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Effect of Marinating Chicken Meat with Lemon, Green Tea and Turmeric Against Foodborne Bacterial Pathogens

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Abstract: Foodborne diseases affect millions of people each year. To reduce the incidence of bacterial foodborne pathogens more effective treatment methods are needed. In this study, we evaluated the effect of marinating chicken breast fillets with extracts of lemon, green tea and turmeric against *Campylobacter jejuni* and *Salmonella enteritidis*. The prepared plant extracts in phosphate buffered saline were added to chicken breast fillets both singly and in combination and were incubated at 4°C. Cell counts were made at regular intervals and the viability percentage was calculated. A combination of green tea, lemon and turmeric was found to be the most effective against *C. jejuni* and *S. enteritidis* killing all the bacteria within 12 hrs of incubation, with a 5-log reduction in growth within 1 hr of incubation and proved to be more effective than any of the extracts used alone. Further, combinations of both lemon and green tea and lemon and turmeric killed all *C. jejuni* isolates, but not *S. enteritidis*, within 24 hrs of incubation. This study showed synergistic effects of lemon, green tea and turmeric extracts as bactericidal agents and that combinations of plant extracts were more effective than using the extracts singly. In summary, it was found that these extracts were effective on chicken meat and should be able to be used as marinates on other meats as well. Since foodborne bacteria are not entirely eliminated using current treatment techniques, plant extracts like lemon, green tea and turmeric could be used as additional treatment options.

Key words: Marinating, tea, turmeric, lemon, plant extract, chicken breast fillet

INTRODUCTION

Bacterial foodborne illness affects 48 million people in United States with 128,000 hospitalizations and 3,000 deaths (CDC, 2010). In addition to affecting millions each year, bacterial foodborne diseases are very expensive causing huge losses to the food industry and consumers. Despite methods to reduce or eliminate bacterial foodborne pathogens, food poisoning is still a major concern (Shan *et al.*, 2007). Due to increasing awareness among consumers and the increasing demand for "organic" foods free of synthetic additives and preservatives, the interest in developing novel antibacterial substances has increased (Suhaj, 2006). Therefore, plant extracts such as extracts of herbs and spices including green tea, turmeric and lemon are becoming more popular. For example, catechin and epicatechin isolated from green tea have been extensively studied and shown to be effective against a wide range of foodborne pathogens (Taguri *et al.*, 2004). There have been numerous studies on polyphenols found in green tea and their health benefits. Not only is green tea an antioxidant, but is also known for its antibacterial, antimycotic, antiviral activity as well as antitoxic activity against bacterial hemolysins (Okubo *et al.*, 1989). A recent study reported that green tea inhibits *Listeria monocytogenes* at concentrations as low as 0.026 to 0.68 mg/mL (Mbata *et al.*, 2008).

Flavonoids are found not only in green tea, but also in turmeric in the form of curcuminoids. Curcumin is the active ingredient of turmeric and is the yellow color pigment of turmeric. In a study conducted by Central Food Technological Research Institute, India, it was reported that turmeric oil showed bactericidal activity against both gram positive and gram negative bacteria, although susceptibility of gram positive bacteria was much higher than that of gram negative bacteria (Negi *et al.*, 1999). Turmeric was found to contain 5-8% essential oil and 3-4% of curcumin (Ruby *et al.*, 1995). Curcumin from turmeric is also an effective antibacterial agent with bactericidal activity against *Staphylococcus aureus*, *E. coli*, *Bacillus cereus* and *Pseudomonas aeruginosa* (Jayaprakasha *et al.*, 2005). In addition to being bactericidal, turmeric also exhibited antifungal activity against *Aspergillus flavus*, *Fusarium liforme* and *Pencillium digitatum*, antiviral activity against Epstein Barr Virus and most importantly, HIV-1 and antiprotozoal activity against *Entamoeba histolytica*, *Leishmania sp* and *Plasmodium falciparum* activities (Chattopadhyay *et al.*, 2004).

In addition to flavonoids, other polyphenolic compounds such as hydrolysable tannins are also found in abundance in plants and are potential antibacterial agents. In a study conducted in Japan (Taguri *et al.*, 2004) plant polyphenols were shown to be effective

against 20 different strains of *Staphylococcus aureus* and 27 different strains of *Vibrio* sp. The same study indicated that *Staphylococcus aureus* and *Vibrio* sp were more susceptible to the polyphenols than *Salmonella* sp and *E. coli*. Other than plant polyphenolic compounds, many household sanitizers such as white vinegar, lime and lemon juices are highly useful as antibacterial agents (Vijayakumar and Wolf-Hall, 2002). A study in Nepal showed that lime juice was very effective in inhibiting *Vibrio cholerae*, *Enterobacter* sp, *Citrobacter* sp and *E. coli* (Jayana *et al.*, 2010). Both limes and lemons contain high concentrations of citric acid, the major organic present in these juices, which is partly responsible for the antibacterial activity of these fruits. Lemon oil, an essential oil, also showed bactericidal activity in addition to the citric acid of lemon juice. Korean researchers found that limonene was the major essential oil found in citrus with a concentration of approximately 82% and the essential oils showed bactericidal effects against *Propionibacterium acnes* and *Staphylococcus epidermidis*. Lemon essential oils also showed antioxidant activity (Kim *et al.*, 2008a). Essential oils are known to penetrate the cell membrane and mitochondrial membranes, thereby increasing permeability of the organelles which lead to ion leakage (Raybaudi-Massilia *et al.*, 2006). Citrus species also contain a group of flavonoids including polymethoxy flavones, flavone glycosides and limonoids which enhance antimicrobial activity (Ladaniya, 2008). A recent study focused on the composition of *Citrus jambhiri* and researchers isolated seven different kinds of polymethoxy flavones and showed that limonin was the most abundant natural product isolated from the plant (Hamdan *et al.*, 2011). Another study in Nigeria also showed that lime juice (both aqueous and ethanol extracts) killed most gram positive and gram negative bacteria at a concentration of 256 mg/mL (Aibinu *et al.*, 2006). The objective of this study was to determine the antibacterial activity of lemon, green tea and turmeric when applied as a marinade on chicken breast fillets. These three plant extracts were tested both alone and in combination against four different isolates of *Campylobacter jejuni* and one isolate of *Salmonella enteritidis* on marinated chicken breast fillets.

MATERIALS AND METHODS

Bacterial cultures and media: *Campylobacter jejuni* 81176, *C. jejuni* from Pre-Chilled Chicken Carcass (PRCC), *C. jejuni* from Post-Chilled Chicken Carcass (POCC), *C. jejuni* from Retail Chicken Carcass (RECC) and *Salmonella enteritidis* (T1B4E). Each of the strains was allowed to grow for 18 hrs. For initial isolation and enrichment of *C. jejuni*, *Campylobacter* Enrichment Broth (Acumedia®) was used. For initial culturing of *S. enteritidis*, Nutrient Broth (DIFCO®) was used. Mueller Hinton Agar (DIFCO®) was used for plating for all the

organisms after serial dilution. For *C. jejuni*, in addition to Mueller Hinton Agar, *Campylobacter* Enrichment Agar supplemented with 5% Horse Blood was used as control.

Plant extracts: All plant extracts were purchased from a local grocery and were prepared in 1X Phosphate Buffered Saline (PBS) and maintained at 4°C until further use.

Turmeric extraction: Turmeric powder (10 g) was boiled in 100 mL of sterile water for 20 min and filtered through sterile gauze to remove impurities. The pH of the extract was adjusted to 7.0±0.2 using 10N NaOH and was autoclaved for 15 min. The extract was maintained at 4°C until further use (Weerasekera *et al.*, 2008).

Lemon extraction: Lemon fruits were washed with sterile water. Each lemon was then cut using a sterile knife and 20 g of the cut pieces were immersed in 100 mL 96% ethanol and ground with a mortar and pestle and held at room temperature for 48 hrs. Held extracts were filtered through Whatman No.1 filter paper into petri dishes and were once again held at room temperature to evaporate the ethanol. After 48 hrs, the dried filtrate was resuspended in 15 ml of PBS. The extract was stored at 4°C until further use (Valtierra-Rodriguez *et al.*, 2010).

Tea extraction: Dried and powdered green tea leaves (Lipton®) were first immersed in 20% w/v PBS and were held at room temperature for 3 hrs. They were then centrifuged at 15,000 rpm for 10 min. The supernatants were transferred to a fresh tube and pH was adjusted to 7.0±0.2 using 10N NaOH and stored at 4°C until further use (Diker *et al.*, 1991).

Preparation of food model: Raw chicken breast fillets (boneless) were purchased at a local supermarket in Fayetteville, AR. The breast fillets were first washed with distilled water 10 times and rinsed with sterile water twice. The fillets were then cut into pieces of approximately 5 cm x 5 cm using a sterile knife. The pieces were kept in sterile open petri dishes and exposed to UV (at 254 nm) for 30 min each side and then frozen at -20°C for 24 hrs to reduce any *Campylobacter* counts. Bacterial counts were taken both after UV exposure and after freezing to determine the initial counts present in the fillets (Valtierra-Rodriguez *et al.*, 2010).

Inoculation of food model: Single pieces of the cut fillets (5 cm x 5 cm size) were each inoculated with 100 µl of the above grown bacterial suspensions of *C. jejuni* and *S. enteritidis* isolates (at 10⁷ CFU/mL) and held at room temperature for 30 min in a well aerated sterile bottle to allow bacterial attachment.

Marinade preparation: To the inoculated breast fillets, 5 ml of the extracts were added both individually and in the following combinations: 1. lemon and green tea (L + G); 2. green tea and turmeric (G + T); 3. turmeric and lemon (L + T) and 4. lemon, green tea and turmeric (L + G + T). The inoculated and marinated meat samples were incubated at 4°C for various times.

Antibacterial activity: The breast fillet pieces of approximately 5 cm x 5 cm were shaken vigorously in a sterile plastic bag with 5mL PBS to remove attached bacteria. Viable cell counts were made from the above samples, by serial dilution, at regular intervals of 1 h, 6 h, 12 h, 24 h and 36 h. The results were expressed in terms of log CFU Vs time for each isolate. One piece of uninoculated chicken breast fillet (approximately 5 cm x 5 cm) marinated with 1X PBS and one piece each of breast fillet inoculated with *C. jejuni* and *S. enteritidis* isolates marinated with 1X PBS served as controls. All tests were done three times to establish statistical significance. Statistical analysis was performed using JMP 8.0 provided by University of Arkansas, Fayetteville. The results were considered statistically significant with $p < 0.001$.

RESULTS AND DISCUSSION

Chicken breast fillets were marinated with lemon, green tea and turmeric both singly and in combination. The effects of these extracts on chicken meat were tested against four strains of *C. jejuni* including 81176, PRCC, POCC and RECC and one isolate of *S. enteritidis*: T1B4E (Table 1). It was found that the combination of lemon, green tea and turmeric ($p < 0.001$) was most effective against all four isolates of *C. jejuni* and the one isolate of *S. enteritidis* (Fig. 1), killing all the strains within 12 hrs of incubation with approximately a 5-log reduction of all bacteria within 1 h of incubation. To our knowledge, this paper is the first of its kind to detect antibacterial activity of various combinations of these three plant extracts against *C. jejuni* and *S. enteritidis* on chicken breast fillets.

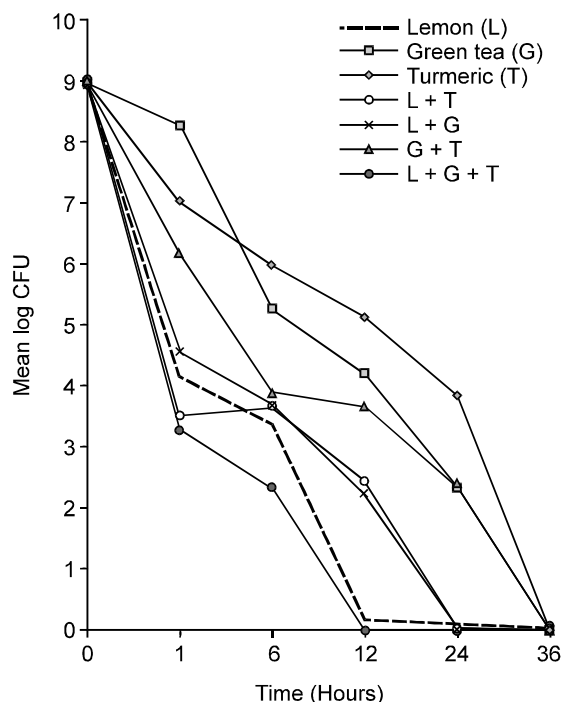


Fig. 1: Log CFU Vs. time for various plant extracts, both singly and in combination against *Salmonella enteritidis*. A combination of lemon, green tea and turmeric was found to be the most effective, killing the isolate within 12 hrs of incubation with a 6.6-log decrease in growth after 6 hrs of incubation ($p < 0.001$)

This study also found that a combination of lemon and green tea ($p < 0.001$) killed all the isolates within 36 hrs. Out of the bacteria tested, *C. jejuni* was found to be more susceptible to lemon green tea combination than *S. enteritidis* strains with a 4-log decrease in growth after 1 hr of incubation. Although the combination killed all the *C. jejuni* isolates within 24 hrs, individually green tea killed only *C. jejuni* strains 81176 (Fig. 2) and RECC (Fig. 3) in 36 hrs while *C. jejuni* strains POCC and

Table 1: An overview of the bactericidal activities of green tea, lemon and turmeric on, *C. jejuni* 81176, PRCC, POCC and RC and *S. enteritidis*, inoculated chicken breast fillets. The table below shows the efficacy of these extracts

	<i>C. jejuni</i> 81176	<i>C. jejuni</i> PRCC	<i>C. jejuni</i> POCC	<i>C. jejuni</i> RECC	<i>S. enteritidis</i>
Lemon (L)	++	++	++	+	+
Green tea (G)	+	-	-	+	-
Turmeric (T)	-	-	-	-	+
L + G	++	++	++	++	+
L + T	++	++	++	++	++
G + T	+	+	+	-	-
L + G + T	+++	+++	+++	+++	+++

Legend: +++ indicates most effective extract killing the bacteria within 12 hrs of incubation. ++ indicates extracts killing the bacterial isolates within 24 hrs of incubation. + indicates bacterial death only at 36 hrs of incubation and - indicates the extracts did not kill the bacteria even after 36 hrs of incubation.

L + G indicates a combination of lemon and green tea; L + T a combination of lemon and turmeric; G + T green tea and turmeric combination and L + G + T indicates a combination of lemon, green tea and turmeric. PRCC - Pre Chilled Chicken Carcass; POCC - Post Chilled Chicken Carcass; RECC - Retail Chicken Carcass

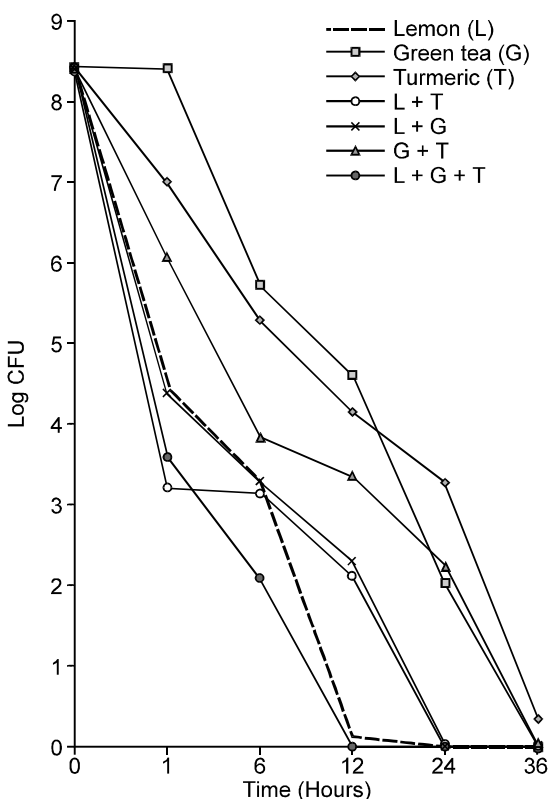


Fig. 2: Log CFU Vs. time for *C. jejuni* 81176 (human isolate) inoculated on chicken meat against plant extracts. Green tea killed *C. jejuni* 81176 in 36 hrs whereas turmeric failed even after 36 hrs. However, green tea and turmeric combination killed the strain in 36 hrs with a 4.5-log reduction in growth after 6 hrs of incubation ($p < 0.001$)

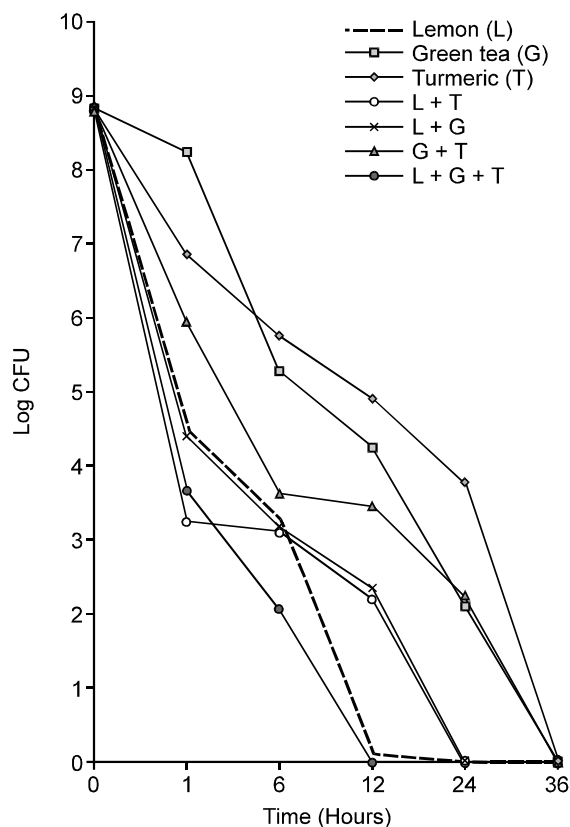


Fig. 3: Log CFU Vs. time of combinations of plant extracts against *C. jejuni* strain RECC (poultry isolate) on meat. Green tea killed *C. jejuni* strain RECC after 36 hrs of incubation, but lemon and turmeric together killed within 24 hrs with a 5.5-log decrease in growth within 6 hrs of incubation ($p < 0.001$)

PRCC were not killed even after 36 hrs. Green tea research has been going on for a long time and the health benefits of green tea are well known. Green tea is known to inhibit bacteria like *Helicobacter pylori* as reported by Takabayashi *et al.* (2004) and Yee *et al.* (2002) in their studies. Furthermore, it has been proven that green tea catechins do not disturb the intestinal microflora which is highly advantageous (Cabrera *et al.*, 2006). Catechins and epicatechins are flavonoids compounds found in green tea and it was found that green tea catechins along with citrus flavonoids inhibit invasion of mouse MO₄ (malignant mouse fibrosarcoma) cells into embryonic chick heart *in vitro* (Bracke *et al.*, 1991). This suggests that these two classes of polyphenols are also anticarcinogenic when combined. A group in Japan discovered that citric acid was effective in inhibiting *Vibrio parahemolyticus* and *Vibrio anguillarum* (Tomotake *et al.*, 2006). Another study by Brock and Ketchum (1951) showed that citrus oil may have an inhibitory effect against the tubercle bacilli.

In addition, a combination of green tea and turmeric ($p < 0.001$) was also tested against all bacterial strains and the combination killed *C. jejuni* PRCC (Fig. 4), POCC and 81176 within 36 hrs with a 5-log reduction in growth after 6 hrs of incubation, but did not kill *C. jejuni* RECC and *S. enteritidis* even after 36 hrs. Out of the three *Campylobacter* strains killed, it was found that POCC was the most susceptible strain with a 6-log decrease in count within 12 hrs of incubation. However, turmeric alone did not kill *C. jejuni* isolates even after 36 hrs of incubation, but killed *S. enteritidis* after 36 hrs. Turmeric contains curcumin, a flavonoid and the active ingredient present. A study in Korea reported that turmeric was effective against *Streptococcus mutans* biofilm reduction and that turmeric might have destroyed the cell wall integrity of *S. mutans*, thereby destroying the biofilm (Kim *et al.*, 2008b). Another study reported that antibacterial efficacy of turmeric was far superior to other plant extracts like oolong tea extracts and catechin (Hirasawa *et al.*, 2006). A study from Nigeria has also

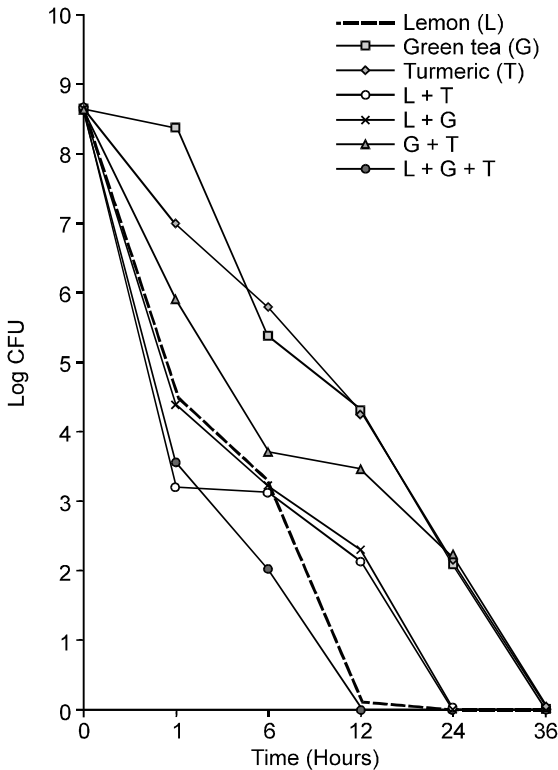


Fig. 4: Log CFU Vs. time for *C. jejuni* PRCC (poultry isolate) on chicken breast fillets against various plant extracts both singly and in combination. A combination of green tea and turmeric killed *C. jejuni* PRCC within 36 hrs with a 5-log reduction in growth after 12 hrs of incubation. In addition, lemon, green tea and turmeric combination killed the isolate within 12 hrs of incubation with 5-log decrease in count within 1 hr of incubation ($p < 0.001$)

shown that turmeric had antibacterial activity against *E. coli* and *Bacillus subtilis*, which was killed within 3 hrs (Ferdinand *et al.*, 2009). Furthermore, curcumin in combination with catechins and epicatechins, apart from being antibacterial, also have other health benefits. For example, in one study done in New York, it was shown that a combination of curcumin and (-) epigallocatechin-3-gallate (EGCG), major phenolic antioxidants found in turmeric and green tea, respectively, inhibited the growth of malignant oral cells (Khafif *et al.*, 1998). Another study group found that EGCG inhibited TNF- α gene expression, a key aspect in cancer prevention and treatment (Suganuma *et al.*, 1999).

A third combination was tested with lemon and turmeric ($p < 0.001$) which killed all the isolates within 24 hrs. Of the *Campylobacter* isolates, POCC (Fig. 5) was the most susceptible and 81176 was the least susceptible. In *S. enteritidis*, a 5-log decrease was seen within 12 hrs

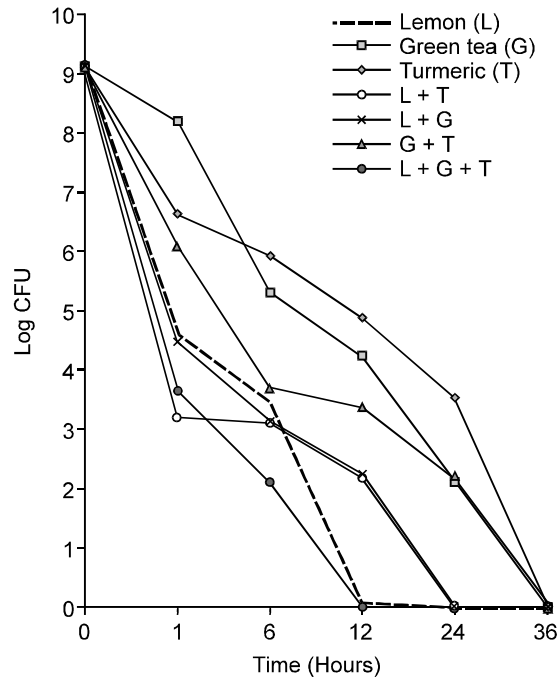


Fig. 5: Log CFU Vs. time for *C. jejuni* strain POCC (poultry isolate) on inoculated meat against turmeric, lemon and tea extracts and their combinations. A combination of lemon and turmeric killed the isolate within 24 hrs with a 5.7-log decrease in count within 1 hr of incubation. Lemon and green tea combination killed the POCC strain within 24 hrs of incubation ($p < 0.001$)

of incubation. Although L + T combination killed *S. enteritidis* within 24 hrs, lemon alone killed *Salmonella* only after 36 hrs of incubation. Of the *Campylobacter* isolates, 81176, POCC and PRCC were killed within 24 hrs of incubation with lemon alone, while RECC was killed only after 36 hrs. Being a common household sanitizer for over a decade, lemon and lime were known antimicrobial agents with a deodorizing scent owing to the presence of essential oils and citric acid. Researchers at Iowa State university showed that citric acid is very effective in inhibiting *Listeria monocytogenes* and *E. coli* O157:H7 (Ko *et al.*, 2008). Surprisingly, the antimicrobial property of lemons and limes were not attributed to their low pH. Lee *et al.* (2002) reported that sodium citrate also had antibacterial properties, thereby, reasoning that the antimicrobial property of lemons and limes were not due to their low pH.

Conclusion: Since food safety is a major concern to the food industry and the consumers, research is ongoing constantly to find more effective methods to reduce or kill foodborne bacterial pathogens. In this study it was shown that some combinations of plant extracts of

lemon, turmeric and green tea were effective in killing or reducing *Campylobacter jejuni* strains and *Salmonella enteritidis* attached to chicken breast fillets. Research is now underway to determine efficacy of other plant extract combinations on other pathogenic and spoilage bacteria on chicken and other meats.

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