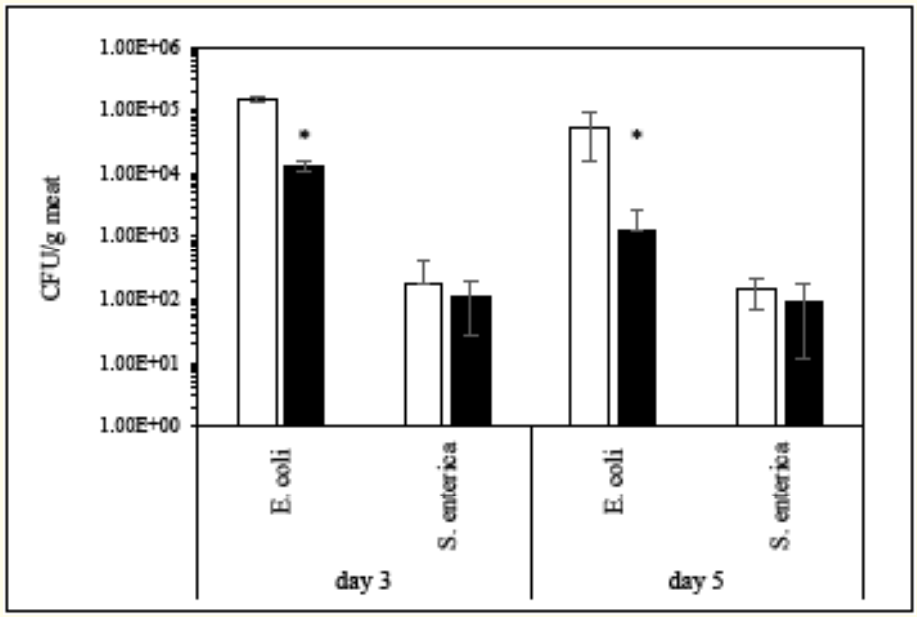


Figure 3: *Bacterial counts of externally added pathogenic bacteria in response to EAA. The Figure contains the CFU/g of meat data for E. coli O157:H7 and S. enterica after 3 and 5 days of incubation for the untreated meat pieces (white bars) and 0.5% of EAA (black bars). Averages and standard errors were calculated as described under Materials and Methods. Asterisks indicate statistically significant differences in live bacterial counts derived from treated and untreated meat pieces.*

PEA reduced live counts of spoilage bacteria

The experiment was repeated, using PEA as anti-microbial treatment. As for EAA, the ANOVA provided evidence that there were statistically significant differences between the $\log_{10}$ CFU/g data from the 5 different concentrations of EAA and the three different days of harvest for all media plates (Table 3). The *post hoc* test revealed that for all plates, data from the two highest concentrations differed from the untreated control with statistical significance. Interestingly, for *Brochothrix*, there was no overlap between the group A and the group B data.

Concentration (p-value)[1]	Day (p-value)[2]	Different from 0%[3]	Different from one another[4]
PCA, total bacterial count			
< 0.0001	< 0.0001	0.5%	5% from 0.25%
		2.5%	5% from 0.5%
		5%	5% from 2.5%
			2.5% from 0.25%
PSA, *Pseudomonas*			
< 0.0001	< 0.0001	2.5%	5% from 0.25%
		5%	5% from 0.5%
			5% from 2.5%
			2.5% from 0.25%

			2.5% from 0.5%
			2.5% from 0.25%
LPT, *Lactobacilli*			
< 0.0001	< 0.0001	0.5%	5% from 0.25%
		2.5%	5% from 0.5%
		5%	5% from 2.5%
			2.5% from 0.25%
VRGB, *Enterobacteriaceae*			
0.0019	0.0251	5%	5% from 0.25%
STAA, *B. thermosphacta*			
0.0011	< 0.0001	0.5%	5% from 0.25%
		2.5%	5% from 0.5%
		5%	5% from 2.5%

Table 3: *Statistical analysis of the data from the PEA treatments.*

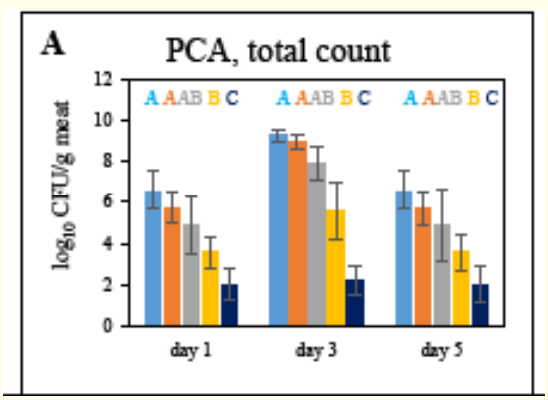
[1]*This is the p-value from the ANOVA, where the means of the* $\log_{10}$ *CFU/g values were compared across the five different concentrations.*

[2]*This is the p-value from the ANOVA, where the means of the* $\log_{10}$ *CFU/g values were compared across the three different days. Fisher's LSD test and Student's t-test were performed as post hoc tests for the concentration comparison.*

[3]*Concentrations are listed that resulted in a grouping that was different from the untreated (0% PEA) control meat pieces.*

[4]*Concentrations are listed that resulted in a grouping that differed from any other concentration (except 0% PEA).*

Figure 4 summarizes $\log_{10}$ CFU/g meat data for the PEA treatments. After 1 day of incubation at 10°C, the total bacterial count from the PCA plates decreased with increasing concentrations of PEA to a maximum log reduction of 4.4 at 5% of PEA (Figure 4A). At 0.5% PEA, a 1.4 log reduction after 3 days was achieved. Log reductions for *Pseudomonads* (Figure 4B) and *Lactobacilli* (Figure 4C) were similar to those for the total plate counts and slightly lower for *B. thermosphacta* (Figure 4D) and *Enterobacteriaceae* (Figure 4E).



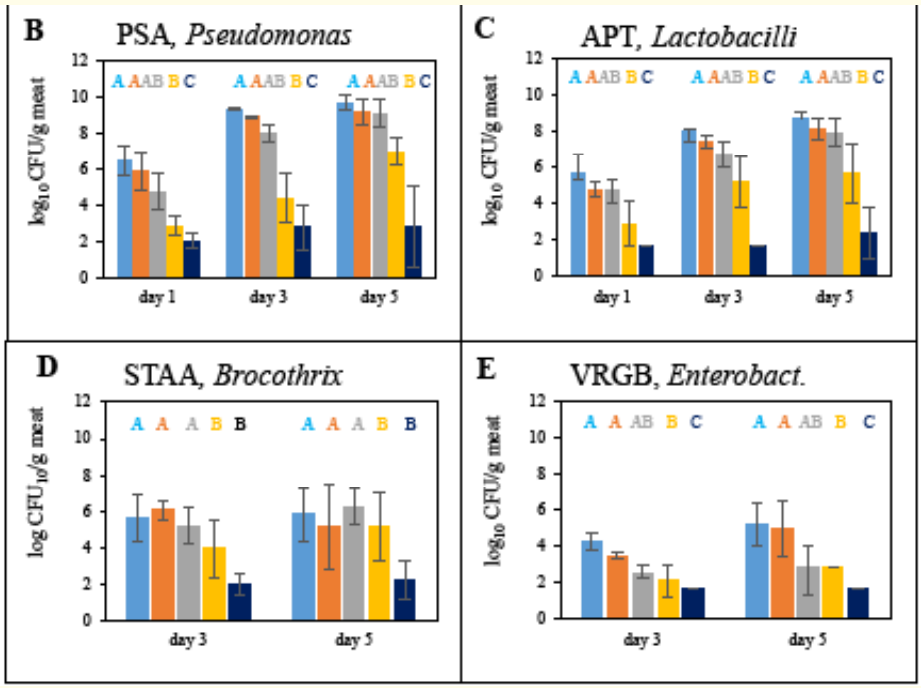


Figure 4: *Bacterial counts of naturally occurring spoilage bacteria in response to PEA. Panel A contains the log10 CFU/g of meat data from the PCA plates, Panel B the data for pseudomonads from the PSA plates, Panel C the data for lactobacilli from the APT plates, Panel D the B. thermosphacta data from the STAA plates, and Panel E the Enterobacteriaceae data from the VRGB plates. Light blue, untreated control; orange, 0.25% of PEA; grey, 0.5% PEA; yellow, 2.5% PEA; dark blue, 5% PEA. The characters on top of the bars are the groupings from the post hoc test that was done as part of the statistical analysis of the data.*

While the spoilage bacteria reacted to PEA in a way that was similar to that of EAA, the responses of *E. coli* O157:H7 to the anti-microbial differed between the two treatments. Adding either *E. coli* or *S. enterica* to the meat samples prior to the incubation at 10°C yielded no statistical significant differences between the untreated control and the meat pieces that had been treated with 0.5% of PEA (Figure 5). Most importantly, however, neither of the pathogens exhibited an increase in growth in response to the reduction in the natural flora (*e.g. Lactobacilli*) of the meat pieces by the PEA treatment.

Altogether, EAA and PEA had dramatic effects on the live counts for spoilage bacteria from the meat samples during the 5 days of the incubation. At day 1 of incubation at abusive temperature, the total bacterial counts for the unsupplemented meat samples (Figure 2A and 4A) were below the 8 logs that were previously defined as spoilage [6,14]. At day 3, however, the total counts had increased to ~8 log in the EAA experiment and ~9 log in the PEA experiment in the untreated samples. At concentrations of 0.5%, EAA reduced this count to about 5.5 log (Figure 2A, yellow bar) and PEA to < 8 log (Figure 4A, grey bar). In addition, neither EAA nor PEA increased the number of two externally added pathogens, *E. coli* and *S. enterica*. We propose EAA and PEA as novel inhibitors of the spoilage microflora of ground beef at concentrations of 0.5% w/w with a maximum storage time of 3 days.

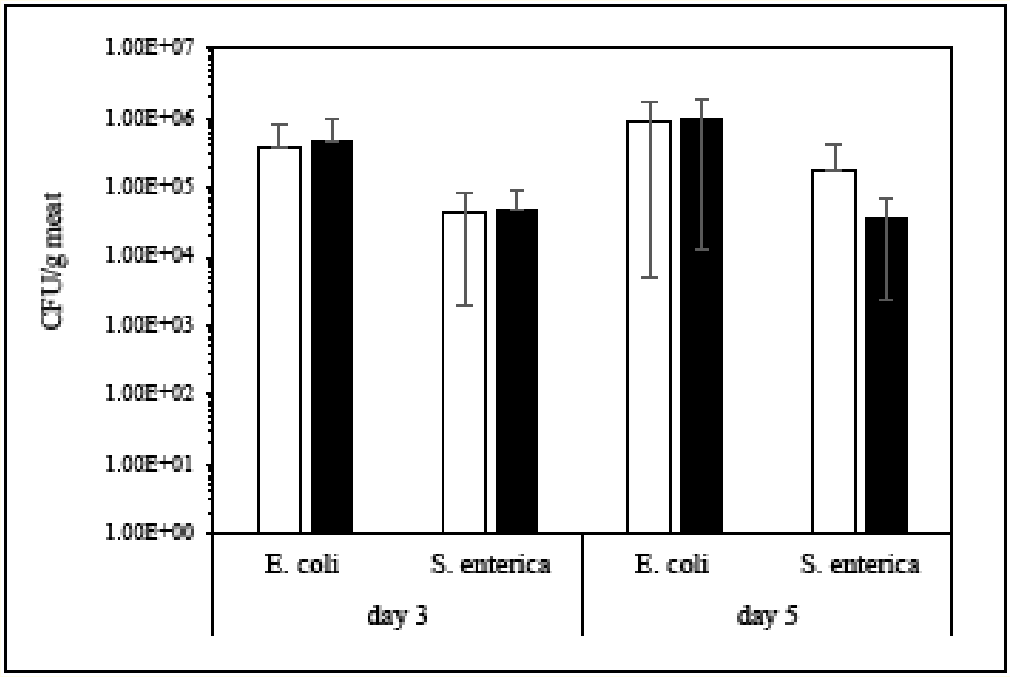


Figure 5: *Bacterial counts of externally added pathogenic bacteria in response to EAA. The Figure contains the CFU/g of meat data for E. coli O157:H7 and S. enterica after 3 and 5 days of incubation for the untreated meat pieces (white bars) and 0.5% of PEA (black bars). Averages and standard errors were calculated as described under Materials and Methods. Differences in live bacterial counts between treated and untreated meat pieces were statistically not significant.*

Whether as agrochemical, food additive, or processing aid, chemicals in food have increased over time and special care has to be taken, when evaluating real and perceived risks [8]. There are several pieces of evidence that lead us to believe that toxicity of EAA should not be a problem at our recommended concentration of 0.5% w/w; i) according to the MSDS by Science Lab, the LD_{50} for the toxicity in rats after oral application is 3.98 g/kg of body weight; ii) a toxicology study with rats demonstrated that feeding rats with up to 300 mg/kg body weight of EAA every day for 28 days did not result in health or hematology changes [5].

As for EAA, there is ample evidence that toxicity of PEA should not be a problem at our recommended concentration of 0.5% w/w; i) the World Health Organization (www.who.org) describes PEA as a flavoring agent of 'no safety concern'; ii) PEA has a half-life of 5 to 10 min in dogs [24]. In humans, the short half-life is due to the metabolic activity of monoamine oxidase B to phenylacetic acid [30]. Given that the human body itself is able to produce PEA in small amounts [1], one might argue that PEA is a natural food additive rather than a chemical.

Conclusion

In conclusion, our two novel anti-microbials were able to reduce spoilage bacteria on ground beef when mixed with the meat. Additionally, EAA reduced live bacterial counts of one *E. coli* O157:H7 strain. We believe that these two treatments will especially benefit consumers and retail businesses who keep their meat at temperatures between the recommended 4.4°C and the 10°C of our study. Whether EAA or PEA may increase shelf life of ground beef is an interesting future perspective.

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Conflict of Interest

The authors declare no conflict of interest.

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