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IN VITRO INHIBITORY EFFECT OF ANTIOXIDANTS ON THE GROWTH OF E. COLI O157:H7

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ABSTRACT

Efforts to control harmful pathogens through the application of various chemicals with proven bactericidal properties have not been adequately effective. Many of these chemicals also have the potential of adverse health impact through reactive effects from their residual presence on products. This study was therefore undertaken to determine *in vitro* inhibition of *E. coli* O157:H7 growth by three GRAS (generally recognized as safe) chemicals (L-ascorbic acid, lactic acid, and propyl gallate).Since *E. coli* O157:H7 is one of the most hazardous pathogenic bacteria and has become a well known pathogen in food and dairy industries. Therefore, five strains of *E. coli* O157:H7 with populations of approximately 3.5 log CFU/ml were individually inoculated into tryptic soy broth (TSB) supplemented with either L-ascorbic acid, lactic acid, propyl gallate or a combination of these chemicals. During an 8 h incubation period at 37ºC, microorganism growth (turbidity via optical density, OD) for each strain was determined at 2 h intervals using a spectrophotometer (610 nm). At the end of incubation, TSB was also serially diluted (1:10) in sterile 0.1% peptone water and surface plated on tryptic soy agar. The results show that concentrations required for L-ascorbic acid, lactic acid and propyl gallate to demonstrate the strongest inhibitory effect on the growth of the microorganisms were 1% (w/v), 0.3% (v/v), and 0.1% (w/v), respectively. Combinations of 0.25% L-ascorbic acid or 0.025% propyl gallate with 0.2% lactic acid completely inhibited the growth of *E. coli* O157:H7 strains tested at an initial inoculum level. There was no specific correlation of OD readings to cfus due to the increase of bacterial populations. These results indicate that antioxidants used in this *in vitro* study may have the potential in controlling foodborne pathogens *in vivo*.

Keywords: antioxidants, *E. coli* O157:H7, inhibition, optical density

INTRODUCTION

Outbreaks of illness associated with foodborne pathogens such as *Escherichia coli* O157:H7 have been an international concern for over a decade .Raw milk was first recognized as a vehicle of transmission of *E.coli* 0157:H7 in 1986(Massa et al., 1999),when children from separate families in Wisconsin, USA, developed haemorrhagic colitis and HUS after drinking raw milk from dairy farms. Since then, there have been further outbreak associated with consumption of raw milk. Fecal contamination of milk is one likely route of transmitting *E.coli* 0157:H7 to humans. .E.coli 0157:H7 was isolated from a milk handling pipe and the bottling machine in a local dairy plant and can be isolated from yoghurt, indicating that inadequate pasteurization or post- pasteurization contamination was the likely factor

responsible for the outbreak (Wang *et al.,* 1997). The Centers for Disease Control and Prevention (CDC) considers *E. coli* O157:H7 to be of great concern because of the severity and number of illnesses it causes (Wilkinson, 1997). Moreover, recently, fresh produce such as spinach (CDC, 2006) and iceberg lettuce (FDA,2006) have been implicated in outbreaks.

Although various chemicals such as iodophor (Best *et al.,* 1990), ozone (Kim *et al*.,,2003), chlorine, and trisodium phosphate (Zhang *et al*.,1996), known to be effective bactericides, have been used to control harmful pathogens, they either have not been adequately effective or have been associated with adverse health impact due to their residual presence on the product. The increase in reports of food related illnesses indicates that there is an urgent need for more effective and safe means of treating fruits , vegetables and dairy products to prevent foodborne pathogen contamination.

Among the most appealing approaches in food preservation is the use of antioxidants due to their benefits, which preserve and stabilize the freshness, nutritional value, flavor and color of foods (Joint FAO/WHO, 1996), their relative safety as a food treatment, and most importantly, their antimicrobial efficacy (Zurita *et al.,* 2007). Antioxidants protect cells against damage by free radicals, which are reactive by-products of normal cell activity, by donating one of their own electrons (Fennema 1996). One potent antioxidant is ascorbic acid, which is the water soluble form of vitamin C. Ascorbic acid is essential for the formation of bone and connective tissue and plays a primary role in collagen formation, which is essential for the growth and repair of tissue cells in all parts of human body Anonymous 2003 and McGee 2007. Another organic acid, propyl gallate, a phenolic antioxidant, has been widely used in foods, cosmetics and pharmaceuticals as a preservative as well as a stabilizer (Joint FAO/WHO, 1996 andVan der Heijden *et al*., 1986). Raghavan and Hultin (2005) reported that propyl gallate prevented or delayed lipid oxidation on fish (cod and haddock) muscle foods. Lactic acid is frequently used in various processed foods, either as an antioxidant or as a preservative (Anonymous 2007). Research (Makras and Vuyst 2006) suggests that lactic acid has a strong inhibitory effect against Gram-negative bacteria and is responsible for the antagonistic activity of bifidobacteria (Fooks and Gibson 2002, Fooks and Gibson 2003and Ibrahim and Bezkorovainy 1993). Gill and Badoni 2004 reported a study treating beef carcass with 2% lactic acid and found the treatment reduced populations of *E. coli* by 1 log as well as enhanced shelf life of ground beef (Jimenez *et al*., 2003).

Although propyl gallate and other organic acids like ascorbic acid and lactic acid are generally recognized as safe (GRAS) ingredients as common preservatives, their ability to control foodborne pathogens as additional hurdles has not been well established. Hence, the purpose of this study was to investigate in vitro the inhibitory activity of L-ascorbic acid, lactic acid, and propyl gallate and their synergistic potential against various strains of *E. coli* O157:H7.

MATERIALS AND METHODS

To investigate the efficacy of the three antioxidants against *Escherichia coli* O157:H7, a series of experiments were conducted comparing L-ascorbic acid alone, lactic acid alone, propyl gallate alone, combinations of either L-ascorbic acid or propyl gallate and lactic acid, and a control condition of no treatment solution. Within each treatment condition (antioxidant), several concentration levels were tested (Tables 1 and3).

Preparation of inocula. Five strains of *E. coli* O157:H7 – 944 (salami isolate), Cider (cider isolate), E0019 (beef isolate), F4546 (alfalfa sprout isolate), and H1730 (lettuce isolate) – associated with foodborne outbreaks were obtained from the Department of Family and Consumer Sciences, North Carolina Agricultural and Technical State University. A loop inoculum of each culture was transferred in tryptic soy broth (TSB, pH 6.9±0.1, Becton Dickinson, Sparks, MD) and incubated at 37ºC for 24 h. A 24-h culture of each strain was then centrifuged (Model 5415 R, Eppendorf North America, Inc., Westbury, NY) twice for 10 min (7,000 × g, 4ºC). The pellets were washed each time with 1 ml of peptone water (1g of peptone per liter, Becton Dickinson). Each pellet was serially diluted in 9 ml of 0.1% peptone water to give an inoculum of each strain containing approximately 3.5 log CFU/ml. Bacterial population in each inoculum was determined by serially diluting in sterile 0.1% peptone water and plating (0.1 ml) duplicate on tryptic soy agar (TSA, Becton Dickinson). The plates were then incubated at 37ºC for 24 h before conducting a bacterial count.

Treatment solutions. Chemicals used for the inhibition of *E. coli* O157:H7 growth were L-ascorbic acid (Sigma-Aldrich, Inc., St. Louis, MO), lactic acid (85%, Thermo Fisher Scientific, Fair Lawn, NJ), and propyl gallate (Sigma-Aldrich, Inc.). Ten ml of TSB was supplemented with sterile filtered either L-ascorbic acid at 0.25, 0.5, 0.75 and 1% (wt./vol.), lactic acid at 0.1, 0.2, 0.3 and 0.4% (vol./vol.) or propyl gallate at 0.025, 0.05, 0.075 and 0.1% (wt./vol.), respectively. In addition, to investigate possible synergistic efficacy with less concentrations of chemicals for the benefits of cost-saving as well as improved effectiveness, ten ml of TSB was also individually supplemented with the combinations of lactic acid and either L-ascorbic acid or propyl gallate. In order to dissolve propyl gallate, a one to one ratio (wt./vol.) of ethyl alcohol (200 proof, Thermo Fischer Scientific) was used. For the preparation of L-ascorbic acid and lactic acid, deionized water was used after filter sterilization through a 0.2-µm Nalgene filtration product (Nalge Nunc International, Rochester, NY). An additional 10 ml of TSB not supplemented with any chemical was used as a control. The pH levels of the treatment solutions were measured immediately after preparation using a pH meter (Accumet® Excel XL15, Thermo Fisher Scientific).

Microbiological analysis. Tryptic soy broth supplemented with the specified concentrations of either L-ascorbic acid, lactic acid or propyl gallate prepared as described above was inoculated with 1 ml of each *E. coli* O157:H7 strain and incubated for 8 h at 37ºC. Microbial growth was monitored by measuring the turbidity (optical density; OD 610nm) of each

TSB at 2-h intervals during 8 h incubation using a Spectrophotometer (Spectronic 21 Miton Roy Spectrophotometer, Thermo Electron Scientific., Madison, WI). At the end of incubation period (8 h), which was determined based on previous unpublished data by the authors, 1 ml of the treated bacterial suspension was serially (1:10) diluted in a sterile 0.1% peptone water and 0.1 ml of each dilution was surface plated in duplicate on Triptic Soy Agar(TSA). The plates were then incubated at 37ºC for 24 h before conducting a bacterial count. In addition, only three strains of *E. coli* O157:H7 were randomly chosen and used for the synergistic efficacy study following the same procedure as described above.

Data analysis. Experiments were replicated twice. All quantitative microbiological data were transformed to log colony-forming units (cfus) per ml of tryptic soy broth prior to statistical analysis. Data were analyzed by the general linear model procedure of the Statistical Analysis System (1999). Optical density readings and bacterial counts (cfus) were compared by ANOVA using Duncan's multiple range test to determine if significant differences ($P<0.05$) in the population of microorganisms existed among mean values due to treatments, respectively.

RESULTS AND DISCUSSION

The results of the pH level assessment show that the addition of L-ascorbic acid and lactic acid comparably reduced pH levels of TSB to considerably lower levels than either the addition of propyl gallate or the control condition (Table 1). Table 1 also indicates that decrease of pH levels associated with L-ascorbic acid and lactic acid may reach the conditions that would seem to likely be inhospitable to *E. coli* O157:H7. However, previous studies (Benjamin and Datta,1995,Brown, et al., 1997, Sainz, et al.2005 and Uyttendaele, *et. al*., 2001) have shown that *E. coli* O157:H7 cells are acid tolerant and can survive at pH level as low as 2 depending on other environmental conditions. This suggests that the ability to change pH level alone is likely insufficient for establishing the efficacy of antioxidants as inhibitors of *E. coli* O157:H7 growth.

The optical density (OD) observations due to survival and growth of five *E. coli* O157:H7 strains in the presence of different concentrations of L-ascorbic acid, lactic acid, and propyl gallate in TSB are shown in Figs. 1-3. When TSB was supplemented with 0.5% or higher concentrations of L-ascorbic acid and incubated for 8 h at 37ºC, significant growth inhibition ($P<0.05$) as represented in the OD readings of 0 was observed in all five *E. coli* O157:H7 strains tested (Fig. 1). In the meantime, the OD reading (0.67) of control sample (TSB alone) for F4546 strain was significantly greater than those (0.26-0.46) of other four strains (statistical analysis not shown). Among *E. coli* O157:H7 strains, OD readings for TSB containing 0.25% and higher concentrations of L-ascorbic acid were not significantly different. Based on the results of the OD readings shown in Fig. 1, 0.5% of L-ascorbic acid appears to have effectively inhibited the growth of the microorganisms and contained their populations at the initial inoculum level (~3.5 log cfu/ml).

Results of OD readings in Fig. 2 suggest that inhibitory effects of propyl gallate at 0.025% or higher concentrations on the growth of *E. coli* O157:H7 were significant ($P<0.05$) compared to control treatment. Fig. 2 also shows that the pattern of *E. coli* O157:H7 strains growth was similar to L-ascorbic acid, from the highest to the lowest OD readings; F4546, E0019, 944, Cider, and H1730. Fig. 3 illustrates some inhibitory effect of 0.1% lactic acid on the growth of *E. coli* O157:H7 compared to control treatment while 0.2% or higher concentrations of lactic acid was associated with OD readings of 0 for all five strains tested (Fig. 3).

Table 1. The pH of TSB supplemented with various concentrations of L-ascorbic acid, lactic acid and propyl gallate used toinhibite the growth of *E. coli* O157:H7.

Treatment		pH
TSB only (Control)		6.92
L-ascorbic acid (%)	0.25	5.93
	0.5	4.82
	0.75	4.54
	1	4.23
Lactic acid (%)	0.1	6.19
	0.2	5.26
	0.3	4.54
	0.4	4.21
Propyl gallate (%)	0.025	6.91
	0.05	6.88
	0.075	6.88
	0.1	6.88

Overall, the results presented in Figs. 1-3 indicate that OD readings of *E. coli* O157:H7 grown in TSB alone (control) were vary ranging from 0.22 to 0.84 depending upon bacterial strain. In the concentrations tested for this study, 0.5% for L-ascorbic acid, 0.025% for propyl gallate and 0.2% for lactic acid were required for the complete inhibition (OD readings of 0) of *E. coli* O157:H7 growth. Our results in OD readings are, however, somewhat different from those of Ibrahim et al. (2006), who showed that *E. coli* O157:H7 strains grown in laboratory medium (brain heart infusion broth) at 37ºC reached the stationary phase within 8 to 10 h with OD readings of 1.00 to 1.10. These differences in the OD readings may be likely the results of differences in laboratory medium as well as the amount of bacterial population inoculated.

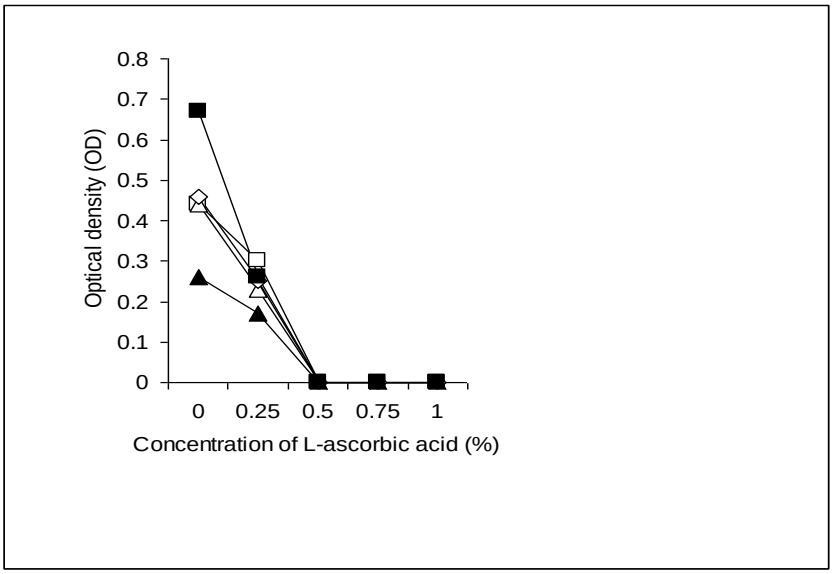

Figure 1. Optical density (OD) due to survival and growth of five *E. coli* O157:H7 strains 944 (□), Cider (Δ), E0019 (◊), F4546 (■) and H1730 (▲) growth in TSB after 8 h incubation at 37ºC with the presence of L-ascorbic acid at different concentrations.

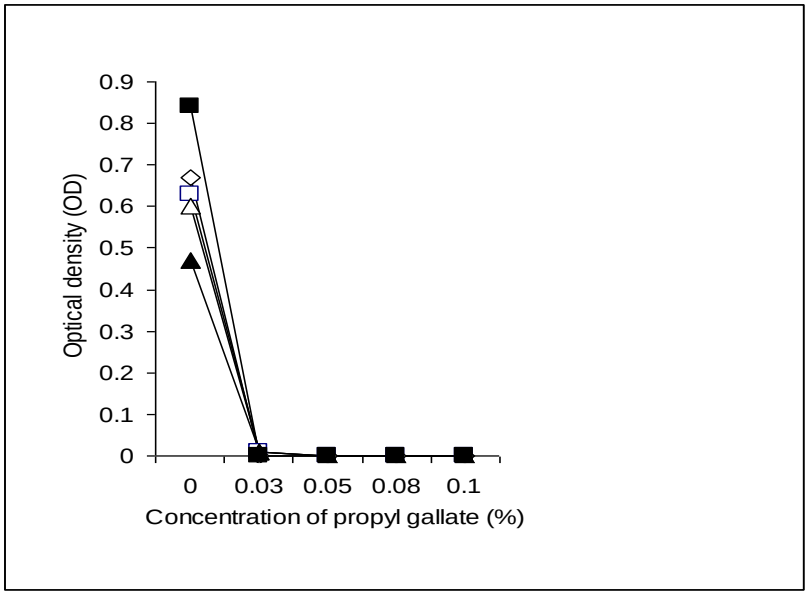

Figure 2. Optical density (OD) due to survival and growth of five *E. coli* O157:H7 strains 944 (□), Cider (Δ), E0019 (◊), F4546 (■) and H1730 (▲) in TSB after 8 h incubation at 37ºC with the presence of propyl gallate at different concentrations.

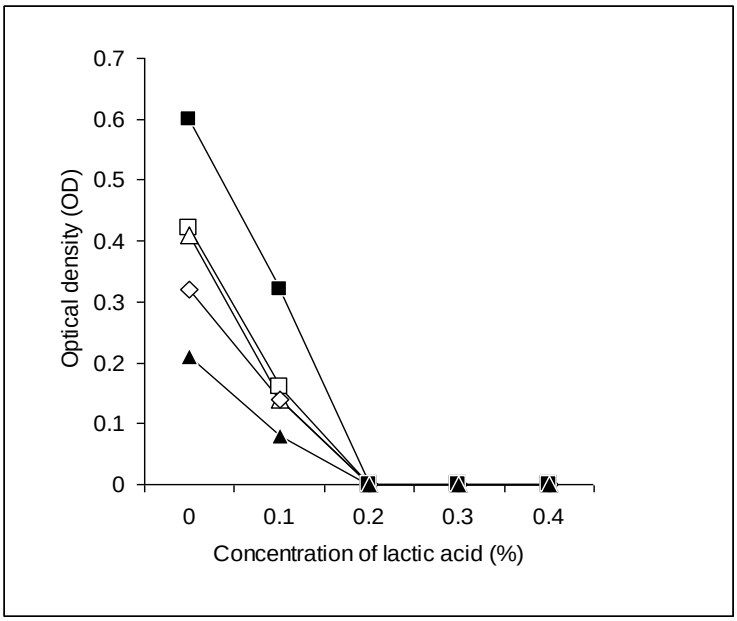

Figure 3. Optical density (OD) due to survival and growth of five *E. coli* O157:H7 strains 944 (□), Cider (Δ), E0019 (◊), F4546 (■) and H1730 (▲) in TSB after 8 h incubation at 37ºC with the presence of lactic acid at different concentrations.

Table 2 shows the efficacy of different concentrations of L-ascorbic acid, lactic acid, and propyl gallate in the survival and growth of five strains of *E. coli* O157:H7. The initial populations of microorganisms inoculated for the study were approximately 3.5 log cfu/ml. When *E. coli* O157:H7 strains were grown in TSB alone (control) without any supplement, bacterial populations after 8 h of incubation at 37ºC ranged from 7.92 to 8.49 log cfu/ml. Although 0.25% L-ascorbic acid inhibited the growth of *E. coli* O157:H7 strains by 0.11-0.57 log cfu/ml, there was no statistical difference in the number of microbial populations compared to control. However, when *E. coli* O157:H7 strains were subjected to 0.5% L-ascorbic acid solution, the bacterial growth was significantly inhibited by 1.54-2.20 log cfu/ml. Increasing the concentration of L-ascorbic acid from 0.5% to 0.75% further enhanced inhibition efficacy by 3.42-4.31 log cfu/ml. At the concentration level of 1.0% L-ascorbic acid, the growth of the microorganisms were completely inhibited and their populations were contained at initial inoculum levels (~3.5 log cfu/ml) for all strains.

Although the mechanism of antimicrobial activity of L-ascorbic acid against *E. coli* O157:H7 strains was not investigated in this study, our results are consistent with those reported by Tabak et al. (2003) and Fujimoto et al. (2006). Tabak et al. (2003) found that ascorbic acid in the concentration ranging from 0.2% to 2% inhibited the growth of several pathogenic microorganisms in a liquid medium. Fujimoto *et al.* (2006)

reported that populations (4.60 log cfu/ml) of *E. coli* were reduced to undetectable levels when subjected to purified water (pH 2.89) containing 0.37% ascorbic acid after 4 h of incubation at 37ºC.

Compared to L-ascorbic acid, lower concentrations (0.2%) of lactic acid were required to significantly inhibit (1.77-2.32 log cfu/ml) the growth of microorganisms (Table 2). Increasing the concentration of lactic acid from 0.2% to 0.3% enhanced its inhibitory effect on the growth of microorganisms showing no significance difference in the number of microbial populations compared to initial inoculum level. Results from Table 2 indicate that since further increasing lactic acid concentration to 0.4% did not show any statistical difference in microbial populations, 0.3% lactic acid could be considered as an optimum concentration for the complete inhibition of bacterial growth in the test media. However, our results contrast with Fujimoto *et al.* (2006), who showed that purified water (pH 2.95) supplemented with 0.07% lactic acid completely inactivated initial population (4.43 log cfu/ml) of *E. coli* within 6 h of incubation at 37ºC. These differences in the antimicrobial activity of lactic acid may be likely the results of differences in laboratory medium (TSB v.s. purified water).

Table 2. Efficacy of various concentrations of L-ascorbic acid, lactic acid and propyl gallate in inhibiting the growth of *E. coli* O157:H7 strains after 8 h incubation at 37ºC.

Treatment	Concentration (%)	Population (log_{10} CFU/ml) of *E. coli* O157:H7 strains*				
		944	Cider	E0019	F4546	H1730
L-ascorbic acid	0	8.34 a	8.34 a	8.26 a	8.49 a	7.92 a
	0.25	8.19 a	8.23 a	8.08 a	7.86 ab	7.79 a
	0.5	6.60 b	6.57 b	6.06 b	6.66 b	6.38 ab
	0.75	4.17 c	4.92 c	3.95 c	4.95 c	4.31 bc
	1	3.63 c	3.53 d	3.55 c	3.51 cd	3.62 c
Propyl gallate	0	8.43 a	8.39 a	8.26 a	8.24 a	8.30 a
	0.025	6.64 b	6.56 b	6.55 b	6.36 b	6.64 b
	0.05	5.44 c	5.45 c	5.75 c	5.76 b	5.73 c
	0.075	4.97 cd	4.84 c	4.88 d	4.60 c	5.28 c
	0.1	3.80 d	3.70 d	3.67 e	3.68 d	3.91d
Lactic acid	0	8.41 a	8.31 a	8.48 a	8.18 a	8.04 a
	0.1	8.14 a	8.11 a	8.09 a	8.18 a	7.78 a
	0.2	6.27 b	6.17 b	6.16 b	6.41 b	5.98 b
	0.3	3.64 c	3.62 c	3.58 c	3.71 c	3.64 c
	0.4	3.54 c	3.58 c	3.40 c	3.40 c	3.62 c

***Values with the same letter in the same column within each treatment are not significantly different (*P*>0.05); initial populations were 3.50, 3.50, 3.63, 3.36 and 3.56 for *E. coli* O157:H7 994, Cider, E0019, F4546, and H1730, respectively.**

Table 2 also indicates significant inhibitions (1.66–1.88 log cfu/ml) on the growth of *E. coli* O157:H7 strains subjected to 0.025% propyl gallate. Increasing concentrations of propyl gallate from 0.05% to 0.075% further enhanced its inhibitory efficacy for *E. coli* O157:H7 strains from 2.48–2.99 to 3.02–3.64 log cfu/ml. For the propyl gallate, 0.1% was the most effective (3.67–3.91 log cfu/ml) in inhibiting the growth of microorganisms. Whereas, Stapleton et al. (2004) reported that 0.03% propyl gallate had no antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) after incubation at 35ºC for 24 h. These differences in the efficacy of propyl gallate

in inhibiting the growth of microorganisms may be the results of differences in bacterial strains and solvent used for dissolving propyl gallate. In our trial study (data not shown), TSB supplemented with 0.1% ethyl alcohol alone, which was used in dissolving propyl gallate, did not affect the growth of *E. coli* O157:H7 strains. Overall, the results in Table 2 indicate that the magnitude of inhibition on the bacterial growth was approximately 5 log greater than the control treatment as well as bacterial populations were contained at initial inoculum level when microorganisms were subjected to 1% L-ascorbic acid, 0.3% lactic acid and 0.1% propyl gallate, respectively. Moreover, bacterial populations presented in cfus in Table 2 were different from the results of OD readings in Figs. 1-3 indicating that OD reading alone may not be the best indicator for the quantitative determination of bacterial growth in TSB. For example, OD readings (Fig. 3) of TSB supplemented with lactic acid at the concentrations of 0.2% and above were the same as 0 while populations of microorganisms in 0.2% lactic acid (6.0-6.2 log cfu/ml) were significantly higher than those (3.4-3.6 log cfu/ml) of 0.4% lactic acid.

In order to investigate any synergistic antimicrobial efficacy of L-ascorbic acid and propyl gallate with the combination of lactic acid, each two concentrations of L-ascorbic acid and propyl gallate, which showed minimal inhibitory effect (referring to Table 2) on the growth of *E. coli* O157:H7, were chosen and their combination impact on the pH of TSB are shown in Table 3. Findings in Table 3 are consistent with those of Table 1 in that addition of 0.25% and 0.5% ascorbic acid lowered pH of control (TSB alone) by approximately 1 to 2. Moreover, the addition of lactic acid generally further lowered the pH of L-ascorbic acid and propyl gallate, respectively. In specific, increasing concentrations of lactic acid from 0.1% to 0.3% lowered pH of L-ascorbic acid (6.03 for 0.25% and 4.99 for 0.5%) and propyl gallate (6.88 for 0.025% and 6.90 for 0.05%) to 5.11–4.12 and 6.20–4.61, respectively. However, pH of TSB was relatively less influenced by the addition of propyl gallate alone than L-ascorbic acid as shown in Table 1.

The effect of lactic acid on the efficacy of L-ascorbic acid in inhibiting the growth of *E. coli* O157:H7 is shown in Table 4. Results in Table 4 indicate that addition of lactic acid idividually at 0.2% and 0.3% to TSB significantly (by 2 to 5 log cfu/ml) inhibited the growth of *E. coli* O157:H7 strains compared to control, whereas OD readings at both concentrations were 0. The addition of 0.1% lactic acid to 0.25% L-ascorbic acid was more effective by about 100-fold than 0.1% lactic acid or 0.25% L-ascorbic acid alone in inhibiting the growth of all three *E. coli* O157:H7 (944, Cider and E0019) strains. Increasing the L-ascorbic acid concentration to 0.5% with the addition of 0.1% lactic acid enhanced inhibitory efficacy of 0.5% L-ascorbic acid or 0.1% lactic acid alone by up to approximately 3 and 5 log cfu/ml, respectively. In other words, combination of 0.5% L-ascorbic acid and 0.1% lactic acid was successful to contain bacterial population at an initial inoculum level even after 8 h incubation at 37ºC. This is probably due to the synergistic effect of lactic acid and L-ascorbic acid. This synergistic effect caused by the addition of lactic acid to L-ascorbic acid was more evident with the addition of 0.2% lactic acid to 0.25% L-ascorbic acid. In specific, a combination of 0.2% lactic acid and 0.25% L-ascorbic acid was 2

to 5 log cfu/ml more effective in inhibiting growth of the microorganisms than 0.2% lactic acid or 0.25% L-ascorbic acid itself. Fujimoto *et al.* (2006) also clearly observed the synergistic effect of the combination of 0.37% ascorbic acid and 0.07% lactic acid in purified water, which reduced initial population (4.46 log cfu/ml) of *E. coli* population to undetectable level within 1 h of incubation at 37ºC. However, differences in the efficacy of our combination treatment from theirs may be the result of differences in laboratory media between TSB and purified water. It was also noteworthy that the Cider strain (2.92 log cfu/ml) was more effectively inhibited by 0.5% L-ascorbic acid alone than strain 944 (1.38 log cfu/ml) and strain E0019 (1.80 log cfu/ml).

Table 3. The pH of TSB supplemented with L-ascorbic acid and propyl gallate only and in combination of lactic acid used for the inhibiting growth of *E. coli* O157:H7.

Treatment			**pH**
		Lactic acid (%, v/v)	
TSB only (Control)		0	6.90
		0.1	5.87
		0.2	4.98
		0.3	4.45
L-ascorbic acid	0.25%	0	6.03
		0.1	5.11
		0.2	4.60
		0.3	4.67
	0.5%	0	4.99
		0.1	4.57
		0.2	4.32
		0.3	4.12
Propyl gallate	0.025%	0	6.88
		0.1	6.20
		0.2	5.20
		0.3	4.67
	0.05%	0	6.90
		0.1	6.03
		0.2	5.04
		0.3	4.61

Although OD readings for TSB alone reached approximately 0.5 after 8 h incubation at 37ºC, there was no specific correlation of OD readings to cfus due to the concentrations of chemicals supplemented and growth of microbial populations (Table 4). For example, OD readings for strains 944 and E0019 grown in TSB supplemented with 0.5% L-ascorbic acid only were 0.00, whereas the microbial populations were 7.06 and 6.68 log cfu/ml, respectively (Table4). Findings in Table 4 indicate that OD readings alone may not be a reliable measure for the quantitative determination of microbial growth.

Synergistic efficacy due to the addition of lactic acid to propyl gallate in inhibiting the growth of *E. coli* O157:H7 strains are shown in Table 5. To effectively contain the growth of microorganisms at initial inoculum level, 0.3% lactic acid was required for all strains, which is consistent with the previous findings (Table 2). Results in Table 5 shows that the presence of propyl gallate at 0.025% inhibited the growth of microorganisms by 1.22 to 1.94 log cfu/ml compared to control. However, in general, increasing the propyl gallate concentration alone to 0.05% did not significantly enhance the inhibitory efficacy. Addition of 0.1% lactic acid to 0.025% propyl gallate significantly (2 log cfu/ml) inhibited the growth of microorganisms compared to 0.1% lactic acid alone while there was no additional inhibitory effect (P>0.05) compared to 0.025% propyl gallate itself. Increasing the concentration of lactic acid to 0.2% in TSB containing 0.025% propyl gallate showed further enhanced inhibitory efficacy of 0.2% lactic acid or 0.025% propyl gallate itself by up to 2 and 3 log cfu/ml, respectively. Overall the magnitude of inhibition was significant with the combinations of 0.025-0.05% propyl gallate and 0.2-0.3% lactic acid. Moreover, OD readings shown in Table 5 confirmed earlier findings (Table 4) that the spectrophotomer used for this particular study may require approximately 7 log cfu/ml of bacterial population in TSB as a detection threshold.

Table 4. Effect of lactic acid on the efficacy of L-ascorbic acid in inhibiting the growth of three strains of *E. coli* O157:H7 after 8 h incubation at 37ºC.

Treatment			OD and population (log_{10} CFU/ml) of *E. coli* O157:H7 strains*					
		Lactic acid (%)	944		Cider		E0019	
			OD	Population	OD	Population	OD	Population
TSB only		0	0.52	8.44 a	0.50	8.62 a	0.56	8.48 a
		0.1	0.17	8.51 a	0.24	8.24 ab	0.25	8.13 a
		0.2	0.00	6.59 b	0.00	6.19 c	0.00	5.60 c
		0.3	0.00	3.63 c	0.00	3.68 d	0.00	3.64 d
L-ascorbic acid	0.25%	0	0.3	8.38 a	0.31	8.40 a	0.36	8.31 a
		0.1	0.00	6.59 b	0.00	6.72 bc	0.00	6.61 b
		0.2	0.00	3.22 c	0.00	4.03 d	0.00	3.75 d
		0.3	0.00	3.44 c	0.00	3.31 d	0.00	3.32 d
	0.5%	0	0.00	7.06 b	0.00	5.70 c	0.00	6.68 b
		0.1	0.00	3.89 c	0.00	4.00 d	0.00	3.73 d
		0.2	0.00	3.37 c	0.00	3.08 d	0.00	3.26 d
		0.3	0.00	3.40 c	0.00	3.25 d	0.00	3.28 d

***Values with the same letter in the same column within each strain are not significantly different (*P*>0.05); initial populations were 3.34, 3.31, and 3.45 for *E. coli* O157:H7 994, Cider, and E0019, respectively.**

Table 5. Effect of lactic acid on the efficacy of propyl gallate in inhibiting the growth of three strains of *E. coli* O157:H7 after 8 h incubation at 37ºC.

Treatment			OD and population (log_{10} CFU/ml) of *E. coli* O157:H7 strains*					
		Lactic acid (%)	944		Cider		E0019	
			OD	Population	OD	Population	OD	Population
TSB only		0	0.59	8.38 a	0.55	8.52 a	0.51	8.41 a
		0.1	0.26	8.13 a	0.20	8.19 a	0.20	7.95 a
		0.2	0.00	6.01 bcd	0.00	6.16 c	0.00	6.28 b
		0.3	0.00	3.79 f	0.00	3.51 e	0.00	3.46 d
Propyl gallate	0.025%	0	0.02	7.16 ab	0.00	6.88 bc	0.00	6.47 b
		0.1	0.00	6.64 bc	0.00	6.15 c	0.00	6.29 b
		0.2	0.00	4.63 def	0.00	4.47 de	0.00	4.28 cd
		0.3	0.00	3.49 f	0.00	3.44 e	0.00	3.39 d
	0.05%	0	0.00	5.49 cd	0.00	5.50 cd	0.00	5.88 b
		0.1	0.00	5.39 cde	0.00	5.65 cd	0.00	5.23 bc
		0.2	0.00	3.99 ef	0.00	3.49 e	0.00	3.46 d
		0.3	0.00	3.58 f	0.00	3.37 e	0.00	3.44 d

*Values with the same letter in the same column within each strain are not significantly different (*P*>0.05); initial populations were 3.52, 3.38, and 3.47 for *E. coli* O157:H7 994, Cider, and E0019, respectively.

CONCLUSION

TSB supplemented with 1% L-ascorbic acid, 0.3% lactic acid or 0.1% propyl gallate was able to contain the population of *E. coli* O157:H7 at an initial inoculum level completely inhibiting their growth even after 8 h of incubation at 37ºC. Significant synergistic inhibition caused by the addition of 0.2% lactic acid for either L-ascorbic acid or propyl gallate was observed for all *E. coli* O157:H7 strains tested. Results of OD readings due to microbial growth were not correlated to actual number of colony forming units of microorganisms in TSB. In addition to their health benefits, L-ascorbic acid and propyl gallate alone or combinations of them with lactic acid may be considered as one of hurdles in controlling harmful microorganisms on food product. Although results from this study may provide guidelines for the development of effective methods in controlling harmful pathogens, additional research is needed to confirm their efficacy on in vivo food product due to the complicated matrix of food system.

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تـاثير بعض المـواد المضـادة للأكسـدة علـى نمـو وحيويـة بكتيريـا *E.coli* 0157:H7

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يعتبر كل من L- ascorbic acid و propylgallate من مضـادات الاكسدة وقد تم استخدام تركيزات مختلفة منهمـا وكذلك lactic acid لمعرفـة تـاثير تلك المواد علـى تثبيط نمو بكتريـا *E.coli* 0 157:H7

ولقد تم استخدام خمس سلالات مختلفة من بكتريـا *E.coli* 0 157:H7 وذلك بتنميتهـا فـى بيئـة tryptic soy broth والتحضين علـى ٣٧م لمدة ٨ سـاعات وتتبـع النمو وذلك بقيـاس optical density كل ساعتين وتلى ذلك العد بعد ٨ ساعات من التحضين.

ولقـد اظهـرت النتـائج ان L- ascorbic acid بتركيـز ١%(وزن /حجـم) و propylgallate ٠,١ %(وزن /حجم) و lactic acid ٠,٣%(وزن /حجم) كان لهـا تـاثير قوى علـى تثبيط كل انواع بكتريا *E.coli*

هذا ولقد لوحظ ان عند الجمع أو الخلط بين هذه المواد معـا كـان لهـا تـاثير اقوي علـى التثبيط حيث وجد ان ٠,٢% من L- ascorbic acid و ٠,٠٢٥% منpropylgallate مضـاف لكل منهما ٠,٢% من lactic acid هى النسب ذات التاثير الاقوى.

ولذا يمكن القول ان اضـافة تلك المواد المضـادة للاكسدة قد ادت الى تثبيط نمو بكتريا *E.coli*